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Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation☆

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Abstract

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three PPARs isoforms have been characterized: PPARα, β/δ and γ. As other nuclear receptors, the PPARs are organized in distinct functional domains: A/B, C or DNA binding domain (DBD), D, E or ligand binding domain (LBD) and F. The A/B domain contains the activation function 1 (AF-1) which is transcriptionally active in absence of ligands. The DBD and the LBD of the PPARs determine the specificity of promoter DNA sequence recognition and ligand recognition, respectively. An activation function 2 (AF-2) is contained in the E domain, which mediates the ligand-dependent activation of the receptor. The transcriptional activity of the PPARs is regulated by post-translational modifications, such as phosphorylation and ubiquitination. Phosphorylation of PPARs is controlled by environmental factors activating different kinase pathways leading to the modulation of their activities. PPARs degradation by the ubiquitin–proteasome system modulates the intensity of the ligand response by controlling the level of PPAR proteins in the cells. PPARs also control the expression of genes implicated in the inflammatory response via negative interference with different inflammatory pathways, such as NFκB, AP-1, C/EBP β, STAT-1 and NFAT. As such, PPARs influence inflammatory cytokine production and cell recruitment to the inflammatory sites. A better understanding of the mechanism of action of PPARs could improve the design of more specific and more efficient novel drugs. Molecules with dissociated effects could be useful for the treatment of lipid disorders or inflammation.

Keywords: Transcription factors; Phosphorylation; Ubiquitination and inflammation

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. PPARα was discovered in 1990 as the mediator of the response to peroxisome proliferators in rodents. Numerous studies performed during the last decade revealed that PPARs are implicated in several physiological processes, such as the regulation of lipoprotein and lipid metabolism, the inflammatory response, glucose homeostasis and cellular differentiation. To date, three PPAR isotypes have been characterized: PPARα, PPARβ (NUC-1 or PPARδ) and PPARγ each encoded by different genes. Each isotype has a specific expression pattern. PPARα is expressed in liver, kidney, muscle, heart and cells from the vascular wall. PPARγ is mainly expressed in adipose tissues where it plays a role in lipid metabolism. PPARβ/δ is expressed in a wide range of tissues and its functions are still unclear [1]. In this review, we will focus on the structure-activity relation of the PPARs and on the regulation of their functions by the post-translational modifications.

1. Structure and activity of PPARs

The PPAR proteins are composed of five different domains: a NH2-terminal region termed domain A/B, a domain C which binds the DNA (DNA binding domain (DBD)), a hinge region (domain D), a domain E which binds the ligands (ligand binding domain (LBD)) and a domain F (Fig. 1).

The A/B domain contains the activation function 1 (AF-1) which operates in absence of ligand, whereas the DBD is composed of two zinc fingers and contains nine cysteines which are conserved across the nuclear receptor superfamily [2]. This domain confers the DNA binding specificity to the PPARs. These nuclear receptors control gene expression by binding to DNA sequences, called peroxisome proliferator response elements (PPRE), after heterodimerization with the nuclear receptor Retinoid X Receptor (RXR) [3]. PPREs consist of the direct repeat of the hexanucleotide DNA

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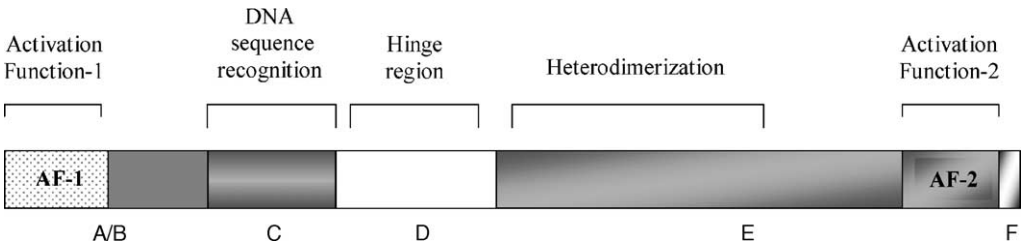

Fig. 1. Schematic representation of the structure of PPARs. PPAR proteins are organized in distinct domain which display specific function. The domain A/B contains the activating function 1 which is independent of the presence of ligand, the domain C is implicated in the DNA binding, the domain D is a hinge region and the domain E is implicated in the ligand recognition, contains an activating function 2 which is dependent of the presence of ligand and is necessary for the heterodimerization with RXR. Concerning the domain F, no function has been identified to date.

sequence AGGTCA separated by one or two nucleotides, respectively, termed Direct Repeat 1 and 2 (DR1 and DR2).

The LBD is formed of 12 α helical regions named H1 to H12. The interacting region of PPARγ with RXRα is localised in its LBD [4]. Moreover, this heterodimer is asymmetrically positioned, since the PPARγ H12 helix interacts with the RXRα H7 and H10 helix [4]. The structure of the LBD is organized in a three-layered, antiparallel helical sandwich [5]. This structure creates a hydrophobic cavity called ligand binding pocket (LBP). The size of the PPARγ LBP is very large and allows the binding of different sized ligands. Ligands of PPARα and γ are natural fatty acids and fatty acid derivatives. Eicosanoid derivatives from the lipoxygenase pathway, such as 8-S-hydroxytetraenoic acid (8-S-HETE) and leukotriene B4 (LTB4), and oxidized phospholipids from oxidized lipoproteins, are activators of PPARα. PPARγ is activated by eicosanoid derivatives from the cyclooxygenase and lipoxygenase pathways, like prostaglandins (PGJ2, PGH1 and PGH2) and 15-hydroxytetraenoic acid (15-HETE). The anti-diabetic glitazones used in the treatment of type 2 diabetes as insulin sensitizers are high affinity ligands for PPARγ. The lipid-lowering fibrates are ligands for PPARα [6]. To date, new synthetic molecules have been discovered with specific high affinity for each PPAR isotype [7,8]. The LBD contains an activation function 2 (AF-2) domain which requires the binding of the ligand to induce a transcriptional activation. Indeed, the binding of the ligand to the nuclear receptor mediates conformational changes leading to the repositioning of the H12 helix, which contains the core of the AF-2, away from the LBD and the H3 and H4 helix in order to create an interacting surface with the co-activators. The recognition of the co-activators is realized by the presence of LxxLL motifs in their sequence. This sequence interacts with two conserved amino acids present in the nuclear receptor C-terminus of the H3 helix (lysine) and in the H12 helix (glutamate). The isotype specific recognition of these co-activators is due the variability of the residues adjacent to the LxxLL motif [9]. Recently, it was demonstrated that interaction between the LBD of PPARα and a SMRT co-repressor motif in the presence of an antagonist of PPARα prevents the C-terminal activation helix to adopt its active position. This effect is due to the three-turn alpha helix structure of the co-repressor motif which prevents the repositioning of the AF-2 helix in its active conformation [10].

These studies have greatly improved the understanding of the structural basis of PPAR transactivation and should help the design of novel molecules with higher selectivity and specificity.

2. Regulation of PPAR activity by post-translational modifications

Recently, it was demonstrated that the transcriptional activities of PPARs are regulated by post-translational mechanisms including phosphorylation and ubiquination.

2.1. Phosphorylation

Environmental changes and extracellular signals modify the phosphorylation status of cell proteins. Phosphorylation of nuclear receptors is a major determinant of their transcriptional activity as shown for the oestrogen receptor (ER), the progesterone receptor (PR) and RXR. Similarly, PPARα is a phosphoprotein and its activity is modulated by its phosphorylation status [11]. Shalev et al. has shown that phosphorylation of PPARα is increased in response to insulin and this correlates with an enhancement of its transcriptional activity [11]. This observation was confirmed in a study realized by Juge-Aubry et al. [12]. Indeed, these authors have shown that PPARα is phosphorylated in response to insulin on serine 12 and 21, depending on the mitogen activated protein kinase (MAPK) pathways leading to increased the AF-1 activity of PPARα. A study realized on rat cardiac myocytes has shown that p38, a MAPK activated in stress conditions like ischemia, hypoxia and hypertrophic stimuli in the heart, phosphorylates PPARα on AF-1 serines and consequently enhances the transcriptional activity of this nuclear receptor by increasing its interaction with the transcriptional co-activator PGC-1 [13]. PPARα is also a target for other kinases. Indeed, mouse PPARα is also phosphorylated by protein kinase A (PKA) and this process increases its transcriptional activity and stabilizes the binding

of the nuclear receptor to the DNA [14]. Moreover, in vitro phosphorylation experiments illustrated that the PPARα DBD is strongly phosphorylated by PKA compared to the A/B domain and the LBD. Recently, it was also shown that cerivastatin, an inhibitor of HMG CoA reductase, increases PPARα transcriptional activity by inhibiting the formation of geranylgeranyl pyrophosphate [15]. The geranylation of small G proteins is necessary for translocation of these proteins to the membrane and for their activation. By inhibiting Rho A small G protein activation, cerivastatin stimulates PPARα transcriptional activity by reducing its phosphorylation [15]. All these studies show that PPARα is an important target protein for different phosphorylation pathways and suggest that PPARα activity is modulated by a variety of extracellular signals linked to different physiological processes.

PPARγ is also a phosphoprotein as it was demonstrated by Zhang et al. in 1996 [16]. In this study, it was shown that insulin treatment increases the ligand-independent transcriptional activity of PPARγ and synergizes with a PPARγ ligand to enhance its transcriptional activity. Moreover, the authors have demonstrated that PPARγ is phosphorylated by the MAPK pathway in vivo. By contrast, it has been shown that phosphorylation of mouse PPARγ by activators of MAPK, like growth factors, induces an inhibition of its transcriptional activity [17]. This effect occurs via the phosphorylation of serine 112 of PPARγ2. This result was confirmed by a study realized by Adams et al. in which it was shown that serine 84 of human PPARγ1 is phosphorylated by the MAPK ERK2 and JNK [18]. Mutation of this serine increases the AF-1 transcriptional activity of PPARγ. Camp et al. demonstrated that mouse PPARγ1 is also regulated by MAPK phosphorylation at a site located on serine 82 [19]. Moreover, these authors demonstrated that JNK, a MAPK, phosphorylates PPARγ2 and decreases its ligand-dependent transcriptional activity. The effect of serine 112 phosphorylation, by the MAPK, on the ligand-activated PPARγ transcriptional activity was explained by a modification of an interdomain communication which reduces ligand-binding affinity [20]. A study performed in adipocytic cell lines demonstrates that cellular growth and adipocyte differentiation is dependent on the phosphorylation status of PPARγ, which is controlled by growth factors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) [21]. In macrophages, a similar pathway of PPARγ regulation is functional [22]. Han et al. demonstrated that treatment of macrophages with transforming growth factor β1 and β2 (TGF β1 and β2) can inhibit PPARγ ligand-induced expression of CD36 via activation of the MAPK pathway. As for PPARα, basal and ligand-induced transcriptional activity of PPARγ is stimulated by activators of PKA [14]. However, phosphorylation by MAPK influences PPARα and PPARγ activity differentially: whereas MAPK phosphorylation activates PPARα it inhibits PPARγ.

Compared to PPARα and PPARγ, the post-translational control of PPAβ/δ has been less studied so far. However, it appears that both cAMP-elevating agents and PKA increase basal and ligand-activated transcriptional activity of PPARβ/δ [14,23,24].

Altogether, these studies clearly demonstrate that PPARs are regulated by kinases activated by numerous extracellular signals (Fig. 2). Therefore, PPAR activity is subject to modulation by a wide variety of physiological changes.

2.2. Ubiquitination

Recent data demonstrate that the ubiquitin–proteasome degradation system affects the activity of several nuclear receptors. This degradation pathway is implicated in the regulation of many short-lived proteins involved in essential cellular functions, including cell cycle control, transcription regulation and signal transduction [25]. The proteins degraded by this pathway are covalently modified on lysine residues by fixation of a 8 kDa polypeptide, called ubiquitin, in a three step process. In the first step, ubiquitin is activated by an ubiquitin-activating enzyme (E1). The activated ubiquitin is subsequently transferred to an ubiquitin carrier protein (E2). Finally, ubiquitin-protein ligase (E3) catalyzes the covalent binding of ubiquitin to the target protein. Following this process, multi-ubiquitinated proteins are rapidly degraded by the 26S proteasome [26]. Ligand-activation of PPARγ results in the degradation of this nuclear receptor via this pathway [27]. Furthermore, the repositionning of the AF-2 helix of PPARγ was shown to be essential for its ubiquitination and therefore, for its degradation. Interestingly, interaction with co-repressor proteins protects PPARγ of degradation whereas interaction with co-activators leads to enhanced degradation [27]. PPARα is also degraded by the ubiquitin–proteasome pathway and this degradation directly regulates its transcriptional activity [28]. In contrast to PPARγ, PPARα ligands protect the nuclear receptor against degradation by decreasing its ubiquitination [28]. These observations suggest that PPARα and PPARγ activity are differently regulated by the proteasome pathway. However, it should be noted that PPARα degradation was studied after 5 h of ligand treatment, whereas, PPARγ degradation was after 15–20 h. It is tempting to speculate that ligands regulate PPAR degradation in a timely manner. In a first stage, the ligand may protect PPAR from degradation in order to increase the ligand effect, whereas in a second stage, agonist-induced AF-2 repositionning and cofactor recruitment may lead to PPAR ubiquitination and degradation as a mechanism to arrest transcriptional activation (Fig. 3). Extracellular signals which activate intracellular phosphorylation pathways can also influence the degradation process, as shown for PPARγ [29]. Indeed, treatment of cells with an inhibitor of MEK kinases inhibits the degradation of PPARγ. In conclusion, PPAR degradation by the ubiquitin–proteasome pathway may be an important mechanism in the regulation of PPAR transcriptional activity by controlling cellular PPAR protein levels.

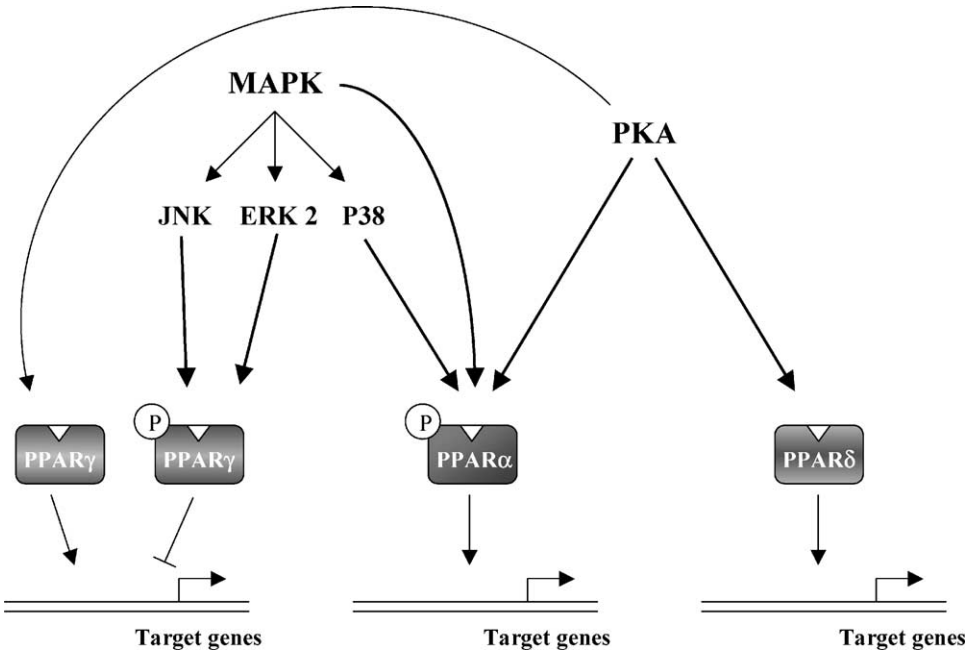

Fig. 2. Representation of kinases pathways implicated in the phosphorylation and in the regulation of PPARs transcriptional activity. The PPARs are targets for kinases. The function of the phosphorylation of PPARs appears to be specific of the kinase implicated and of the PPAR isotype phosphorylated. Indeed, MAPkinase phosphorylation increases the activity of PPARα and decreases the activity of PPARγ. However, PKA induces an increase of the transcriptional activity of the three PPAR isotypes. Since the kinases are activated by numerous extracellular signals and since the kinases modulate the PPARs activities, it appears that PPARs are regulated by physiological changes leading to the production of kinase activators. (P): described phosphorylation by kinases.

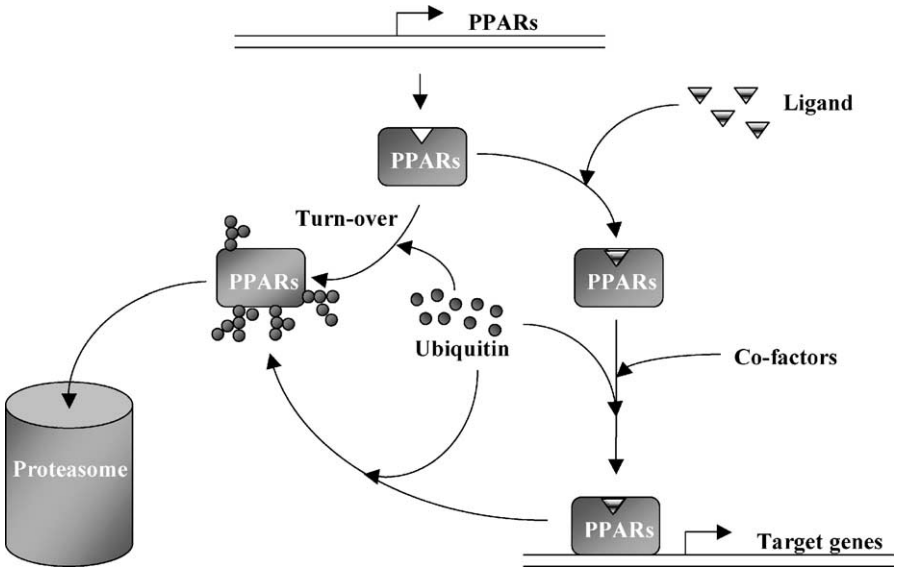

Fig. 3. Possible mechanisms of PPAR degradation by the ubiquitin–proteasome system. PPAR proteins are degraded by the ubiquitin–proteasome pathway. This system controls the PPAR proteins level in cells and then the intensity of the response to the ligand. However, in a first time the ligand stabilizes the PPAR proteins by decreasing its ubiquitination and in a second time, the ligand induces the degradation of the PPAR proteins as the consequence of the cofactors recruitment and in order to stop the response.

3. Transrepression by PPARs as a mechanism contributing to the control of the inflammatory response

The role of PPARs in the control of inflammation was first demonstrated for PPARα. PPARα-deficient mice treated with LTB4 were shown to exhibit a prolonged inflammatory response. The initially proposed mechanism was based on the fact that LTB4 is a PPARα-ligand, that, as a consequence, induces its own degradation by stimulating the β-oxidation pathway [30]. To date, several studies have confirmed the

anti-inflammatory properties of PPARs in vitro and in vivo. For example, administration of fibrate to patients with a moderate hyperlipidemia decreases plasma concentrations of interleukin-6 (IL-6), tumor necrosis factor-α (TNFα interferon-(IFNγ), fibrinogen and C-reactive protein (CRP) [31,32] whereas treatment of type 2 diabetics with rosiglitazone results in lowered plasma concentrations of MMP-9 (gelatinase B) and CRP [33].

Aorta from PPARα-deficient mice display a stronger inflammatory response to lipopolysaccharide (LPS) stimulation [34]. A similar observation was made in splenocytes of PPARα-deficient mice in which the production of IL-6 and IL-12, in response to LPS, is two to three times higher than in wild type splenocytes [35]. In liver, fibrates repress fibrinogen expression via PPARα [36]. Molecular studies of the anti-inflammatory effects of PPARs have given new insights into the action mechanism of this nuclear receptor in this process. This repression of fibrinogen is due to the inhibition of the C/EBP β pathway possibly through interaction of the co-activator glucocorticoid receptor-interacting protein-1 (GRIP-1) with PPARα [37]. In addition, PPARα can repress the NFκB and AP-1 pathways [34]. These effects occur via an interaction of PPARα with the Rel homology domain of the p65 subunit of NFκB, and via an interaction of the N-terminus DBD-containing domain of PPARα and the N-terminus of c-Jun, respectively [34]. The interaction of PPARα with p65 is not the only mechanism by which this nuclear receptor represses the NFκB pathway. PPARα also induces the expression of IκB, the major inhibitor of NFκB in smooth muscle cells and hepatocytes [38].

By inhibiting these inflammatory pathways, PPARα can repress the expression of inflammation mediators induced by extracellular inflammatory stimuli. For example, PPARα ligands repress cytokine-induced expression of vascular cell-adhesion molecule-1 (VCAM-1) [39,40], thrombin-induced endothelin-1 expression [41] and TNFα-induced intercellular adhesion molecule-1 (ICAM-1) expression in endothelial cells [40]. Moreover, in T lymphocytes, PPARα decreases the secretion of IL-2 and TNFα [42]. In hepatocytes, PPARα represses CAAT/enhancer-binding protein (C/EBP) activity which regulates fibrinogen β and CRP expression (Fig. 4) [37,43].

The role of PPARγ in the control of inflammation is more controversial. On the one hand, glitazones reduce colonic inflammation in a mouse model of bowel disease [44] and inhibits the production of TNFα and gelatinase B in LDL receptor-deficient mice [45]. By contrast, Thieringer et al. demonstrated that glitazones do not affect the LPS-dependent induction of IL-6 and TNFα expression in *db*/*db* mice and that glitazones have no effect on the secretion of certain cytokines in monocytes and macrophages [46]. Molecular studies have shown that PPARγ can interfere in vitro with inflammatory pathways, such as NFκB by physically interacting with p50 and p65 [47]. Similar as PPARγ, PPARα can also inhibit the AP-1 signalling pathway by interacting with c-Jun [34]. Moreover, glitazones inhibit the expression of c-fos in vascular smooth muscle cells which could be an additional mechanism for the repression of the AP-1 pathway by PPARγ [48]. PPARγ also inhibits the expression of inducible nitric oxide synthase (iNOS) via interference with the STAT-1, AP-1 and NFκB pathways [49]. Finally, in T lymphocytes, PPARγ ligands reduce IL-2 secretion due to an interaction of this nuclear receptor with the nuclear factor of activated T-cells (NFAT) [50] (Fig. 4).

Overall, PPARs can interfere with different steps of the inflammatory response by modulating the expression of chemokines, chemokine receptors and adhesion molecules in endothelial cells, smooth muscle cells, monocytes/macrophages and T cells [51].

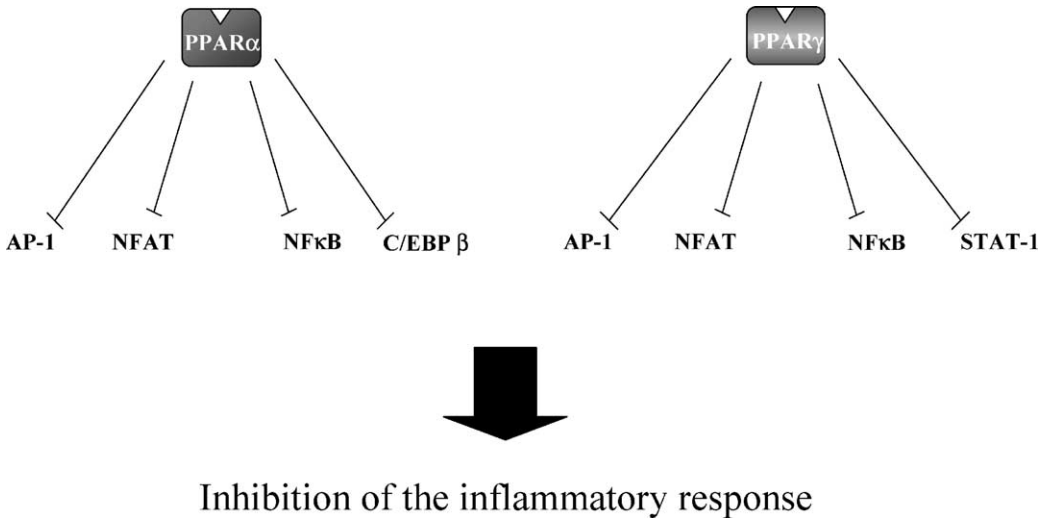

Fig. 4. Inhibition of the different inflammatory pathways by the PPARs. By interfering with major inflammatory pathways, the PPARs display anti-inflammatory functions. These properties of the PPARs lead to the modulation of the expression of chemokines, chemokine receptors and adhesion molecules and then, inhibit the inflammatory response.

4. Conclusion

Since the discovery of the PPARs, their role in the regulation of the metabolism of lipids and lipoproteins, and in the inflammatory response as well as the action mechanisms involved have been extensively studied. Moreover, the elucidation of the three-dimensional structure of the PPARs will allow the design of new ligands which are more specific and active. Several studies have shown that the regulation of the PPAR activity is under control of environmental changes and pathophysiological conditions. Further studies are necessary to improve our knowledge on mechanism governing PPAR transactivation and transrepression as well as the regulation of their expression and activity. The combination of such novel data along with knowledge of the structure of these receptors will undoubtedly improve the design of molecules with selective activity.

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