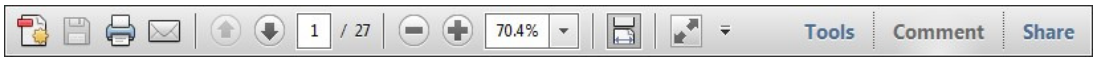
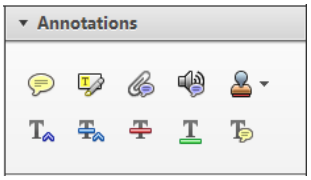
Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

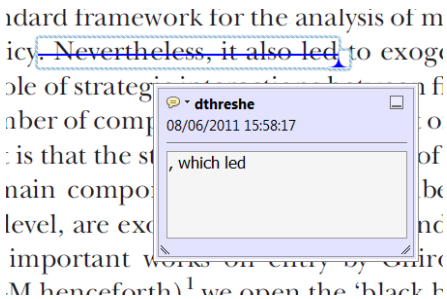


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

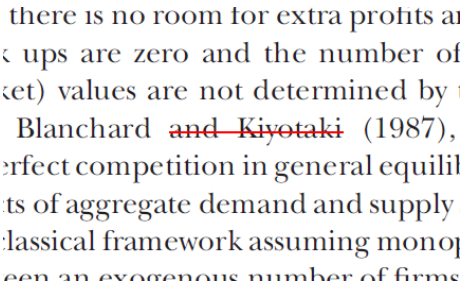


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

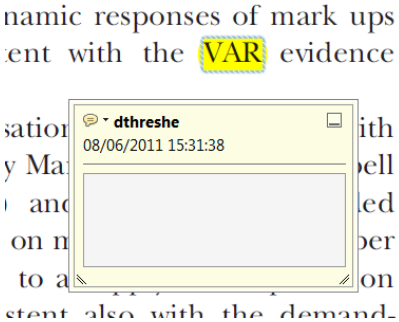


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

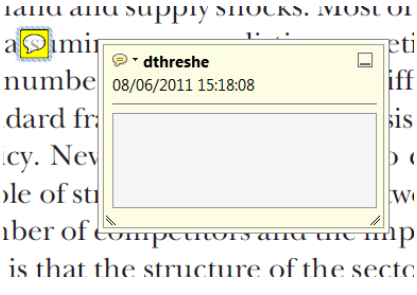


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

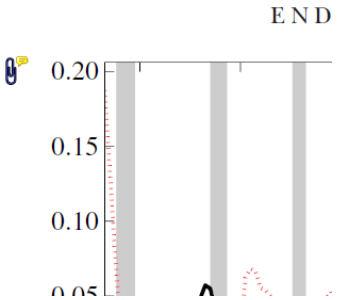


5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

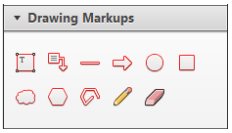
How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



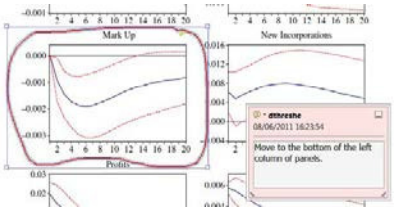
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



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REVIEW

Genomewide effects of peroxisome proliferator-activated receptor gamma in macrophages and dendritic cells – revealing complexity through systems biology

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ABSTRACT

Systems biology approaches have become indispensable tools in biomedical and basic research. These data integrating bioinformatic methods gained prominence after high-throughput technologies became available to investigate complex cellular processes, such as transcriptional regulation and protein–protein interactions, on a scale that had not been studied before. Immunology is one of the medical fields that systems biology impacted profoundly due to the plasticity of cell types involved and the accessibility of a wide range of experimental models. In this review, we will focus on peroxisome proliferator-activated receptor γ in macrophages and dendritic cells to highlight the crucial importance of systems biology approaches in establishing novel cellular functions for long-known signalling pathways.

Keywords Genome-wide studies, immune cells, peroxisome proliferator-activated receptor gamma, systems biology.

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Introduction

Our body is protected against intruders by multiple layers of defence, each part of a highly ordered, hierarchical and integrated immune system. Decades of research have led to the identification of several key molecular components that define the activity and communication of immune cells; however, our understanding is still far from complete.

Importantly, traditional research strategies lacking high-throughput technologies ignored the complexity of biological systems, screening gene functions on individual targets rather than focusing on biomolecular networks. Introduction of novel systems biology approaches opened the way to investigate the consequences of manipulation of a gene on the entire molecular network and has revolutionized the way we think about biological systems and their complexity.

In this review, we will summarize the recently available peroxisome proliferator-activated receptor gamma (PPARγ)-related 'omics' data in the context of macrophages and dendritic cells to demonstrate how a single protein can play such a diverse role in multiple biological processes. We will describe how genomewide studies from the recent years redefined the function of the receptor in these cell types. Moreover, we will demonstrate that re-analysis of existing genomewide studies can provide further insights into the biological role of a factor and identify novel cellular signalling pathways.

Systems biology approaches in immunology

Systems biology is the study of a complex entity using a holistic approach; which enables the prediction of the system's behaviour through the complex relationships among its components [1]. Such prediction of a system's behaviour can, and should be, later validated experimentally. It is clear that a biological signal is not generated by a particular molecule alone, but instead by the complex interaction of several factors [2]. A main driving force behind holistic approaches has been the development of novel high-throughput methods, such as microarray, RNA-seq, proteomics, ChIP-seq, RNAi screens and 3D imaging. These techniques are now indispensable in biological and medical research. Analysis of data generated through these methods requires multidisciplinary research

teams, which include experts from medicine to mathematics [3].

In the context of the immune system, a complex system of cells and signalling molecules, the use of mathematical models and bioinformatics approaches holds great potential in the identification of novel therapeutic targets [4–6]. Comparing the transcriptome and proteome of immune cells isolated from different stages of disease development and progression could have a significant clinical relevance [3].

Gardy *et al.* [1] summarized the system biology workflows available for immunology research, which includes the analysis of transcriptome, gene expression networks, proteomics, miRNAs, genetic polymorphisms as well as network inference and modelling. Visualization of the high-throughput output information is also crucial for understanding and interpreting data. Advances in this segment can be already seen in the literature [7]. Using a wide variety of software, published data can be reanalysed and gene lists or networks can be generated as a starting point for further experimental research [8–10].

This approach can also be used for studies aiming to identify drugs that may have potential indications in immunology (drug repurposing). Data obtained from large data sets can be utilized to generate networks that associate drugs to genes and diseases [11]. As an example, PPARγ agonists have been predicted, based on omics data, to have a therapeutic effect in colorectal cancer [12]. This effect has been confirmed *in vivo* experiments [13].

Genomewide studies of transcription factor binding sites have been performed in a variety of immune cells, including macrophages and dendritic cells. These approaches pinpoint the fact that lineage-determining transcription factors take part in defining the transcriptomic response of the cell upon stimulation [14].

General characteristics of PPARγ

PPARγ is a ligand-activated transcription factor that directly binds to specific DNA sequences and can respond to various ligands [15,16]. Many putative endogenous PPARγ ligands have been identified, most of them derived from unsaturated fatty acids hydroxyeicosatetraenoic acids and hydroxyoctadecadienoic acids (HODEs), such as 9- and 13-HODE [17,18]. PPARγ appears to be a sensor of a group of oxidized lipids, too. Selective synthetic ligands of PPARγ, called thiazolidinediones are also available, some of them being used in the treatment of diabetes [19].

PPARγ is expressed primarily in adipose tissue and kidney and is essential for adipogenesis [20–22]. However, PPARγ expression is not limited to metabolic tissues, as it is also expressed in various cells of the immune system such as macrophages, dendritic cells, eosinophils, T cells, and B cells [23–26].

PPARγ forms a permissive heterodimer with RXR, another member of the nuclear receptor superfamily. The PPARγ/RXR heterodimer can recognize specific sequences (PPAR response element) in the DNA and can initiate transcription of a group of target genes.

Despite the large amount of data available regarding the role of PPARγ in various model systems, it is safe to say that novel 'omics' methodologies allow us a more systematic characterization and detailed investigation of PPARγ function in different cell types. Transcriptomic data that are already available helped us to identify PPARγ-regulated target genes in different immune cell types. Cistromic data are also available for PPARγ and RXR and suggest a striking cell type-dependent binding pattern of PPARγ/RXR heterodimer (Fig. 1) [27]. In the following sections, we attempt to systematically summarize our recent knowledge of PPARγ-mediated macrophage and dendritic cell functions, including those novel insights that we could obtain with the application of 'omics' technologies.

PPARγ in macrophages

Macrophages are a highly heterogeneous population of immune cells residing throughout the body that participate in pathogen clearance, inflammatory response and tissue regeneration among others. PPARγ in macrophages is involved in lipid metabolism but also in regulation of immune functions. As mentioned above, PPARs bind various fatty acids and fatty acid by-products, regulating aspects of lipid metabolism, differentiation and cellular growth by transcriptional control of several downstream genes [28]. The receptor has well-documented accessory roles in the differentiation of the monocytes to macrophages in both mouse and human [29,30].

Macrophages, after the exposure of certain cytokines, can be driven to classical or alternative activation. Although this is a fairly simplistic view of macrophage polarization, it serves well to the development of system level concepts, which obviously would need to be validated for *in vivo* relevant cell types as well.

Classical macrophage activation (resulting in the so-called M1 macrophages) is driven by certain primary stimuli, such as IFNγ and as a second signal, bacterial LPS or cytokine TNF-α, leading to an inflammatory phenotype secreting TNFα, IL-12, IL-1, IL-6 and NO [31,32]. As a result, M1 macrophages acquire phenotypic changes making them capable of fighting a bacterial infection [33]. On the other hand, alternative activation of macrophages, mediated by IL-4, leads to an anti-inflammatory phenotype (M2 macrophages), participating primarily in tissue remodelling and regenerative processes. M2 macrophages have decreased levels of inflammatory cytokines and produce large amount of anti-inflammatory molecules including IL-10 and TGF-β [34]. PPARγ has been implicated in influencing the

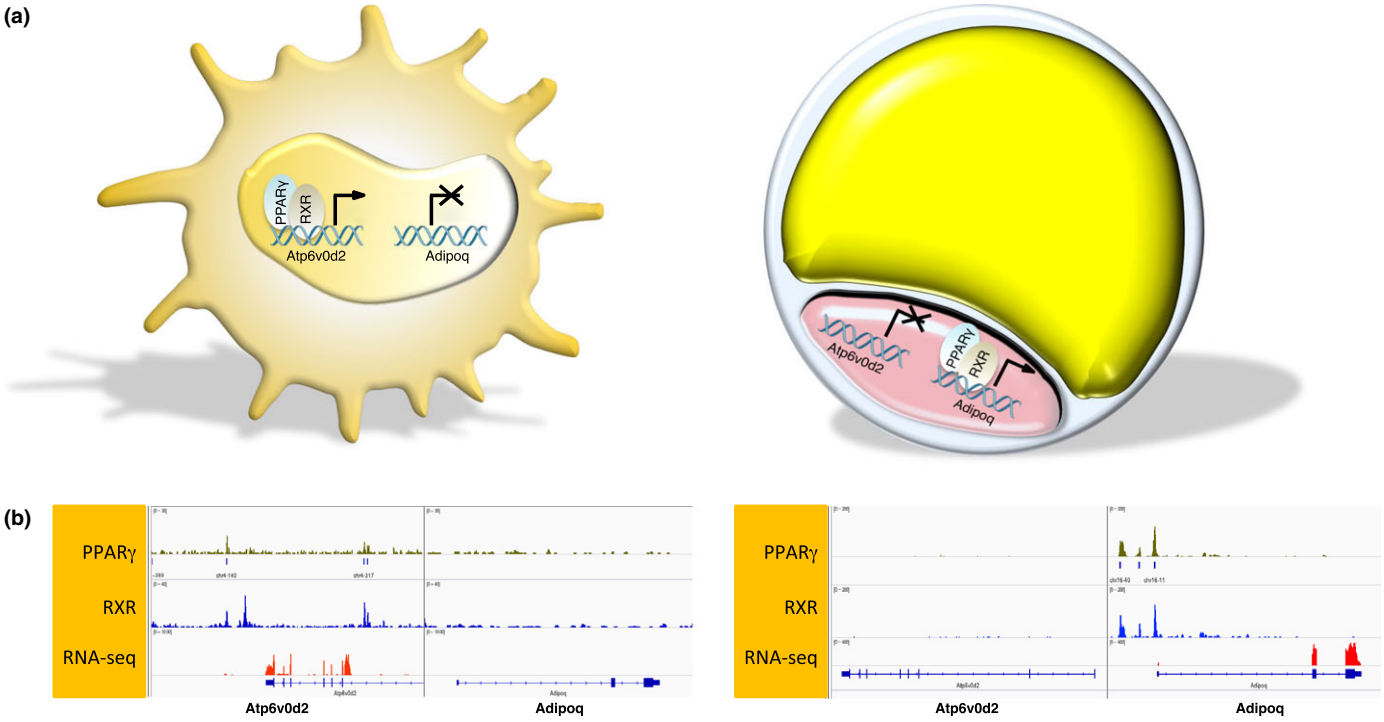

Figure 1 Cell type specificity of peroxisome proliferator-activated receptor gamma (PPARγ) transcriptional activity. (a) Reductionism point of view of PPARγ molecular genetics in macrophages (left) and adipocytes (right) on genes Atp6v0d2 and Adipoq. (b) IGV Browser snapshot showing genes Atp6v0d2 and Adipoq with PPARg, RXR ChIP-seq and RNA-seq data in macrophages and adipocytes (data obtained from Lefterova *et al.* 2010, Daniel *et al.* 2014, Nielsen *et al.* 2008 and I. Cuaranta-Monroy unpublished data) [27,87,88]. 17 7

phenotype of both macrophage subsets. Its natural and synthetic ligands inhibit the expression of a wide range of inflammatory molecules, alleviating the inflammatory response of M1-like macrophages [35–39]. Furthermore, PPARγ agonists inhibit the transcription of monocyte chemoattractant protein-1 and its receptor CC chemokine receptor 2 (CCR2), affecting the recruitment of macrophages to inflammatory sites [40–43].

The proposed molecular mechanism, by which PPARγ can repress pro-inflammatory cytokines, is called transrepression. Corepressor complexes Ncor/HDAC3/TBL are bound to the promoter of inflammatory genes (i.e. iNOS); this association is disrupted in response to inflammatory stimuli (e.g. LPS). In macrophages treated with PPARγ agonists, however, the repressor complexes remain in association with the promoter even in the presence of LPS. This model proposes the ligand-dependent SUMOylation of PPARγ preventing the ubiquitination-/proteasome-mediated degradation of the corepressor complexes [44]. Nevertheless, this model is poorly understood and does not explain how PPARγ can exert two different actions in the same cell type; in other words, how PPARγ induces the dissociation of the corepressor complexes from the targeted promoters in order to lead to gene induction and on the other hand, bind to promoters that are involved in transrepression [45]. Interestingly, Chawla *et al.* [46] showed that thiazolodinediones were able to inhibit INFγ and IL-6 secretion both in wild-type and PPARγ-deficient macrophages, indicating that transrepression by PPARγ ligands has a receptor independent component, as well. However, the mechanistic details of this aspect of transrepression still need to be elucidated. In addition, it has been shown that certain nonthiazolidinedione PPARγ agonists do not have anti-inflammatory effects in macrophages, suggesting that transrepression might be dependent on the structure and chemical nature of the ligand [47].

Aiming to elucidate the role of PPARγ in the M2 macrophage subset as well, Szanto *et al.* [48] performed a comparative transcriptome analysis in human and mouse macrophages and demonstrated that pro-inflammatory molecules inhibit, whereas the treatment with IL-4 increases the expression of PPARγ and its previously known target genes. It was also shown, using global gene expression analysis in PPARγ-deficient mice, which the alternative macrophage activation could

be achieved in the absence of PPARγ. Furthermore, the gene expression of alternative activation markers such as Ym1, Arg1 or Fizz1 was not affected upon rosiglitazone treatment. However, the authors could show in a genomewide analysis that IL-4, through STAT6, augments PPARγ activity by increasing the magnitude of transcriptional responses orchestrated by the receptor both in macrophages and in dendritic cells [48].

Analogous to this approach, Scotton *et al.* [49], Syed *et al.* (50) and Czimmerer *et al.* (51), distinguished IL-4-specific target genes in human macrophages with the aim to identify alternative macrophage activation markers. Applying microarray technology: CD1b/c/e, TLR1 and C-type lectin superfamily member 6, IL-1RI, IL-1RII, IL-1Ra, CCL17, CCL22, CCL23, CCL26, WNT5A, CD180, SLA, MS4A4A among others could be identified as novel markers. *In silico* analysis of the macrophage transcriptome in the presence of IL-4 showed that the cytokine not only regulates immunological processes but also is potentially implicated in cellular metabolism, cell proliferation and apoptosis. Transcriptome-based prediction of pathway-specific transcription factors suggested the importance of PPARγ and nuclear factor erythroid derived 2-like 2 (NFE2L2) in alternative macrophage activation, among others [49–51]. In addition, IL-4 induces the expression of 15-lipoxygenase, autotaxin and monoamine oxidase, enzymes capable of producing ligands for PPARγ [51,52].

As part of an effort to translate findings obtained primarily using mice to human macrophages, genomewide analysis of regulator regions occupied by PPARγ in murine macrophages and the human macrophage-like cell line THP-1 was performed [53]. Interestingly, this study revealed that human and murine PPARγ binding sites are only 5% shared between the two species; however, 30% of PPARγ downstream genes appear to be conserved through evolution. As part of this analysis, 4302 PPARγ/RXR binding sites were found in THP-1 cells. Within these enriched sequences, up to 60% were co-occupied with the well-established myeloid lineage specific transcription factor PU.1 [54], similar to the murine macrophage data. This led to the proposed model where PU.1 assists PPARγ functional binding both in murine and human macrophages, possibly through facilitating the remodelling of the chromatin structure.

PPARγ expressed by various tissue-specific macrophages has also been shown to play important roles in the pathophysiology of diseases ranging from autoimmune disorders to atherosclerosis. An *in silico* analysis predicted macrophages as a potential therapeutic target in inflammatory bowel disease. Accordingly, PPARγ deletion in macrophages exaggerated clinical and pathological outcomes in a mouse model [55]. Global gene expression analysis of PPARγ-deficient colonic macrophages showed altered expression of 124 genes compared to control littermates. An IPA analysis of these genes showed two main pathways compromised: (i) carbohydrate metabolism and (ii) immunological disease suggesting that both alterations in the metabolism and immune function of macrophages are responsible for the observed disease phenotype [56].

Berthier *et al.* performed a large-scale interconnection analysis in human and murine lupus nephritis renal biopsies using an algorithm called Tool for Approximate Subgraph Matching of Large Queries Efficiently to identify related networks affected in both species. Surprisingly, both in human and murine samples, macrophage/DC-related genes were affected. With this approach, the authors could identify an enhancement of PPARγ-dependent regulation in glomerular macrophages [57]. Supporting these results, Rőszer *et al.* found that macrophage-specific deletion of PPARγ in mice resulted in autoimmune kidney disease resembling certain aspects of human lupus nephritis. It was proposed that the pathological autoimmune response was a result of impaired phagocytosis of apoptotic cell debris throughout the body [58].

As stated above, PPARγ is involved in macrophage lipid metabolism as well [59]. One of the best-studied examples of this effect is the PPARγ response element in the promoter of the scavenger receptor CD36, by which this gene responds to PPARγ activation [60,61]. This receptor uptakes the oxidized low-density lipoprotein (LDL) in a cell type- and context-dependent manner [46]. Oxidized LDL is formed during the early stages of atherosclerosis, when oxidative modifications in the components of LDL complex are initiated driving the formation of fatty streaks in the endothelium of large arteries. This is accompanied by continuous macrophage accumulation with oxidized LDL uptake that serves as a crucial step in the course of atherosclerotic lesion formation [62]. Accordingly, in a murine model of mild and severe hypercholesterolemia the macrophage-specific deletion of PPARγ exacerbated atherosclerosis. The anti-atherogenic effect of the receptor in macrophages is probably attributable to its role in activating the LXR-ABCA1/ABCG1 cholesterol efflux pathway as well as to its inhibitory effect on CCR2, thereby presumably decreasing monocyte recruitment to plaques [63]. Corroborating these observations, PPARγ agonist thiazolidinedione treatment alleviated atherosclerosis in LDL deficient murine models and decreased the expression of inflammatory markers. Furthermore, atherosclerotic lesions were increased in size in PPARγ null bone marrow transplanted LDL-deficient mice [46,63,64]. In the same *in vivo* model, it has been observed that rosiglitazone treatment reduced cholesterol accumulation in part independently from the LXR-regulated cholesterol efflux pathway [65]. A transcriptome study on human stable and ruptured atherosclerotic plaques showed that the most upregulated pathway in ruptured plaques was the PPAR/Adipocytokine signaling pathway, suggesting a so far unexplored contribution of the receptor in the formation of unstable plaques [66]. It is clear, based on the studies above, that macrophage-expressed

PPARγ has a significant impact on the pathogenesis and progression of atherosclerosis. Nevertheless, crucial genome-wide studies are still missing that could describe the entire spectrum of PPARγ-responsive macrophage gene networks in the context of atherosclerosis. PPARγ ChIP-seq together with RNA-seq, and mapping of active enhancers using a combination of histone modification-specific ChIP-seq and ATAC-seq [67] in macrophages isolated from different stages of disease progression could identify novel PPARγ targets, potentially representing targets for pharmacological intervention as well. In addition, inflammatory reactions can be inhibited in numerous cell types, including endothelial cells, using PPARγ agonists [68], making PPARγ an interesting target for prevention and treatment of atherosclerosis.

It has been proposed that PPARγ acts as an important regulator in adipose tissue macrophages as well. Adipose tissue macrophages are responsible for maintaining an anti-inflammatory equilibrium in adipose tissue, thereby influence metabolic processes at a systemic level. It is thought that this equilibrium becomes disturbed in obesity. It has been shown using a combination of transcriptome analyses and immunohistological studies that there is an increased number of macrophages in adipose tissue of obese individuals, correlating with the body mass [69]. These macrophages are the primary source of TNFα, IL-6 and iNOS expression in adipose tissue. It is proposed that these macrophages secreting inflammatory mediators participate actively in maintaining a chronic low-grade inflammation in obese individuals, which will lead to insulin resistance and type 2 diabetes mellitus [70]. Odegaard *et al.* [34] found that mice lacking PPARγ in their macrophages were predisposed to diet-induced obesity and insulin resistance. Moreover, macrophage PPARγ has been shown to be indispensable for the full antidiabetic effect of thiazolidinediones [71]. It is important to note, however, that these results could not be confirmed using a different mouse strain and experimental model [72].

As PPARγ serves as an essential transcription factor during adipocyte differentiation, it is an intriguing question whether the PPARγ-regulated gene networks in macrophages and adipocytes share similarities. It turned out that indeed the receptor influences overlapping pathways in macrophages and adipocytes including genes regulating lipid metabolism such as lipoprotein lipase, acetyl coenzyme A acetyltransferase and phospholipase A [73]. At the same time, the receptor exhibits functions, which are cell type specific and involve different signalling pathways in each cell type (Fig. 1). To elucidate the mechanisms responsible for the cell type-specific effects, a genomewide study using ChIP-seq technologies has been performed. Lefterova *et al.* [27] investigated the differences between the PPARγ-binding sites in macrophages and adipocytes. According to this study, the PPARγ cistrome shows cell type specificity; however, 30% of the binding sites overlap between macrophages and adipocytes [27]. These data also support that tissue-specific PPARγ function is modulated by the accessibility of binding sites determined by chromatin conformation, and concurrent binding of tissue-dependent transcription factors (e.g. PU-1 in macrophages and C-EBP in adipocytes).

Schneider *et al.* [29] recently found that PPARγ is also essential for the differentiation of foetal monocytes into alveolar macrophages, but not for other tissue-resident macrophages. The authors used microarray data to identify the unique transcriptional signature that PPARγ grant to alveolar macrophages [29]. Lavin *et al.* [67] confirmed these observations by mapping the enhancer landscapes of macrophages with different tissue origins using a combination of RNA-seq, ChIP-seq and ATAC-seq methods. They found that the PPARγ-binding motif was overrepresented among enhancers specific for lung macrophages [67].

Therefore, based on the recent advancements in our understanding regarding the function of the receptor in tissue macrophages, it is clear that more genomewide transcriptome and epigenome analyses are needed in mouse knock-out models and in human diseases to decipher the entire complexity of PPARγ activity.

PPARγ in dendritic cells

Dendritic cells (DCs) constitute a system of cells bridging innate and adaptive immune responses through their ability to orchestrate a wide range of lymphocyte responses according to signals perceived in the periphery. In most tissues, DCs are present in an immature state, unable to stimulate T cells, but specialized to capture and process antigens. Various stimuli (e.g. microbial products, lymphocyte-derived signals, endogenous mediators) are capable of inducing a maturation process, characterized by a switch from antigen sampling to antigen presenting and lymphocyte stimulatory activity, which requires enhanced surface expression of MHC and costimulatory surface molecules (CD40, CD80, CD86). After migration to secondary lymphoid organs, activated DCs induce antigen- and tissue-specific adaptive responses. DCs also control the nature of T-cell response by driving the differentiation of naïve T cells into different effector T-cell subsets through exhibiting distinct secreted cytokine profiles. In addition to their role in adaptive immunity, DCs also elicit innate immune responses by producing cytokines involved in host defence, such as IL-12 and interferons, and by activating NK and NKT cells [74]. In the course of their lifecycle, DCs encounter continuously changing microenvironments containing a diverse range of lipids. The lipid microenvironment can influence DC phenotypes; therefore, inducing DC elicited immune responses via

G-protein-coupled receptors and nuclear hormone receptors. Among the latter, PPARγ is increasingly appreciated as a sensor capable of translating lipid signals into gene expression changes, thereby shaping DC function. It seems though that the gene networks orchestrated by the receptor in macrophages and DCs are distinct (Fig. 2).

Microarray analyses revealed that during *ex vivo* DC differentiation from human monocytes, a group of genes involved in lipid metabolism is upregulated. These genes encode proteins implicated in the production, uptake, transport and solubilization of cholesterol and fatty acids [75]. Several of them produce lipid metabolites capable of activating PPARγ, such as monoamine oxidase A-produced serotonine metabolites or 15-lipoxygenase-produced eicosanoids [52,76]. PPARγ shows a marked and rapid upregulation during this differentiation process, as well. Thus, it seems that DC differentiation induces a switch of PPARγ signalling pathway to a state when the receptor is, at least, ready to be activated by its intracellular ligands or lipids of extracellular origin, such as 15d-PGJ_2, LPA or oxLDL [77–79]. Research from the last decade has shown that activation of this signalling pathway affects both the metabolism and immune phenotype of DCs [80].

The complexity of the downstream effects of PPARγ activation has been revealed in its entirety by the global gene expression analysis of human monocyte-derived DCs differentiated in the presence of rosiglitazone for different time spans [81]. The results derived from this analysis indicated that PPARγ does not act as a general inhibitor of DC development, as the vast majority of genes regulated upon *ex vivo* DC differentiation showed unaltered expression in the presence of

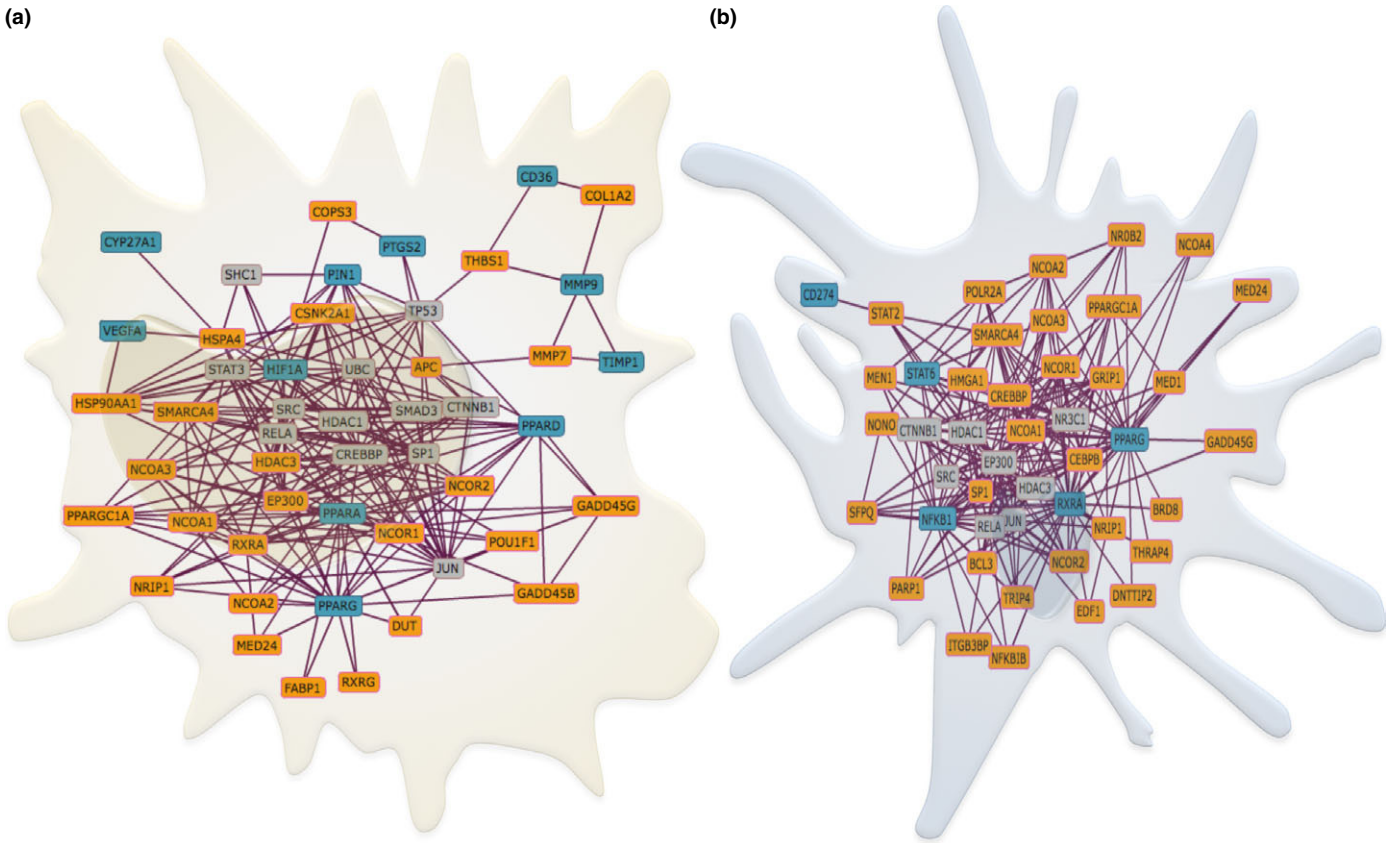

Figure 2 PPARγ signalling networks in macrophages and dendritic cells. List of genes affected by PPARγ published in The National Center for Biotechnology Information in macrophages (a) and dendritic cells (b) were generated by the online access software Genes2FANs. Next, using the same software, networks for PPARγ signalling were obtained in these two different cell types [10]. Nodes which were obtained from the seed list (list of genes directly linked to PPARγ based on publication data) are coloured in blue, intermediated nodes (genes linked to the components of the seed list) with a significant *z*-score are coloured in orange, intermediated nodes with nonsignificant *z*-score are coloured in grey (significance cutoff = 2·5). Already published data can be used to generate gene lists and networks for identifying novel regulated pathways in different cell types.

rosiglitazone. Rather, PPARγ activation appears to skew DC differentiation, creating a unique DC subset in terms of its transcriptional profile and consequential functional properties. Human serum enhances PPARγ activity in *ex vivo* differentiated DCs, suggesting that continuous low-level activation of PPARγ signalling might be an integral part of the differentiation process *in vivo*. The dynamic temporal changes in PPARγ-regulated genes reflect the two-faced role of the receptor in immune regulation, connecting metabolic and immune functions (Fig. 3). After 6 h, the category of lipid and fatty acid metabolism was markedly overrepresented among the genes upregulated by rosiglitazone. These include genes involved in fatty acid oxidation (e.g. ACOX1, HADHSC), phospholipid and triacylglycerol synthesis (e.g. DGAT2, PPAP2B) and lipid uptake/transport (e.g. CD36, FABP4), showing that the receptor simultaneously regulates antagonistic metabolic processes. The net effect of these transcriptional changes seems to be the enhanced capacity of DCs to metabolize and redistribute lipids, as rosiglitazone-treated DCs accumulated fewer lipid droplets.

After 24 h, among the genes regulated by rosiglitazone immune response-related genes showed a marked enrichment. Amid these, the CD1 group of surface protein encoding genes showed coordinate regulation by PPARγ characterized by decreased expression of CD1a, CD1b, CD1c together with CD1e and elevated expression of CD1d molecules [79]. These MHC class I-like molecules are implicated in lipid antigen presentation to T cells. Group I CD1 molecules (CD1a, CD1b, CD1c) present self-lipid antigens and microbial lipid antigens, whereas Group II (CD1d) molecules primarily bind self-lipid antigens [82]. In contrast to Group I CD1 molecules, which are recognized by TCRs showing diverse rearrangements, CD1d proteins are able to interact with T cells carrying invariantly rearranged TCRs, as well. These CD1d-restricted T cells are referred to as invariant natural killer T (iNKT) cells [83]. Enhanced expression of CD1d in PPARγ-instructed DCs endows them with the ability to selectively induce $V\alpha24^{+}V\beta11^{+}$ iNKT cell expansion in the presence of certain lipid antigens [79]. iNKT cells have cytolytic activity and rapidly induce the expression of IL-4, IL-13 and IFNγ

COLOR

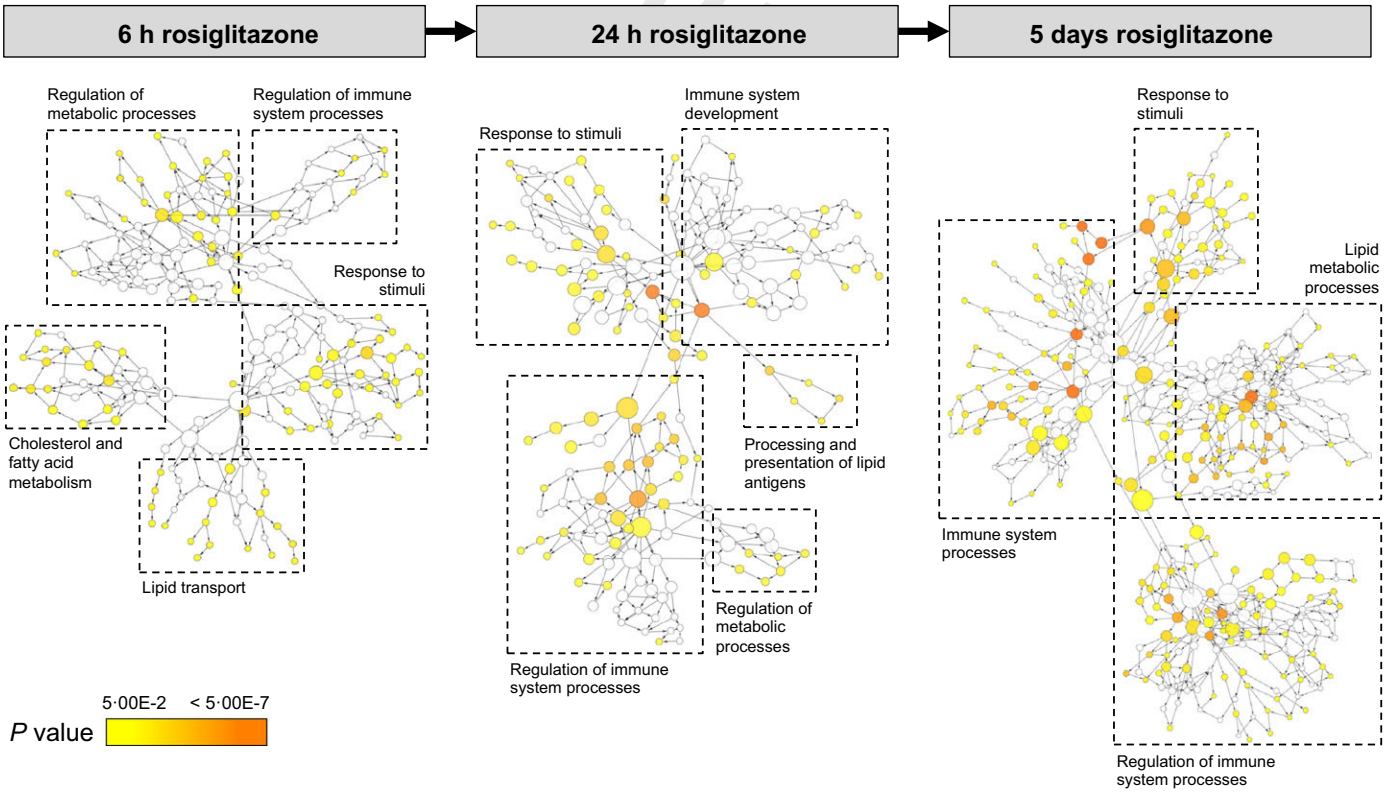

Figure 3 Network visualization of Gene Ontology Enrichment Analysis of human monocyte-derived dendritic cells differentiated in the presence of rosiglitazone using BiNGO Cytoscape plugin [89]. Nodes represent enriched Gene Ontology terms, and node colours represent corresponding Benjamini-Hochberg FDR corrected enrichment *P* values. Analysis is based on the microarray experiment of Szatmari *et al.* (GEO ID: GSE8658) [81].

Box 1 Glossary of large-scale data acquisition technologies

Microarray

Based on hybridization of complementary nucleic acid strands and one of the most widely used technology to identify dynamic transcriptomes in normal and disease states, gives poor performance in quantification of less abundant transcripts.

Next-generation sequencing (NGS)

Uses massive parallel analysis of individually amplified DNA fragments and produces short (35–400 bp) but tremendous amount (up to 10^7) of sequence reads.

Targets of NGS analysis can be not only the genomic, but methylated DNA, expressed mRNA (RNA-seq), DNase I hypersensitive DNA regions, regions bound by a specific transcription factor (ChIP-seq), or open chromatin regions (ATAC-seq).

ChIP-seq

Based on chromatin immunoprecipitation, where a protein specific antibody is used to purify DNA fragments that are associated with the protein of interest, for instance PPARγ or its heterodimeric partner RXR. ChIP-seq has already revolutionized our understanding of how transcription factor binding and epigenetic modifications control gene expression on a system level.

RNA-seq

Through deep-sequencing of cDNA, this approach allows us to determine the expression levels of whole transcripts and isoforms at a given physiological or developmental stage. These transcripts include the following: noncoding RNAs, small RNAs and mRNA.

ATAC-seq

The assay for transposase-accessible chromatin using sequencing (ATAC-seq) is based on direct transposition of sequencing adaptors into native chromatin. This way, ATAC-seq identifies open chromatin regions throughout the genome and revealing the interplay between genomic locations of open chromatin, DNA-binding proteins, individual nucleosomes and chromatin compaction at nucleotide resolution.

3D imaging

Fluorescent microscopy or contrast imagining technics sections can be processed by computational specific software's. The data obtained from this approach can be used to draw models from cells to whole organism behaviour after a stimulus or during developmental stage.

after TCR stimulation, which, in turn, can activate NK cells, T cells, B cells, macrophages and recruit DCs. The rapid activation of iNKT cells potentially renders them powerful effectors of the early phases of immune response.

The regulation of CD1d expression and subsequent iNKT cell expansion by PPARγ takes at least 24 h to develop that suggested an indirect mechanism instead of direct transcriptional activity of the receptor. By studying the global gene expression profile of PPARγ-activated human DCs, Szatmari *et al.* found that activation of PPARγ induces the retinoid pathway responsible for controlling CD1d expression. Specifically, activated PPARγ induces the expression of retinal- and retinol-metabolizing enzymes leading to increased intracellular generation of all-trans retinoic acid (ATRA) from retinol. ATRA activates retinoic acid receptor-α (RARα), which, in turn, induces CD1d transcription. ATRA-induced CD1d expression confers the ability to selectively activate iNKT cells [84].

Microarray analyses of DCs treated with the combination of rosiglitazone and an RARα antagonist made it possible to determine what portion of PPARγ-regulated genes are controlled indirectly via induced retinoid synthesis and subsequent RAR activation. Unexpectedly, this analysis revealed that approximately 30% of PPARγ ligand-responsive genes in DCs are regulated through the induction of the retinoid pathway [84].

Activation of PPARγ in human monocyte-derived DCs also upregulated several genes that are normally downregulated during DC differentiation (e.g. PDK4, CD1d, TLR4, CD36, leukotriene B4 receptor, transcription factor ATF3), possibly contributing to the development of a distinct PPARγ-instructed DC phenotype. At the same time, 66·7% of genes, which were negatively regulated by rosiglitazone treatment were induced during DC differentiation (group I CD1s, IL1R1, IL1R2, IRF4, CD80, DCNP1), showing that the receptor inhibits certain aspects of the differentiation process [81].

The microarray data of human *ex vivo* differentiated DCs, described above, were compared recently to the trascriptomic profiles of $CD1a^+MHCII^+$ lung myeloid DCs from smokers with and without emphysema. Interestingly, this analysis revealed an opposite gene expression pattern between the two data sets, namely, genes induced by PPARγ activation were decreased in emphysema, and genes downregulated by PPARγ activation showed upregulation in emphysema. These findings suggested an important negative regulatory role for the receptor in myeloid DCs in chronic lung inflammation. Confirming the genomewide analyses, mice exposed to smoke showed decreased expression of PPARγ in their lung DCs. Furthermore, animals with PPARγ-deficient DCs developed spontaneous emphysema revealing a crucial role for the receptor expressed by myeloid cells in controlling the inflammatory response in the lung triggered by cigarette smoke [85].

PPARγ forms a permissive heterodimer with RXR that can be potentially activated both by PPARγ and RXR ligands. By comparing the transcriptome of human monocyte-derived DCs after specific PPARγ and RXR activation, Szeles *et al.* found that only a subset of PPARγ target genes possesses dual ligand regulation. The majority of these genes were regulated to a lesser extent by RXR activation; thus, the permissiveness of PPARγ/RXR heterodimer seems to be only partial in DCs [86].

In conclusion, global gene expression profiling made it possible to decipher PPARγ-regulated transcriptional changes throughout the genome and identify new lipid-responsive pathways that shape the functional properties of DCs. It is important to note though that studies on PPARγ-regulated transcriptome were all carried out using *ex vivo* differentiated DCs. Thus, it would be crucial to extend studies on the cistrome and transcriptome of the receptor to DCs of *in vivo* origin in the steady state of the immune system and also in the context of different disease challenges.

Concluding remarks

In the last four decades, many molecular components of the cell have been identified in the hope that it helps to understand how individual cells work and in particular, how various cells work as a well-orchestrated system in our body. In particular in the recent years, microarray, next-generation sequencing technologies, and more recently mass spectrometry, have made possible the generation of comprehensive data sets from a variety of biological systems.

In this review, we have attempted to demonstarte how high-throughput methods changed our view on the biological function of PPARγ. We emphasized that integrating data from high-throughput data collection methods (e.g. microarray, RNA-seq, ChIP-seq, mass spectrometry) is essential to develop a deeper understanding of how the immune system operates at various scales and what functions its individual components execute. Our summary of the multiple functions of PPARγ in macrophages and dendritic cells clearly demonstrate the existence of crosstalk across multiple pathways, feed-forward and feedback loops present in the PPARγ network. Thus, representation of the PPAR signalling pathway as linear cascades funnelling signals from cell membrane to the nucleus is clearly an oversimplification of reality.

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Competing financial interests

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