SUMOylation and PPARγ: Wrestling with inflammatory signaling

The molecular mechanisms whereby PPARγ inhibits inflammatory gene expression in macrophages are poorly understood. In a recent *Nature* paper, Pascual et al. (2005) provide a new model for *trans*-repression in which ligand-dependent SUMOylation of PPARγ results in its recruitment to the promoters of inflammatory genes where it inhibits transcription by preventing clearance of corepressor complexes.

Studies over the last decade have identified members of the nuclear receptor superfamily of transcription factors as important regulators of gene expression in macrophages. Given the established and emerging roles for macrophages in metabolic diseases, including atherosclerosis and diabetes, the ability to target macrophage gene expression using nuclear receptor ligands has suggested new strategies for intervention in such disorders. For example, the observations that synthetic agonists for PPARs and LXRs reduce atherosclerosis in mice have stimulated widespread interest in these receptors as potential targets for cardiovascular therapeutics (Castrillo and Tontonoz, 2004). In addition to exerting beneficial effects on macrophage lipid metabolism, nuclear receptors such as PPARγ (Jiang et al., 1998; Ricote et al., 1998), PPARα (Staels and Fruchart, 2005), PPARδ (Lee et al., 2003), and LXR (Joseph et al., 2003) have also been reported to attenuate the inflammatory component of atherosclerosis by inhibiting the production of inflammatory mediators. A recent *Nature* study from Pascual et al. (2005) has provided new insight into potential mechanisms whereby nuclear receptors exert these effects. The authors propose that ligand-dependent SUMOylation directs PPARγ to the promoters of inflammatory genes where it inhibits transcription by stabilizing corepressor complexes.

The inhibitory action of nuclear receptors on inflammatory pathways has been best studied in the case of the glucocorticoid receptor (GR). Antagonism of the NF-κB and AP1 signaling pathways by GR occurs by several distinct mechanisms, including direct interactions with p65 and cJun, competition for limited amounts of transcriptional coactivators, chromatin remodeling, and by induction of the NF-κB inhibitor, IκB (Smoak and Cidlowski, 2004). In contrast to GR, the mechanism whereby RXR heterodimeric receptors such as PPAR inhibit inflammation is less clear. Although significant controversy exists in the literature and several distinct hypotheses have been proposed, the preponderance of evidence points to intranuclear crosstalk between PPARγ and transcription factors such as NF-κB on the promoters of inflammatory genes, a phenomenon known as *trans*-repression.

In their recent work, Pascual et al. (2005) propose a novel and provocative mechanism to explain how PPARγ *trans*-represses inflammatory gene expression in macrophages. The authors started from the longstanding observation that induction of iNOS expression by LPS is attenuated by PPARγ agonists. Using a combination of siRNA and ChiP methodology they demonstrated that in the basal state transcription of iNOS is repressed by a complex that contains TBL1, TBLR1, NCoR, and HDAC3. LPS stimulation was shown to causes a rapid clearance of NCoR and HDAC3 from the promoter by a ubiquitin-dependent mechanism and the replacement of these corepressor complexes with coactivator complexes. When the cells were treated with LPS in the presence of a PPARγ agonist, however, PPARγ was recruited to the iNOS promoter, and NCoR and HDAC3 were not cleared. Recruitment of PPARγ to the iNOS promoter was not dependent on sequence-specific DNA binding, since a PPARγ DNA binding mutant was also recruited and still inhibited iNOS expression. Surprisingly, recruitment of PPARγ to the iNOS promoter was inhibited when expression of the corepressor NCoR was reduced by siRNA. Similar effects were observed for four additional LPS-responsive genes indicating that this phenomenon of NCoR-dependent PPARγ recruitment is not specific for the iNOS promoter.

The involvement of NCoR in *trans*-repression by PPARγ presents an apparent paradox. On the one hand, ligand-dependent recruitment of PPARγ to promoters and repression of LPS-responsive genes requires NCoR, but on the other hand, ligand binding to PPARγ has been shown previously to disrupt PPARγ/NCoR interactions and result in PPARγ target gene induction. Pascual et al. (2005) hypothesized that PPARγ/NCoR interactions during *trans*-repression were distinct from the NcoR interactions that occur during activation and that another factor may be involved. They carried out a yeast two-hybrid screen and identified protein inhibitor of activated stat 1 (Pias1) as a PPARγ interacting protein. As its name suggests, Pias1 was identified in a screen for inhibitors of Stat1 signaling and has been since shown to be a repressor of the interferon-γ and NF-κB signaling pathways (Liu et al., 2004, 2005). Pias1 also belongs to the SUMO E3 ligase family and is able to SUMOylate target proteins (e.g., P53). Pascual et al. (2005) show that PPARγ and Pias1 physically interact and that knocking down Pias1 abolishes the ligand-dependent recruitment of PPARγ to the iNOS promoter and *trans*-repression. Knocking down the rate limiting E2 SUMO ligase, Ubc9, has a similar effect.

Does SUMOylation of PPARγ explain its ability to *trans*-repress inflammatory genes? PPARγ contains two SUMOylation sites (K77 and K365). The SUMOylation of K77 has been previously demonstrated to inhibit PPARγ-dependent gene induction but to have no effect on *trans*-repression. In stark contrast, Pascual et al. (2005) found that mutation of K365 eliminated the ability of agonist-activated PPARγ to repress iNOS and to be recruited to its promoter. How does modification of PPARγ by SUMOylation exert this effect? One possibility that the authors propose is that ligand-dependent SUMOylation of PPARγ strengthens its direct interaction with NCoR and prevents its clearance, thus maintaining a

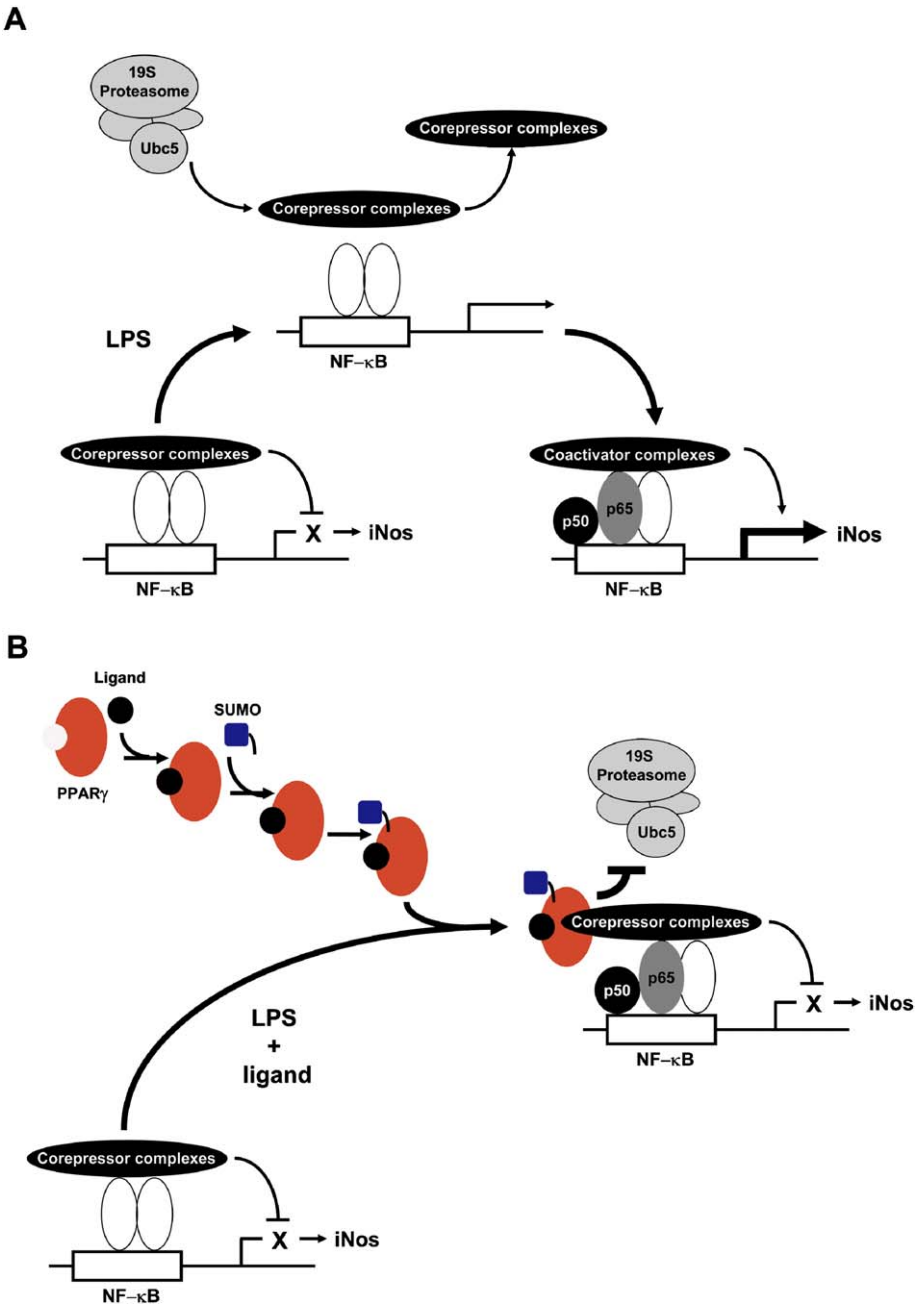

Figure 1. A model for PPARγ-mediated *trans*-repression of iNOS expression

A) LPS stimulation promotes the ubiquitin-dependent proteasomal degradation of corepressor complexes and their exchange for coactivator complexes resulting in iNOS expression.

B) LPS stimulation in the presence of ligand results in SUMOylation of PPARγ on position K365. SUMOylated PPARγ interacts with NCoR present in corepressor complexes and prevents the clearance of these complexes, keeping the promotor in a repressed state.

Adapted from Pascual et al. (2005).

repressed state at inflammatory promoters.

These findings have opened the door to the study of a completely new pathway for nuclear receptor action. As one would expect with any new idea, a number of issues remain to be addressed in future studies. A basic assumption of this model (Figure 1) is that ligand binding enhances the SUMOylation of PPARγ. The observed effect of ligand on PPARγ SUMOylation, however, is modest and it is unclear what fraction of PPARγ in the cell is SUMOylated. Moreover, it remains to be established that SUMOylated PPARγ is present in corepressor complexes associated with macrophage inflammatory genes. The consequence of SUMOylation of PPARγ at K365 for transcriptional activation is also not yet clear. Are such SUMOylated receptors still competent to participate in activation complexes?

The proposed involvement of Pias1 in the PPARγ *trans*-repression mechanism will also be an interesting area for future investigation. Previous studies implicated Pias1 in the control of a small subset of interferon-γ- and LPS-responsive genes (Liu et al., 2004, 2005). Such genes were found to be hyperresponsive to inflammatory induction in Pias1 KO macrophages compared to wt controls. Interestingly, however, iNOS expression was not altered in Pias1 knockout cells. The importance of the E3 SUMO ligase activity in Pias1 for its effects on cell signaling is also not well defined. It is therefore unclear at present whether the Pias1-dependent regulation of inflammatory gene expression observed by Liu et al. (2004, 2005) is related to the findings of Pascual et al. (2005) on PPARγ repression of iNOS. Ultimately, it will be important to address whether SUMOylation contributes to the anti-inflammatory effects of PPARγ in disease contexts such as atherosclerosis and inflammatory bowel disease. A knockin of the K365R mutant into the PPARγ locus would be predicted to abolish the anti-inflammatory action of PPARγ ligands in these settings.

A particularly interesting question is whether the model of Pascual et al. (2005) is a general mechanism that also governs the *trans*-repression of inflammatory gene expression by other nuclear receptors. The fact that LXRs, PPARγ, and GR repress an overlapping yet distinct set of inflammatory genes (Ogawa et al., 2005) suggests a high degree of complexity in the mechanisms that control *trans*-repression. SUMOylation may be a key part of this regulatory network.

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