Analysis of PPAR sub-types with selected ligands using biochemical and cellular assays

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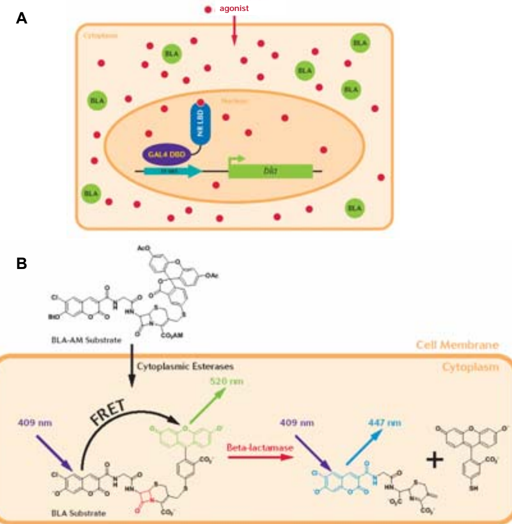
Introduction

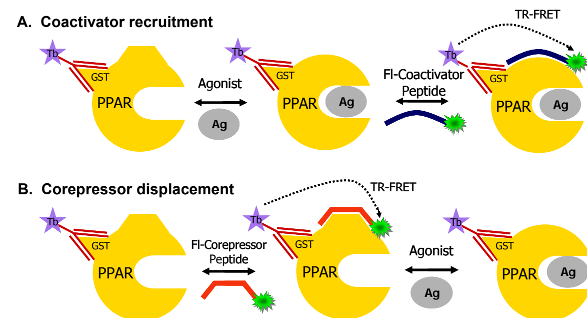
The peroxisome proliferator-activated receptor (PPAR), an important regulator of lipid metabolism, has three sub-types with specific biological functions: PPARα, δ (β), and γ. The different effects of each can be explained in part by the different tissue distributions and by the fact that each sub-type responds differently to specific ligands. Studies have shown that different sub-types bound to the same compound preferentially associate with distinct coregulator proteins that ultimately control transcription. An understanding of these coregulator interactions provides insight into the behaviors of the different sub-types and can potentially aid in the design of targeted therapies. We chose a subset of specific PPAR agonists and an antagonist and compared the coregulator peptide recruitment pattern for all sub-types and compounds. We then evaluated each compound in cell-based reporter assays for all three sub-types. Additional follow-up ligand dose responses were performed with the coregulator interaction assays.

Figure 1 – PPAR-GAL4 Chimeric Reporter Cellular Assays



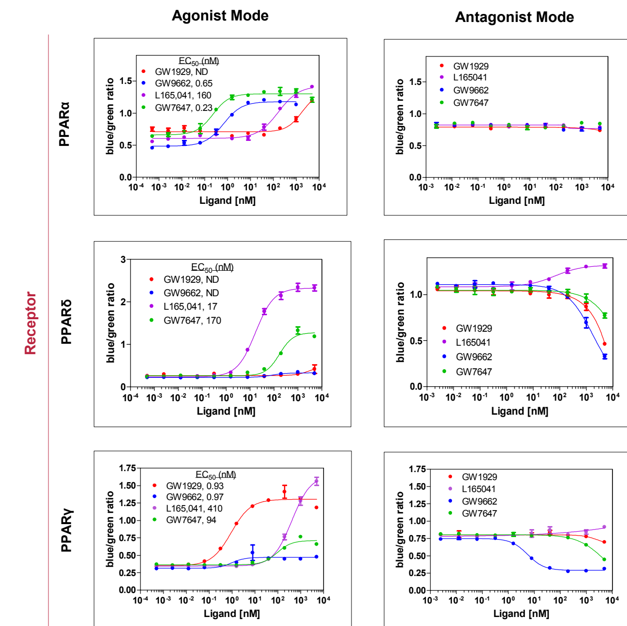
The PPAR-GAL4 GeneBLAzer® cell-based assays (**A**) use HEK293 cells engineered with β-lactamase (*bla*) cDNA under transcriptional control of an Upstream Activator Sequence (UAS). These cells are then either transduced with baculovirus containing the GAL4-DNA binding domain fused to the PPAR ligand binding domain for PPARα or are stably integrated with a similar construct for PPARδ and PPARγ. β-lactamase (BLA) expression is detected using a cell permeable FRET based substrate (LiveBLAzer™- FRET B/G substrate) (**B**). Intact substrate undergoes FRET and fluoresces green while cleavage of the substrate by BLA results in the loss of FRET (blue fluorescence).

Figure 2 – Schematic of the Coregulator Interaction Assays



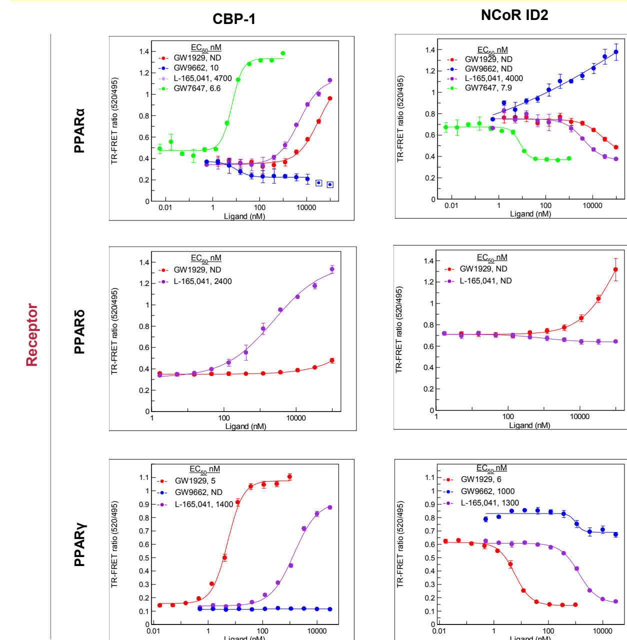
Upon binding of agonist, helix 12 of the receptor undergoes a conformational change that results in an increased affinity for coactivator proteins and decreased affinity for corepressor proteins. Using LanthaScreen™ technology, recruitment of labeled coactivator peptides (**A**) is detected by an increase in the TR-FRET signal between the Tb-anti-GST antibody and the fluorescein of the coactivator peptide while displacement of corepressor peptides (**B**) is observed by a decrease in the TR-FRET signal.

Figure 3 – Selected Ligands in the PPAR-GAL4 Chimeric Cellular Assays



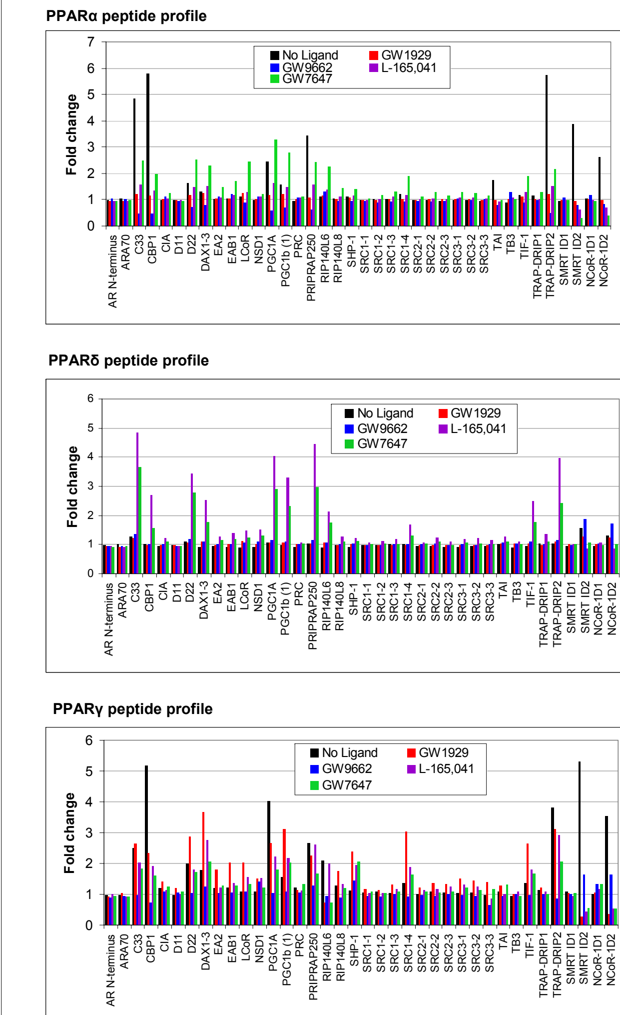
PPAR-GAL4 cell-based assays were performed with serial dilutions of selected ligands in agonist and antagonist mode; GW1929 (γ agonist), GW9662 (irreversible γ antagonist), L-165,041 (δ agonist), and GW7647 (α agonist). In agonist mode, cells were incubated with compounds for 20-24 hours before loading with the LiveBLAzer™-FRET B/G substrate. Antagonist assays included pre-incubation of the cells with compounds for ~30 minutes before addition of the EC_{80} concentration of primary agonist.

Figure 4 – Interactions of the PPARs with a Corepressor and a Coactivator Peptide in the Presence of Selected Ligands



Serial dilutions of selected ligands were analyzed for their effect on ligand bound receptor interactions with NCoR ID2 corepressor peptide and CBP-1 coactivator peptide in the TR-FRET coregulator assays. Generally, sub-type specific agonists recruited CBP-1 and displaced NCoR ID2, however, the PPARγ agonist, GW1929, recruited NCoR ID2 with PPARδ. This effect may be related to the weak antagonist activity observed with PPARδ in the cell-based assay.

Figure 5 – Coregulator Peptide Profiles with the PPARs



A panel of fluorescein labeled coregulator peptides was screened against all three PPAR sub-types in the presence and absence of ligands. Data for ligand bound receptor is reported as the fold change of the receptor with ligand compared to the no ligand control. Ligand independent recruitment is captured in the "no ligand" data set where receptor without ligand is reported as the fold change relative to the no receptor control. Values greater than one indicate peptide recruitment while values less than one indicate displacement. The corepressor peptide, NCoR ID2, and the coactivator peptide, CBP-1, were chosen for further analysis.

Conclusions

- All three sub-types show recruitment of similar coactivator peptides with their preferred agonists.
- Both PPARα and γ show comparable ligand independent recruitment of corepressors and a sub-set of coactivators, whereas PPARδ shows negligible ligand independent recruitment.
- The PPARγ agonist, GW1929, appears to be a weak antagonist for PPARδ in the cell-based assay, which correlates with the recruitment of NCoR ID2 corepressor peptide in the coregulator assay.
- Rank order potency shows good correlation between the coregulator interaction assays and the GAL4 chimeric cell-based assays.
- The combination of the GeneBLAzer® PPAR-GAL4 chimeric cell-based assays and the LanthaScreen™ PPAR coregulator assays provide different but complimentary information that can be used to further characterize the effects of a particular ligand on all three PPAR sub-types.

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