Transcriptional induction of the gene encoding cytochrome P450 3A oxygenase (CYP3A) causes a prominent class of dangerous drug-drug interactions wherein one drug accelerates the metabolism of another. In our 1998 JCI paper, we reported the cloning of the human nuclear receptor PXR and demonstrated that it mediates CYP3A induction. We determined that PXR is expressed in liver, acts through a DNA response element located in the CYP3A promoter, and is activated by a structurally diverse collection of drugs that induce CYP3A. Our findings revealed the molecular basis for the CYP3A induction class of drug-drug interactions and provided a high-throughput means for screening out drug candidates with this activity.
Nuclear receptor PXR: discovery of a pharmaceutical anti-target

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CYP3A inducers and drug interactions

In the 1960s, it was shown that repeated administration of “catatoxic” steroids to rodents conferred resistance against the harmful or anesthetic effects of structurally unrelated drugs (reviewed in ref. 1). For example, rats treated for several days with the anti-mineralocorticoid spironolactone were almost completely protected against otherwise fatal doses of the cardio-toxin digitoxin. Among the most potent of these catatoxic steroids was pregnenolone (now GlaxoSmithKline) came across an expressed sequence tag that encoded part of a novel nuclear receptor. We went on to clone this receptor and show that it was abundantly expressed in the liver. As part of our group’s ongoing campaign to identify ligands for orphan nuclear receptors, we screened the receptor against a large library of compounds. Among our initial hits was PCN, which bound and activated the receptor at concentrations that were consistent with that required for CYP3A induction. We named this protein the pregnane X receptor (PXR) based on the efficient activation of the receptor by PCN and other natural and synthetic C21 steroids (pregnanes). We further demonstrated that PXR acted through a DNA response element located within the rodent Cyp3a gene promoter (3).

While we had identified the long-sought-after PCN receptor, this work did not address the broader issue of the overall contribution of PXR to CYP3A induction and drug-drug interactions. This work did set the stage for our 1998 study published in the JCI, in which we cloned and characterized the human ortholog of PXR (7), which was subsequently reported by two other groups and referred to as steroid and xenobiotic receptor (SXR) (8) and PAR (9), respectively. The DNA-binding domain of mouse and human PXR shares 96% amino acid identity, and both orthologs bind the same response elements within CYP3A promoters. However, the ligand-binding domains are only 76% identical, which is much lower than the identity between orthologs of other nuclear receptors. This divergence provided the first hint that mouse and human PXR may have distinct pharmacologic activation profiles.

In cell-based reporter assays, human PXR was activated by a structurally diverse set of established CYP3A inducers, including the drugs rifampicin, dexamethasone, RU486, spironolactone, cyproterone acetate, clotrimazole, and lovastatin. Importantly, there were striking differences in
the response profiles of mouse and human PXR that mirrored those for CYP3A induction in the two species. From these studies, we reached the surprising conclusion that most CYP3A induction was mediated by this single, divergent nuclear receptor (Figure 1). This conclusion was subsequently confirmed in an elegant study by the Evans laboratory that showed introduction of human PXR into mice results in a “humanized” CYP3A induction profile (10). Structural studies later revealed that PXR has a large, hydrophobic ligand-binding pocket that changes shape to accommodate different ligands (reviewed in ref. 11). Thus, PXR evolved to serve as a generalized xenobiotic sensor rather than a receptor for an endogenous ligand. This was a new concept in the nuclear receptor field.

The immediate impact of our JCI study was that high-throughput PXR assays, such as the one we used, could be applied prospectively to identify and eliminate CYP3A inducers early in the drug-discovery process. In fact, PXR assays are now widely used by pharmaceutical companies for this purpose. Notably, PXR is not only activated by prescription drugs, but also by herbs such as St. John’s wort, which interacts with many drugs (reviewed in ref. 12).

Thus, PXR assays are also useful for assessing the potential of unregulated remedies to interact with prescription drugs.

In closing, our JCI paper identified PXR as a molecular basis for drug-drug interactions and introduced the concept of a nuclear receptor acting as a generalized xenobiotic sensor. Ironically, while our nuclear receptor group at Glaxo Wellcome set out to identify new drug targets, our pharmaceutical legacy is the discovery of an important anti-target!

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Figure 1. PXR causes drug-drug interactions. Due to a large, hydrophobic ligand-binding pocket, PXR is activated by a wide variety of drugs, including dexamethasone, RU486, rifampicin, and hyperforin (a constituent of St. John’s wort). Once activated, PXR binds to the CYP3A promoter and induces transcription. Elevated levels of CYP3A result in increased metabolism of CYP3A substrates, which include most prescription drugs.