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"Competition: Physical Interactions between CYP2D6, NADPH-Cytochrome P450 Reductase, and Heme Oxygenase-1"

The human cytochromes P450 (CYPs or P450s) are a superfamily of 57 hemeproteins that play an essential role in the metabolism of numerous endogenous and exogenous compounds. P450 families 1, 2, and 3 are responsible for metabolizing xenobiotics and account for the phase I biotransformation of approximately 75% of the most commonly prescribed drugs. P450s obtain electrons from their redox partner NADPH-cytochrome P450 reductase (POR) for the catalytic reaction, requiring P450s to form physical complexes with POR. Another enzyme known as heme-oxygenase 1 (HO-1) also interacts with POR to obtain electrons for the metabolism of free heme. HO-1 is expressed in most tissues and is induced by stress. Given that both P450s and HO-1 interact with POR, a complex network of protein-protein interactions is possible. Induction of HO-1 as a result of the stress response has the ability to interfere with the P450-POR interactions by forming complexes with either POR or P450. This study was focused on the physical interactions of CYP2D6 in particular. Previous work has shown that POR has ~7-fold higher affinity for HO-1 than for CYP2D6. The effects of CYP2D6 on HO-1 activity, and vice versa, were inconsistent with simple competition between HO-1 and CYP2D6 for POR binding. The goal of this study was to gain further insight into the network of interactions between CYP2D6, POR, and HO-1.

Bioluminescence resonance energy transfer (BRET) was used to observe the physical interactions between CYP2D6, POR, and HO-1 (tagged with Rluc and GFP) within the ER membrane of transfected 293T/17 cells. The BRET signal is used to measure formation of protein complexes tagged with luciferase and GFP, and the potential disruption of these complexes by untagged proteins.

We were able to detect a physical complex formed between HO-1 and CYP2D6 that was stable in the presence of co-transfected unlabeled POR. Both CYP2D6 and HO-1 were capable of disrupting the other's interaction with POR, though more CYP2D6 expression was needed to break up the POR/HO-1 complex than vice versa. A specific homomeric CYP2D6 interaction was also detected, but was somewhat disrupted in the presence of excess POR.

Together, these results point to a network of interactions that likely play a role in balancing CYP2D6 and HO-1 activity when both proteins are expressed in excess of POR. Finer mechanistic details and potential *in vivo* effects remain to be determined. Nevertheless, the potential of a stress response to effect drug metabolism via HO-1 induction and protein-protein interactions merits further study.