530c Directed Evolution of Specific Receptor-Ligand Pairs for Use in the Creation of Gene Switches

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Despite their versatility and power in controlling gene regulation in nature, nuclear hormone receptors (NHRs) have largely eluded utility in heterologous gene regulation applications such as gene therapy and metabolic engineering. The main reason for this void is the pleiotropic interference of the receptor–ligand combination with regulatory networks in the host organism. In recent years, numerous strategies have been developed to engineer ligand–receptor pairs that do not cross-interact with host regulatory pathways. However, these strategies have either met with limited success or cannot be readily extended to other ligand–receptor pairs. Here, we present a systematic approach for reengineering NHRs to respond specifically to a selected synthetic ligand. The method involves generation of genetic diversity by stepwise individual site saturation mutagenesis of a fixed set of ligand-contacting residues and random point mutagenesis, followed by phenotypic screening based on a yeast two-hybrid system. As a test case, this method was used to alter the specificity of the NHR human estrogen receptor α in favor of the synthetic ligand 4,4'-dihydroxybenzil, relative to the natural ligand 17β-estradiol, by $>10^7$-fold. The resulting ligand–receptor pair is highly sensitive to the synthetic ligand in human endometrial cancer cells and is essentially fully orthogonal to the wild-type receptor–natural ligand pair. This method should provide a powerful, broadly applicable tool for engineering receptors/enzymes with improved or novel ligand/substrate specificity.