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Towards a less toxic, ligand-inducible Cre recombinase

The bacteriophage P1 Cre/*lox*P system has become an important tool for the analysis of gene function. *In vitro* and *in vivo* studies revealed that expression of the Cre recombinase in cells lacking exogenous *lox*P sites caused severe toxic effects, which were displayed by decreased growth, cytopathic effects, and/or chromosomal aberrations. It has been shown that toxicity of Cre correlates with the duration of Cre activity. Although the exposure of the target cells to Cre is limited in ligand-regulated Cre systems, the tamoxifen-inducible Cre estrogen receptor (ER) fusions (CreERT-type recombinases) were shown to be highly toxic when expressed in cultured MEFs, B and T cells progenitors or c-Myc-transformed B-cells. We assume that the ER^{T2} ligand-binding domain (LBD) exacerbates the toxicity of the Cre fusion protein, at least in part, by inducing its recruitment into transcriptional complexes. This interaction is mediated by the transcriptional activation domain (TAF2) within the LBD. To minimize the toxic effect of Cre, TAF2 activity was obliterated by changing three amino acids in the carboxyl-terminus of the ER domain of $CreER^{T2}$ fusion protein. The constructed $CreER^{T3}$ transgene, whose expression is driven by the cytomegalovirus enhancer/chicken β-actin promoter (CAG promoter), was introduced into the *Rosa26* locus of embryonic stem (ES) cells and a knock-in mouse line was generated. The $CreER^{T3}$ transgene will soon be investigated in respect of a ubiquitous expression, the ability to be induced by tamoxifen and the recombination efficiency. The crucial question, whether $CreER^{T3}$ provides a less-toxic ligand-inducible Cre system, needs to be evaluated in future experiments.