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Gene Regulation by Tetracyclines: Mechanisms and Applications

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Gene regulation by the tetracycline (tc) inducible Tet repressor (TetR) is not only a paradigm for understanding the properties of a regulatory protein, but also a widespread tool for conditional gene expression in most organisms, including bacteria, fungi, plants, invertebrates and mammals (1). TetR is a homodimer of about 46 kDa containing one helix-turn-helix DNA recognition motif and one tc binding site per subunit. Extensive X-ray analysis has produced detailed models of the conformational changes associated with induction (2). *In vitro* evolution approaches yielded TetR mutants with altered DNA sequence and inducer recognition properties (1), and also a TetR inducing oligopeptide. Recently we have also succeeded in reversing the TetR allosterical response by obtaining TetR variants for which anhydro-tc, the inducer of the wild type, is a corepressor (3). The implications for binding site plasticity and conformational changes in allosterical proteins will be discussed.

For eukaryotic applications a suitable TetR variant is usually fused with a readout domain. We have used fusions with VP16 and KRAB to construct HeLa cell lines in which procaspase-2 or t-Bid can be expressed under doxycycline (dox) control. Although both cell lines show dox inducible apoptosis, a rather large amount of procaspase-2 must be expressed to induce apoptosis, whereas the much more active t-Bid must be tightly controlled to avoid uninduced apoptosis. Two different regulatory strategies accomplishing these needs are described (4).

To analyse the influence of DNA structure on enhancer activity, we have fused TetR with various oligomerisation domains and flanked an SV40 enhancer with binding sites for that construct. As a result, we can completely regulate SV40 enhancer activation of a downstream TATA box by reversibly separating it from the promoter in a DNA loop.

For random gene targeting, we have constructed an insertion DNA element carrying a tc regulated outward promoter and used it for random mutagenesis of *E. coli*. Transposase driven insertion of this element turns out to be random and yields about 4% of the mutants with a tc inducible gene knockout phenotype. Thus, this construct allows the random generation of conditional knockout mutants throughout bacterial chromosomes.

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