The F.A.S.T.™ Gene Modulating System

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ingenious targeting laboratory (ingenious) has acquired exclusive rights to a new gene modulating technology that allows various genetic outcomes to be produced from a single mouse model. In many experimental settings, the targeted inactivation of a gene in the form of a knockout mouse is the starting point for the generation of additional mouse strains with variant genetic alterations of the gene in question. Based on the tetracycline operon, the F.A.S.T.™ (Flexible Accelerated STOP TetO-knockin) system allows multiple mouse lines to be derived from a single targeted locus, comprising distinct genetic modalities such as: 1) a constitutive knockout; 2) Cre-mediated tissue-specific rescue; 3) selective tTA-directed ectopic target gene expression within the knockout environment; 4) selective tTA-mediated target gene overexpression within the wild-type environment; and 5) tTS-induced conditional knockout/knockdown of the target gene. Here, we summarize the versatility of multiple gain- and loss-of-function mouse lines of various genes, were produced using this integrative model approach.

Introduction/Problem

Commonly, probing for gene function in health and disease states and supporting drug discovery/testing are aided by various sets of mutant mouse lines, which model gain and/or loss of gene function in a flexible manner. Especially, Tet mouse models have served this role in basic and biomedical research for many years now (Schoenig et al., 2010; Stieger et al., 2010). This fact is founded on unique features of components that regulate gene expression either by tetracycline-dependent induction or tetracycline-dependent inhibition. The respective control circuits involve transactivators which are switched on (rtTAs) or are switched off (tTAs) in the presence of tetracycline derivatives. In addition, transcriptional silencers (tTS) are capable of repressing gene expression in the absence of tetracycline-related modulators. The common core of the genetic switch in all of these systems is the Tet operator (tetO) in the vicinity of a promoter. Based on the need to achieve a spectrum of controllable expression levels for single genes and to allow for the parallel production of variant mouse lines originating from one basic design, we describe here a further development of the Tet technology by employing a unique combination of genetic elements as originally described by Tanaka et al., 2010.

Materials, Methods and Data

The general design feature underlying this technology is a loxP-FRT-Neo-STOP-FRT-TetO-loxP cassette (later on referred to as STOP-tetO), which is inserted immediately upstream of the translation initiation site of the gene of interest by gene targeting (Fig. 1B). This produces an initial constitutive knockout phenotype.

Upon mating with Flippase-expressing mice or FLP transient expression at the ES cell level, wildtype-like tetO knockin mice are produced as a result of the removal of the STOP (later referred to as tetO; Fig. 1C). Both knockin variants, either with a STOP cassette or without (Phenotype 1), allow different scenarios to take place as a result of further matings with tetracycline transactivator, silencer or Cre strains, thus contributing locality of gene function (Phenotype 2). In addition, dosage with doxycycline allows reversal of the initial phenotype and investigation of gene function in a time-dependent manner (Phenotype 3, Fig. 1A). As a proof of principle study, such manipulations were undertaken for the Mlc1 gene, and functional consequences were evaluated in selected regions of the mouse brain. Specifically, the following outcomes were generated:
1. Constitutive knockout: the knockin of the STOP-tetO cassette efficiently terminates transcription from the endogenous Mlc1 promoter as documented by *in situ* hybridization of cells of the astrocyte lineage in the cerebellar lobe (blue signal, Fig. 2A). This is in agreement with previous usage of STOP cassettes.

**Fig. 1 Applications of the F.A.S.T.™ system**

(A) Diagram of functional outcomes originating from two distinct knockin alleles (STOP-tetO, tetO) and respective phenotypes relating to no and/or further matings with Cre recombinase/tTA/tTS mouse lines and dosage with doxycycline. (B) Composition of the original knockin cassette. A STOP terminates both transcription and translation and is placed down-stream of the endogenous promoter of a given gene. The gene's ATG is fused to the cassette to gain control over its expression. LoxP and FRT sites allow for genetic modifications to take place. (C) Removal of the neomycin selection cassette via FRT-mediated recombination results in a functional tet operator sequence regulating the gene of interest independently or in combination with the wildtype promoter.

2. Cre-mediated selective rescue was achieved by removal of the STOP via Cre recombinase expression, either randomly in the cerebellum (via an Emx1-Cre line) or in general (via HSP70-Cre line), resulting in a partial or a complete rescue phenotype. Consequently, gene function can be recovered spatially by directing the distribution of Cre recombinase which restores the native promoter activity (Fig. 2B).

3. Selective tTA-directed ectopic target gene expression within the knockout environment: as a consequence of the presence of the STOP, it is possible to drive gene expression independently of the native promoter via the transactivator (tTA) only. If the respective tTA displays a divergent expression pattern from the targeted gene, ectopic expression of the latter is possible and reversible by dosage with doxycycline as demonstrated using an alphaCamKII-tTA mouse line as a mating partner of the STOP-tetO line (Fig. 2C, hippocampal CA1 and striatum medium spiny neurons).

4. Selective tTA-mediated target gene overexpression from within the wildtype environment was realized by mating the Mlc1 tetO line with an astrocyte-specific Mlc1-driven tTA BAC transgenic line. The offspring was evaluated by *in situ* histochemistry of the cerebral cortex and cerebellum in the absence and presence of doxycycline resulting in wildtype-like and overexpression phenotypes, respectively (Fig. 2D, 2E). Importantly, tetO knockin mice per se, are indistinguishable from wildtype mice when dosed with doxycycline, as tTA can not bind to tetO and the native promoter controls transcription. In the absence of doxycycline, an additional activation occurs via tTA interacting with tetO.

5. For the tTS-induced conditional knockout/knockdown of the Mlc1 target gene a beta Actin-tTS line was used, providing a very wide spread and strong silencing in homozygous Mlc1 tetO offspring within the mouse brain (Fig. 2G). Again, this effect was reversible with dosage of doxycycline (Fig. 2F).
In addition, F.A.S.T.™ technology was successfully employed by Baudouin et al., 2012 in a rodent model of autism.

**Conclusions**

Validation of the F.A.S.T.™ system has shown considerable robustness and flexibility in the regulation of gene expression. The STOP-tetO knockin model provides a strong termination signal that can be overcome by Cre-mediated recombination or by tTA-directed expression in a reversible manner (doxycycline). The tetO knockin variant generally does not affect basic expression levels driven by the native promoter nor does it affect native splicing reactions. These characteristics in combination with the application of tTA and tTS lines allow a spectrum of reversible phenotypes to be generated for investigations which demand a considerable degree of specificity and flexibility.

**References**


**Fig. 2** Examples of five distinct F.A.S.T.™ manipulation strategies (A) STOP-tetO knockin mice produce a knockout phenotype of the Mlc1 gene compared to wildtype mice. ISH of the Mlc1 transcript in the cerebellar lobe. (B) Cre-directed rescue of the knockout phenotype (via Emx1-Cre/HSP70 Cre lines) which remove the F.A.S.T.™ cassette. (C) Ectopic expression of Mlc1 driven by an alphaCamKII-tTA line in the absence of doxycycline, see arrows, versus its presence. (D, F) tetO knockin mice display wildtype expression patterns. Neither tTA nor tTAs can bind to tetO when dosed with doxycycline, see Mlc1 ISH of the cerebral cortex and the cerebellum. (E) Binding of tTA in the absence of doxycycline leads to overexpression of Mlc1. (G) tTS mediates a reversible knockout phenotype in the absence/presence of doxycycline. Please note, for some applications a heterozygous or homozygous F.A.S.T.™ knockin allele is required.

The usage of tTS lines was further supported by investigations regarding the serotonin 1A receptor knockout/knockdown in an inducible and reversible tissue-specific manner in the raphe nuclei and the hippocampus (Richardson-Jones et al., 2010; 2011).