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# Inducible Site-Specific Somatic Mutagenesis in Mouse Hepatocytes

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To perform ligand-dependent site-specific mutagenesis in the liver, we established transgenic mice expressing the tamoxifen-inducible Cre-ER$^T$ recombinase (Feil *et al.,* 1996; Brocard *et al.,* 1997) under the control of the human α1-antitrypsin (αAT) promoter (Fig. 1) that is specifically active in hepatocytes (Jallat *et al.,* 1990). Out of 20 transgenic founder animals, three lines expressing Cre-ER$^T$ in the liver, but not in the other tested organs, were obtained (Fig. 2A and data not shown). The expression pattern of Cre-ER$^T$ in the liver was analysed in the transgenic line exhibiting the highest Cre-ER$^T$ expression level. In situ hybridization as well as immunohistochemistry analysis revealed that Cre-ER$^T$ was expressed in approximately 40%–50% of the hepatocytes (Fig. 2B,C). Mosaic transgene expression using the αAT promoter was previously reported (Ponder, 1996; Veniant *et al.,* 1996). To analyse the recombinase activity, the αAT-Cre-ER$^T$ transgenic line was crossed with reporter mice containing a loxP-flanked (floxed) allele of a target gene (Feil *et al.,* 1997; Mascrez *et al.,* 1998), and offspring harboring both the Cre-ER$^T$ and the floxed reporter allele were treated with tamoxifen. Excision occurred for 40%–50% of the reporter allele in the liver and was undetectable in all other organs analysed, as well as in oil-treated animals (Fig. 2D). Thus, recombination occurred in almost all hepatocytes expressing Cre-ER$^T$.

Taken together, our results show that the αAT-Cre-ER$^T$ transgenic mice allow conditional site-specific somatic mutagenesis in the liver. The mosaic Cre-ER$^T$ expression in hepatocytes should be useful to produce genetic chimeras to study gene function during postnatal liver growth, regeneration, and carcinogenesis, and to perform genetic marking studies (Ponder, 1996).

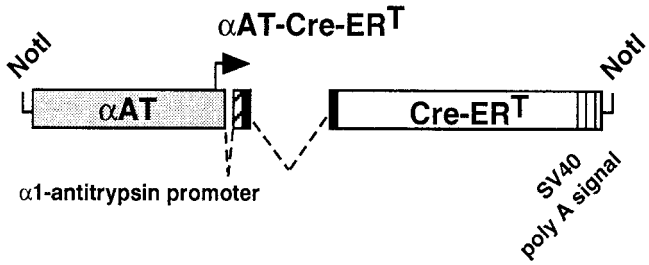


**FIG. 1.** Schematic diagram of the αAT-Cre-ER$^T$ transgene. The blunt-ended 1.8 kb EcoR I-Sal I fragment, containing the human α1-antitrypsin promoter and the first non-coding exon (grey box), the 5′ sequences of the first intron (dotted line), and the c-myc splice acceptor site (hatched box), was isolated from pTG3925 plasmid (Jallat *et al.,* 1990) and cloned into the blunt-ended Sall site of pGS-Cre-ER$^T$ (Indra *et al.,* 1999), resulting in pαAT-Cre-ER$^T$. The black boxes represent rabbit β-globin exonic sequences. Transgenic mice were established with the 4.6 kb Not I fragment of pαAT-Cre-ER$^T$, as described (Feil *et al.,* 1996).

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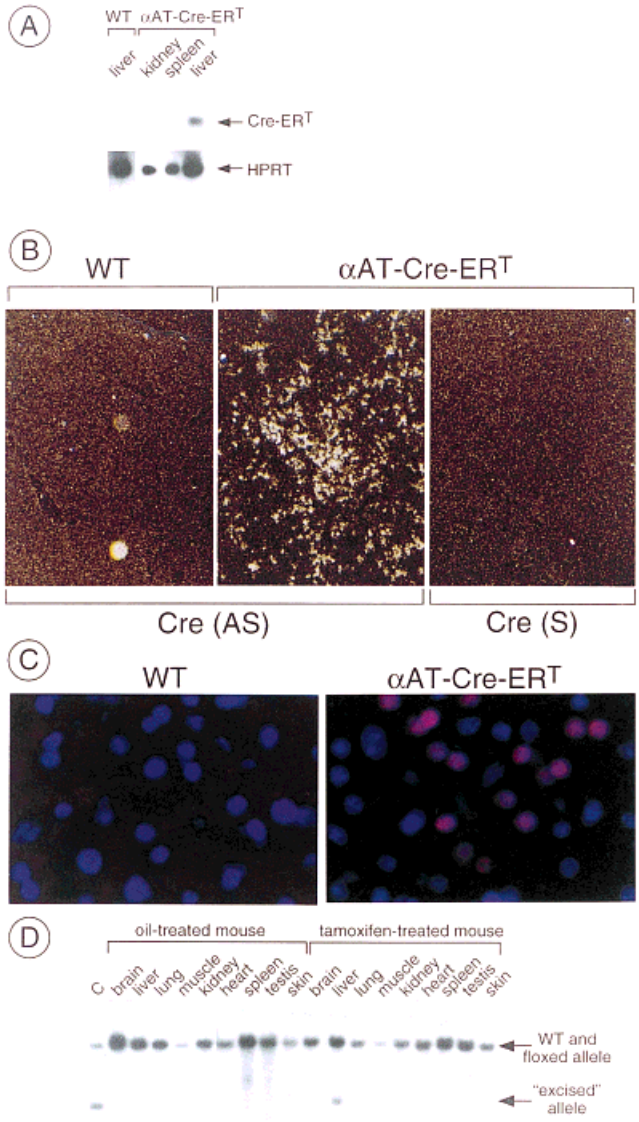


**FIG. 2.** Characterization of the αAT-Cre-ER$^T$ transgenic mouse line. **(A)** RT-PCR analysis of Cre-ER$^T$ expression was performed on RNA extracted from liver of WT mice and from kidney, spleen, and liver of αAT-Cre-ER$^T$ transgenic mice, as described (Feil *et al.,* 1996). The PCR products corresponding to Cre-ER$^T$ and HPRT mRNA are indicated. **(B)** In situ analysis of Cre-ER$^T$ expression was performed as indicated on cryosections of liver from WT or αAT-Cre-ER$^T$ transgenic mice, hybridized with Cre antisense (AS) and sense (S) riboprobes, according to Dollé and Duboule (1989). Magnification, 160×. **(C)** Cre-ER$^T$ immunohistochemistry was performed with the biotinylated mouse monoclonal antibody 2Cre1A1 (unpublished) on cryosections of liver from tamoxifen-treated WT and αAT-Cre-ER$^T$ transgenic mice as indicated, according to Brocard *et al.* (1997). Staining of Cre-ER$^T$ with 2Cre1A1 (red) and nuclear DNA with DAPI (blue) were superimposed (pink). Magnification, 400×. **(D)** Cre-ER$^T$-mediated DNA excision was determined by Southern blot analysis, performed on genomic DNA isolated from organs of αAT-Cre-ER$^T$/RXRα$^{+/af2(I)}$ double-transgenic mice three days after the last tamoxifen or oil injection and digested with BamHI and hybridized with probe B, as described (Feil *et al.,* 1996; Mascrez *et al.,* 1998). C, control DNA from RXRα$^{+/af2(II)}$ mice. The WT, floxed [af2(I)], and "excised" [af2(II)] RXRα alleles (Mascrez *et al.,* 1998) are indicated by arrows.

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