Control of Memory Formation Through Regional and Temporal Regulation of Gene Expression with the Tet-Off™ System

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Transgenic mice are an ideal model for the investigation of eukaryotic gene expression in vivo. However, the tissue-specific and temporal control of gene expression in whole animals poses special challenges. Here, the Tet-Off™ Gene Expression System was used to regulate forebrain-specific expression of an activated Ca^{2+}-independent form of calcium-calmodulin-dependent kinase II (CaMKII). Expression of the transgene results in a reversible deficit in spatial memory but not in other types of memory.

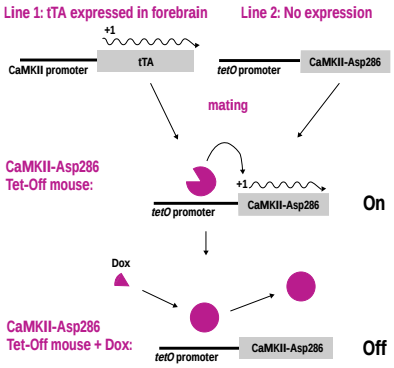

Figure 1. Regulation of the CaMKII-Asp286 transgene with the Tet-Off System. To obtain forebrain-specific Dox-regulated transgene expression, two independent lines of transgenic mice were established. The two transgenes were then introduced into a single line by mating. In the Tet-Off mice, transgene transcription is activated in the absence of Tc or Dox and repressed in their presence.

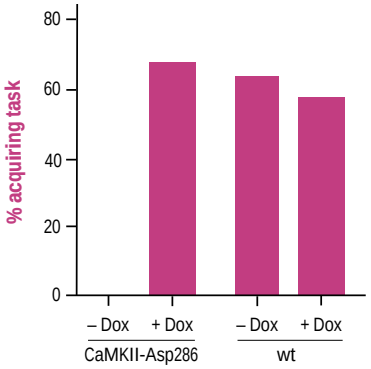

Figure 2. Reversible learning defect in mice expressing the CaMKII-Asp286 transgene. The percentage of mice that met the learning criterion for the Barnes circular maze was determined for the indicated classes of mice. A chi-square analysis revealed a significant difference ($P < 0.0001$) between the percentage of transgenics expressing the CaMKII-Asp286 transgene (–Dox) that acquired the task (0%), and the transgenics receiving Dox (1 mg/ml in drinking water for 4 wk) or the wild-type (wt) groups.

Studies with genetically modified animals have sought to relate specific genes to specific forms of memory storage. However, it is difficult to distinguish between direct effects on synaptic mechanisms and indirect effects on neuronal circuitry development. To analyze the effect of expression of CaMKII on specific forms of memory, we used the tetracycline (Tc)-controlled Tet-Off System to regulate forebrain-specific transgene expression in mice (1, 2).

CaMKII is a serine-threonine protein kinase expressed primarily in neurons of the forebrain. The ability of CaMKII to become persistently active in response to a transient Ca^{2+} stimulus indicates its potential involvement in memory. Furthermore, targeted inactivation of the CaMKII gene leads to deficits in long-term potentiation and certain memory tasks. Mutation of Thr286 to Asp in CaMKII (CaMKII-Asp286) produces a calcium-independent form that mimics the autophosphorylated form. Transgenic expression of CaMKII-Asp286 leads to a shift in response to stimulation as well as a severe defect in spatial memory.

Construction of Tet-Off mice

The strategy employed to obtain tissue-specific, tetracycline-regulated transgene expression is shown in Figure 1. One line of mice was generated expressing the tetracycline-controlled transactivator tTA under control of the CaMKII promoter, which limits expression to neurons of the forebrain. tTA binds to and activates transcription from the *tetO* operator element in the absence of Tc or the Tc derivative doxycycline (Dox). In additional lines, the *tetO* promoter was linked to either the *lacZ* or CaMKII-Asp286 gene. When these lines were mated with the tTA-expressing mice, the *tetO*-linked gene was activated only in those cells that express tTA. Examination of several lines of mice expressing β-gal or CaMKII-Asp286 revealed forebrain-specific expression. The CaMKII-Asp286 transgene was shown to be functionally expressed and regulated in response to Dox. In one line, Ca^{2+}-independent CaMKII activity was increased seven-fold relative to wild type, but returned to wild-type levels in response to treatment with Dox.

Inducible expression of gene control

Severe defects in spatial memory were observed in response to CaMKII-Asp286 expression using the Barnes circular maze. This maze consists of a lighted disk with 40 open holes around the perimeter. Mice are averse to brightly lit open areas and must learn to use cues in the room to find the one hole that leads to a dark escape tunnel. One line of CaMKII-Asp286 mice was incapable of learning this memory task, despite 40 consecutive days of training. However, this profound memory impairment was reversed by suppression of transgene expression in response to treatment with Dox (Figure 2).

We used the Tet-Off Gene Expression System to obtain tightly regulated expression of the CaMKII-Asp286 transgene in restricted regions of the mouse forebrain. This system allowed study of the underlying cellular and behavioral functions dependent on temporal CaMKII expression. Use of the Tet Systems in transgenic mice represents an advancement in the genetic study of cognitive processes and indicates the potential benefits of the Tet Systems for other areas of research.

Product	Size	Cat. #
Tet-Off Gene Expression System*	1 kit	
Tet-Off & Tet-On Cell Lines and Vectors		many

* Kit modified for use described here.

References

1. Mayford, M. *et al.* (1996) *Science* **274**:1678–1683.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.