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REVIEW

The Ons and Offs of Inducible Transgenic Technology: A Review

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Classical transgenic and gene-targeted mouse mutants are powerful model systems in which to study the pathogenesis of neurodegenerative diseases. However, a number of issues of fundamental importance to neurodegenerative research cannot be addressed using classical techniques. These include identification of the earliest events in disease pathogenesis and a determination of whether a particular pathogenic protein produces a inexorable or a reversible disease process. Both of these issues have profound implications for the rational development of new therapies. To address these questions, genetic techniques that allow pathogenic proteins to be expressed or knocked out with temporal and regional specificity have been developed. We have reviewed these systems, highlighting the tetracycline-regulated system because of its demonstrated utility in mice and its reversibility. These regulatable systems are a new and powerful tool for the neurobiologist and allow one to address a new set of important questions in an *in vivo* setting. © 2001 Elsevier Science

INTRODUCTION

Genetically altered mice are powerful model systems in which to study the pathogenesis of neurodegenerative disease. The identification of pathogenic mutations underlying a number of these diseases has allowed investigators to create transgenic mouse models, and their characterization has given us insights into the role of the pathogenic proteins in these disorders (Dawson, 2000; Gurney, 2000; Janus *et al.,* 2000; Sommer *et al.,* 2000). Despite these advances, however, mice made with traditional transgenic techniques are not ideally suited to address many questions of fundamental importance in neurodegenerative research. For example, classical transgenic mice do not allow the identification of the earliest events in disease pathogenesis. Furthermore, constitutively active promoters cannot address whether the progressive nature of these diseases requires continuous expression of the mutant protein, or allow one to determine if the pathogenic protein leads to a reversible or irreversible disease process. These types of questions can only be addressed in a system in which the expression of the pathogenic protein can be turned on or off at defined times.

To address these issues, regulatable promoters have been designed to allow precise control over transgene expression. In this review, we will discuss the most recent advances made in inducible technology and provide a brief summary of how they have been applied in transgenic mice.

EUKARYOTIC-BASED INDUCIBLE SYSTEMS

Regulatable mammalian systems have been designed around the heat shock, metallothionein, interferon-γ, and hormone-dependent promoters. However, their use has mostly been limited to cell culture and has met with little success in mice due to pleio-

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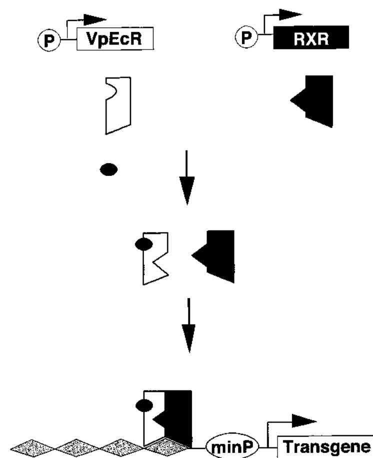

FIG. 1. Eukaryotic-based systems. Schematic diagram of the ecdysone inducible system. VpEcR, a fusion protein between EcR and the VP16 activation domain, is expressed together with RXR. In the presence of ecdysone or its synthetic analogue, murA, the two receptors can heterodimerize, and translocate to the nucleus, and bind to its response elements (EcREs). For optimum induction, four EcREs are placed in front of a minimal promoter (minP) to drive expression of the transgene.

tropic effects from inducers, inefficient regulation, and high basal expression (For review, see Gingrich and Roder, 1998).

A promising eukaryotic-based system worth note, however, is the ecdysone-inducible system, first described in 1996 (Fig. 1) (No *et al.,* 1996). Ecdysone, a Drosophila steroid hormone, acts in concert with its nuclear receptor, the ecdysone receptor (EcR). The ecdysone-bound EcR then can heterodimerize with ultraspinacle (USP), the insect homologue of the vertebrate retinoid X receptor (RXR). Heterodimerization leads to nuclear translocation and transcriptional activation of a transgene linked to the EcR/USP DNA binding domain. Since the insect steroid hormone ecdysone and its synthetic analog muristerone A (mur A) are inert in mammalian systems, this system does not appear to suffer from unwanted pleiotropic or toxic effects from the inducer. Furthermore, ecdysone and mur A are lipophilic, allowing efficient penetration of tissues and have a short half-life. Thus far, the ecdysone-inducible system has been applied only *in vitro,* and the most recent studies used in this system in neuronal cells to examine the role of apolipoproteins in Alzheimer's disease (Niikura *et al.,* 2000), as well as aggregation and toxicity in Huntington's Disease (Jana *et al.,* 2000). Although No and colleagues demonstrated the system's effectiveness in mice, other investigators have not reported its use *in vivo.* Perhaps the requirement for coexpression of three transgenes (EcR, USP, and the DNA binding sequence plus the transgene of interest) is limiting the use of this technique. Nevertheless, the potential of this system for in the study of neurodegeneration is promising.

PHAGE-BASED SYSTEMS: THE CRE/LOXP RECOMBINATION SYSTEM

These systems and the prokaryotic-based ones listed below are designed to allow for expression of a transgene to be induced and abolished in a reversible manner. Although a continuously regulatable promoter is ideal, a number of important biological questions can be addressed simply by turning on a transgene at a defined time point in the adult mouse. The Cre/loxP recombination system allows for this type of control. The *cre* gene of the P1 bacteriophage encodes a 38-kDa site-specific recombinase of the integrase family, that catalyzes intra- and intermolecular recombination between two of its recognition sites termed *loxP* (for review see Nagy, 2000). This 34-bp consensus sequence consists of two Cre binding sites around a core sequence at which recombination can occur. Recombination occurs when two of these core sequences are brought together by Cre recombinase as illustrated in Fig. 2A. The DNA sequence flanked by loxP sites is said to be "floxed," and will be excised when acted on by Cre recombinase. First, two Cre recombinase molecules bind to each *loxP* site. The recombinase molecules then form a tetramer to bring the two loxP sites together, allowing the core regions to be in close enough proximity to recombine, excising the floxed region. Cre-mediated recombination was shown to work in eukaryotic cells in culture experiments in the late 1980s (Sauer and Henderson, 1988) and has since then have been shown to work *in vivo* when Cre is expressed from a transgene in mice (Lakso *et al.,* 1992; Orban *et al.,* 1992).

To gain temporal control over Cre-mediated recombination, several groups have demonstrated that the activity of Cre recombinase can be controlled when fused to the ligand binding domains of nuclear receptors. As noted above, however, the use of nuclear receptors can lead to pleiotropic effects. Therefore, ligand binding domains mutagenized to selectively bind synthetic ligands have been created. Two examples of this approach involve mutating the progesterone receptor, to bind the synthetic steroid RU486, or mutating the estrogen receptor to bind the receptor antagonist tamoxifen. Modifications have also been

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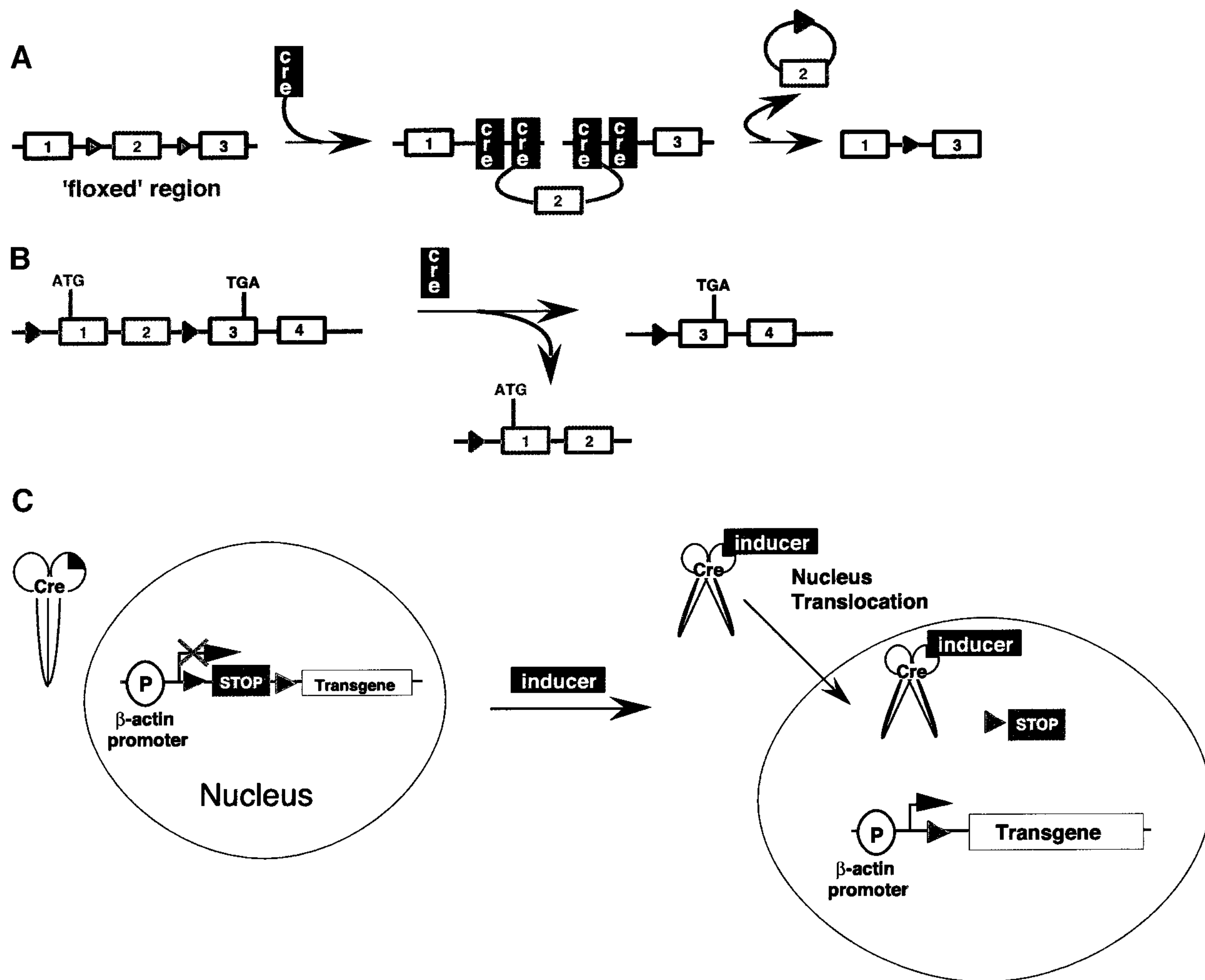

FIG. 2. Cre/loxP system. (A) Cre recombinase-mediated excision. "Box 2" is "floxed" by two head-to-tail oriented lox P sites. Two Cre recombinase molecules bind to each loxP sequence. The four Cre molecules come together to form a tetramer, recombination occurs and "box 2" is excised. (B) Scheme of Cre-mediated gene knockouts. In this strategy, *loxP* sites are "knocked-in" to flank a critical coding region of the gene, and inducible Cre is introduced as a transgene. Since the floxed region can only be excised in the presence of RU486 or tamoxifen, the gene can be "knocked-out" at any time. (C) Scheme of inducible Cre-mediated transgene expression. A "stop fragment," flanked by two loxP sites, is placed between the transcriptional start site and the coding region of the transgene. In presence of the inducer, the fusion Cre translocates to the nucleus, and Cre-mediated recombination between the two loxP sites removes the "stop fragment." In the absence of this sequence, transgene expression can occur.

made to the glucocorticoid receptor in a similar manner. The Cre recombinase is fused to the mutated ligand binding domain, and remains in the cytoplasm (sequestered from 'floxed' DNA) until bound by synthetic ligand (Fig. 2C). Ligand binding translocates the fusion protein into the nucleus, where it can excise the floxed DNA.

The inducible Cre/loxP system can be used in several ways, the most common of which is inducible knock out designs (Fig. 2B). In this strategy, *loxP* sites are "knocked-in" to flank a critical coding region of the gene, and inducible Cre is introduced as a transgene. Since the floxed region can only be excised in the presence of RU486 or tamoxifen, the gene can be "knocked-out" at any time. This method is especially useful to prevent developmental compensations, and to introduce null mutations in the adult mouse that would otherwise be lethal. Two examples of this are the assessment of the retinoid X receptor alpha in skin maintenance (Li *et al.,* 2000), and the function of hun-

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tingtin protein in the adult mouse brain (Dragatsis *et al.,* 2000). The inducible Cre/loxP system may also be used in a two mouse system to irreversibly induce a transgene at a defined time (Kellendonk *et al.,* 1999) (Fig. 2C). In the first transgenic line, the inducible Cre is expressed at high levels in a tissue specific manner. In the second line, the gene of interest is placed under control of a universal promoter such as β-actin. To prevent constitutive expression of this gene, a "stop fragment" flanked by two loxP sites is placed in the 5′ UTR. "Stop fragments" contain several signals such as a polyadenylation signal and a 5′ splice donor site that terminate transcripts. To obtain recombination, the inducer is administered for up to 8 days. Binding of the inducer causes nuclear translocation of inducible Cre and leads to excision of the "stop fragment" and expression of the gene of interest.

The Cre-loxP system allows for a spatial and temporal control over transgene expression, and takes advantage of inducers with minimal pleiotropic effects. However, there are a number of weaknesses with the current technology. The available inducible Cre fusion proteins display a low level of uninduced baseline activity, resulting in nonregulatable excision of the "stop fragment." High levels of recombinase expression are required, and several transgenic inducible Cre lines may need to be generated to obtain adequate expression in the desired pattern. Although promising, the system requires further modifications to achieve better regulatability.

PROKARYOTIC-BASED SYSTEMS: THE LAC AND TET OPERONS

The difficulties of pleitropic effects and toxicity encountered with eukaryotic-based systems have led scientists to investigate regulatable systems derived from prokaryotic organisms. Two well-defined regulatory systems of *Escherichia coli,* the *lac* and *tet* operons have been extensively investigated for their ability to direct inducible transgene expression.

The Lac-Based System

The *lac*-dependent system is based on the well-characterized *E. coli* lac operon (Fig. 3A). In *E. coli,* the lac structural genes are suppressed by the *lac* repressor (lacR). The lacR functions by binding to an operator sequence (lacO), located between the promoter and the regulated genes. When bound, the lacR prevents RNA polymerase from initiating transcription at the promoter. β-galactoside or the synthetic nonmetabolizable isopopyl-β-d-thiogalactopyranoside (IPTG) are able to induce expression of the lacR-repressed genes by binding to the lacR, preventing its binding to lacO.

There have been several attempts to inducibly express lacR-dependent transgenes in mammalian cells, and most have used a modified lacR molecule. In one strategy lacR was fused to the eukaryotic activation domain of herpes virus protein VP16, and multiple lacO sites were placed in tandem in front of a minimal promoter (Fig. 3B) (Labow *et al.,* 1990). This alteration changed the lacR from a repressor to an allosteric activator of transcription, such that IPTG binding induced transcription. Although induction was efficient, temperature-dependence of the system led to pleiotropic effects similar to those encountered when using heat shock dependent regulation. Fusion of lacR with a nuclear localization sequence (Hu and Davidson, 1991) eliminated this temperature-sensitivity, but the NLS led to high basal levels of expression resistant to IPTG regulation (Liu *et al.,* 1992). Moreover, the concentration of IPTG required for full induction is cytotoxic (Figge *et al.,* 1988).

Attempts have also been made to introduce the lacR system in the mouse (Scrable and Stambrook, 1997; Wyborski *et al.,* 1996). However, in contrast to expression in cultured cells, the expression of the lac repressor molecule (Fig. 3A) was either sporadic or absent, thus limiting the degree to which IPTG-mediated expression could be achieved. The inefficient expression was found to be due to heavy methylation of the bacterial sequence, inhibiting transcription (Scrable and Stambrook, 1997). To overcome such epigenetic modifications, the DNA sequence encoding the lac repressor was modified to utilize mammalian amino acid codons, and other structural changes were made to make the transgene resemble a typical mammalian gene (Cronin *et al.,* 2001). This system displays fully reversible regulation, both during embryogenesis and in the adult animal, and is reversible with nM to mM concentrations of IPTG (in the drinking water). The newly modified lacR system appears promising, although its utility for neuroscience research awaits a demonstration that this system works in the brain.

The Tet Regulatable System

The second prokaryotic based system uses the transposon-10 (Tn10) specified tetracycline-resistance (*tet*) operon of *E. coli.* The *tet* operon works similarly to the *lac* operon; a *tet* repressor (tetR) binds to an operator

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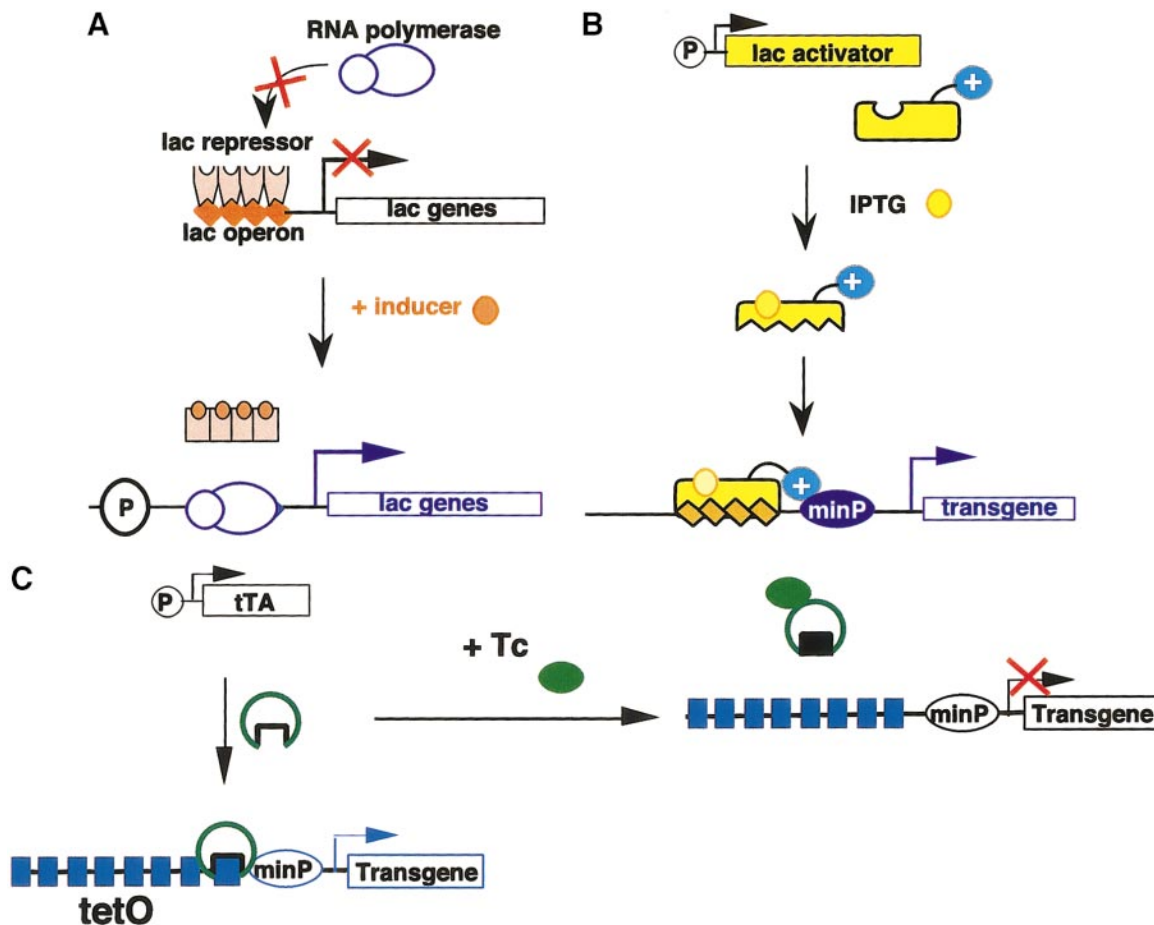

FIG. 3. Prokaryotic-based systems. (A) Schematic representation of the *lac* operon system. The *lac* repressor (lacR) in tetrameric form binds to the lac operon sequences, inhibiting RNA polymerase from binding to initiate transcription of the lac structural genes. (A) In the presence of an inducer (β-galactoside or the synthetic IPTG), lacR can no longer bind to the *lac* operon sequences. RNA polymerase can now bind, and transcription can begin (Adapted from Lewin, 1997). The lac repressor can also be used in a transgenic design (Cronin *et al.,* 2001). (B) The lac activator system. The lac activator, a product of a fusion between the lacR and VP16 activation domain, is expressed with a tissue specific promoter. When IPTG binds, the lac activator can bind to the lac operon sequences and drive transcription of the transgene through a minimal promoter. (C) The tetracycline transactivator system as created by Bujard and colleagues (Gossen and Bujard, 1992). The tTA molecule is a heteromeric molecule derived from the tetR and the VP16 activation domain. This molecule can bind to the tet operon sequence to drive expression of the transgene through a minimal promoter. In the presence of tetracycline (Tc), the binding is allosterically inhibitted, thus shutting the system off in a reversible manner. (Adapted from Yamamoto *et al.,* 2000).

sequence (tetO) and suppresses transcription. Repression is lifted in the presence of tetracycline (Tc), which prevents tetR from binding to tetO (Gossen and Bujard, 1992). In 1992, Hermann Bujard and colleagues reported modification to the tet operon leading to the creation of the tetracycline transactivator (tTA) regulatable system (Fig. 3C). The regulatable molecule, tTA, is constructed from a combination of the tetR DNA binding domain and the VP16 activation domain. tTA binds to a series of tandemly arranged tetO sequences allowing for transcriptional activation through a minimal promoter. In the presence of Tc, however, tTA is unable to bind DNA, blocking transgene expression. The earliest experiments in HeLa cells demonstrated that very low intracellular concentrations of Tc were sufficient to abolish expression of a luciferase reporter gene. No temperature dependence was encountered, and the inhibition was achieved in a rapid, dose-dependent manner.

In order to adapt this system to mice, the generation and crossing of two different transgenic mouse lines is required. The first line expresses the tTA under con-

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trol of a strong promoter while the second line carries a transgene of interest linked to the tetO sequence (tetO-transgeneX). Using β-galactosidase and luciferase as marker genes, Furth *et al.* (1994) found that the Tc-mediated regulation seen *in vitro* also was seen *in vivo,* and full suppression of transgene expression was reached within 7 days of Tc treatment. After treatment with a more lipophilic analog of Tc, doxycycline (dox), mRNA levels are reduced 50% within 2 days of treatment. Furthermore, this repression was reversible, with full induction 7 days following removal of dox (Tremblay *et al.,* 1998).

Although this system overcomes many of the problems encountered in other strategies, the requirement to combine two transgenic lines often leads to unexpected patterns of transgene expression. For example, different tetO-transgene X lines may yield quite different expression patterns when bred with the same tTA line (Furth *et al.,* 1994). Different tetO-transgene X lines bred to the same calcium/calmodulin dependent kinase II α (CamKII α) tTA line yielded a number of different expression patterns, including isolated striatal expression (Ghavami *et al.,* 1999), expression in extra-CamKII regions (unpublished results), and mixed patterns (Yamamoto *et al.,* 2000). The regional variability from line to line may be advantageous or disadvantageous, depending upon the goal of the study. In addition to regional variability, a line may also give chimeric expression within a given region (Furth *et al.,* 1994); we have seen this in an unpublished line of mice. Although the reasons for chimeric expression are not understood, they could conceivably result from uneven expression of tTa or accessibility of the tet-O transgene. As was demonstrated in the lac system noted above, methylation and silencing events in individual cells might contribute to this type of variability. To overcome the unpredictability of the binary system, a strategy that involves targeting by homologous recombination a single cassette containing both the tTA coding region and tetO sequences to promoters of interest has been performed (Bond *et al.,* 2000). By using a knock-in strategy as opposed to a transgenic one, a transgene can be expressed in the normal pattern of a native promoter. Expression levels may differ from wild-type levels due to an amplification effect of the tet system.

Another difficulty of the tet system arises from the exposure to tetracycline. The effect of long-term or prenatal exposure to Tc is unclear. Furthermore, Tc, which is stored in the bone, clears slowly from the animal, thus delaying transgene expression after long-term exposure. These characteristics make tet system unsuitable for the investigation of acute transgene effects.

To address these concerns the reverse Tc transactivator (rtTA) was created (Gossen *et al.,* 1995). rtTA requires Tc to bind to tetO to activate transgene transcription, eliminating the need for long-term Tc exposure for transgene suppression. This system also allows rapid transgene induction at a defined time. In HeLa cells this system displayed rapid, regulatable induction. In primary neuronal cultures there has been significant background expression (Brusa, 1999). Reported regulation in mice has been equally variable, with maximal induction requiring dox administration periods from 24 h (Kistner *et al.,* 1996) to 6 days (Mansuy *et al.,* 1998) for maximal induction. Moreover, the amount of dox required for rtTA-induction is 100 times the levels necessary for tTA-suppression. In light of these findings, Bujard and colleagues have recently reported an improved version of rtTA, termed rtTA2s-M2 (Urlinger *et al.,* 2000). In HeLa cells, this transactivator demonstrates greater sensitivity to the inducer (dox or Tc), increased stability and negligent background expression. The rtTA2s-M2 system has yet to be used in mice.

Bujard and colleagues further enhanced the power of the tet system by creating a bidirectional tetO transcription unit (BiTetO), which allows for coexpression and coregulation of two genes in stoichiometric amounts in the same cells (Krestel *et al.,* 2001) of the same tissues (Baron *et al.,* 1995). A useful application of the BiTetO system is coexpression of the gene of interest with a marker such as β-galactosidase (lacZ). This allows the effectiveness of regulation to be quickly assessed by an easily detectable marker. This is especially useful when the mRNA of the transgene is difficult to monitor, as is the case of a small protein containing a polyglutamine tract (Yamamoto *et al.,* 2000). Green fluorescent protein (GFP) is another useful marker that allows identification of transgene-positive cells in culture or in tissue. The ability to differentiate transgene-positive cells is particularly useful for studying dynamic processes in living cells. The BiTetO thus clearly enhances the already flexible tet-regulatable system allowing one to maximize the use of transgenic animals created under this design.

THE TET-REGULATABLE SYSTEMS APPLIED

The many advantages of the tet regulatable system have made this system the most widely applied strat-

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egy in mice. Another advantage of this system is the ease of Tc administration. Although intravenous or intraperitoneal injections, or subcutaneous time-release pellets can be used, administration through drinking water or food pellets is generally adequate (Tremblay *et al.,* 1998). Recommended doses and routes of administration are summarized in Table 1. Several groups have reported success by expressing transgenes in the CNS using the tet system, including two which focused on neurodegenerative disease (summarized in Table 1).

Memory and Plasticity

Kandel and colleagues were the first to clearly demonstrate the power of the inducible transgenic systems in mice (Mayford *et al.,* 1996). Using classical transgenic animals, these authors had previously shown that expression of a constitutively active mutant form of the calcium/calmodulin dependent kinase II (CamKII) resulted in a selective deficit in hippocampal plasticity (Mayford *et al.,* 1995) and severe impairments in a spatial memory task (Bach *et al.,* 1995). To demonstrate unequivocally that these effects were due to the presence of the mutant CamKII acting in the adult animal and not the consequence of a developmental defect, the transgene was inducibly expressed during adulthood using the tet-based system. The deficits reported in the classical transgenic mouse were replicated when the transgene was expressed in adult mice, but the deficits were fully reversed when the transgene was suppressed (Mayford *et al.,* 1996). Because expression of the transgene was limited to the hippocampus, Kandel and colleagues were further able to prove this structure as the site responsible for the behavioral effects. Using a similar approach, Mansuy *et al.* expressed a constitutively active form of the calcium dependent phosphatase calcineurin under the control of rtTA. The inducible control allowed this study to probe the effect of calcineurin induction on temporally distinct components of memory in adult mice (Mansuy *et al.,* 1998).

Prion Disease

Studies of transmissible spongiform encephalopathy, or prion disease, have demonstrated that conversion of the cellular form of prion protein (PrP^c) into a pathogenic, protease-resistant form (PrP^{Sc}) is crucial for disease pathogenesis. However, disagreement existed about whether PrP^{Sc} was an integral component of the infectious agent (For review, see Chesebro, 1999). To test this protein-only hypothesis, PrP^c knockout mice were inoculated with PrP^{Sc}. PrP-deficient mice did not develop disease, and abnormal accumulation of PrP^{Sc}, a hallmark of prion disease, was not found (Bueler *et al.,* 1994; Prusiner *et al.,* 1993; Sailer *et al.,* 1994). Although these studies were impressive, it remained possible that the absence of pathology in PrP^c null mice was due to a developmental compensation resulting from the lack of PrP^c. If this were the case, adult mice inoculated with PrP^{Sc} after induced loss during adulthood of PrP^c expression would be expecteed to develop prion pathology. To test this, mice in which PrP^c expression could be controlled with the tet system were generated on a PrP^c knockout background (Tremblay *et al.,* 1998). In these mice, tTa expression was driven by the prion promoter, and the PrP^c cDNA was place downstream of tetO. tTA mediated expression of PrP^c (200 to 300% endogenous levels) was maintained throughout development until adulthood. To shut-off transgene expression, dox was administered in several ways including 7 days of dox in the drinking water (2 mg/ml), 7 days of a subcutaneous pellet (50 or 200 mg dox or minocycline), or 3 days daily intravenous injection (25 mg/kg). Of the four tet-transgene lines created, three had 5 to 15% residual expression, while one line had 10% residual expression even after 30 days of oral treatment. Despite this nonregulatable expression, mice expressing 15% of PrP^c levels did not develop prion pathology after inoculation with PrP^{Sc}, even after 1 year of observation. In contrast, mice that received innoculations without the preceding dox suppression developed progressive ataxia within 2 months, and had clear signs of neurodegeneration where PrP^c was expressed. Taken together, these findings clearly indicate that the absence of PrP^c, not developmental compensation, accounts for the absence of pathology in inoculated knockout mice.

Huntington's Disease

Recently, we used the tet-inducible system to study the pathogenesis of Huntington's Disease (HD), a member of the triplet repeat expansion disorders. This inheritable neurodegenerative disease is caused by a CAG expansion mutation in exon 1 of the huntingtin gene (For review, see Leavitt *et al.,* 1999). The mutation results in a polyglutamine (polyQ) stretch in the N-terminus of the huntingtin protein, which can lead to novel protein-protein interactions (For review see Petersen *et al.,* 1999), as well as form insoluble aggregates (Perutz *et al.,* 1994). Transgenic mice that express exon

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TABLE 1
Summary of Published Tet Regulatable Systems in Mice

Citation	Application	tTA line	TetO-transgene expression pattern	TetO-transgene expression level	Dox administration	Dox regulation
Mayford *et al.* (1996)	The role of CamKII in memory and storage	CamKIIα-tTA Line B 8.5-kb genomic fragment of promoter region.	LacZ Endogenous CamKIIa pattern but no CA3 CamKII-Asp286 B13: FB, no CA3. B21: Hippo, stria, amyg, little in ctx. No CA3. B22: Stria. amyg, olf tub.	Up to sixfold of WT levels of expression.	1 to 2 mg dox per 1 ml 5% sucrose solution in lieu of drinking water	Dox treatment returned protein to wild-type levels 2–3 week dox treatment led to reversal in LTP deficits; 4 weeks led to reversal in memory tasks
Chen *et al.* (1998) Nestler *et al.* (1999) Kelz *et al.* (1999)	The role of ΔFosB in sensitivity to cocaine and neural and behavioral plasticity	NSE-tTA Lines A, B, C, D 1.8-kb promoter fragment	Luciferase (TetOp-luciferase/ TetOp-tTA) × Line A, D: stria × Line B: cereb, stria × Line C: ctx > stria, hippo ΔFosB × Line A*, B* CREB × Line A* (*regions as seen with luc)	Dose response of dox vs luciferase activity showed 0.25 μg/ml led to a 12-fold reduction, while 2.5 μg/ml was as effective as 200 μg/ml	200 μg dox per 1 ml of 5% sucrose solution in lieu of drinking water	Transgene reexpression dependent upon amount of dox used; after 2 mg/ ml dose during gestation and life, 8 weeks were required for full reinduction
Trembley *et al.* (1998)	The necessity of PrPC for prion disease	Prion-tTA F973, F959, F966	F973 > F959, F966: expression throughout brain	TetO-PrP F966 × E6740: 200% WT F959 × E6740: 400% WT F959 × E6550: 800% WT F959 × E7655: 100% WT	2 mg dox per 1 ml 5% sucrose solution 21-day time release s.c. 50 or 200 mg pellets dox or mino. 25 mg/kg i.v. for 3 days	Leaky expression. 2-day treatment for 50% repression of protein levels, 7 d for 100%. Protein reexpression at 50% after 2 d, 100% after 7 d.
Yamamoto *et al.* (2000)	The necessity of continuous expression of mutant huntingtin fragment in HD-like pathology	CamKIIα-tTA Line E 8.5-kb genomic fragment of promoter region	BiTetO (lacZ-biTetO-exon1CAG94) LacZ and exon1CAG94 expression patterns overlapped. Stria, hippo > ctx >amyg, hypo. No expression in cereb.	Prenatal expression as early as E14 detected Fetal lethality reversed via 0.1 to 2 mg/ml of dox. 50 μgml was insufficient	50 μg to 2 mg dox per 1 ml 5% sucrose solution	16-week treatment led to aggregate and behavior reversal LacZ suppression seen after 7 day dox treatment of 2 mg/ml
Mansuy *et al.* (1998)	The role of calcineurin in memory	CamKIIa-rtTA Line 1237 Line 1076 8.5-kb genomic fragment of promoter region.)	LacZ lacZ: Hippo (CA1, CA2), ctx, stria, sept. ΔCam-Al CN279: Hippo (all), ctx and stria.	2-week induction led to approximately 77% increased calcineurin activity in hippo 2 weeks after dox removal, no transgene mRNA could be detected.	6 mg dox per 1 g of food; made into food pellets 12 mg/g yielded similar results	Full induction at 6 days partial induction at 3 days. No expression without dox.

Note. Abbreviations: amyg, amygdala; cereb, cerebellum; ctx, cortex; dox, doxycycline; FB, forebrain; hippo, hippocampus; hypo, hypothalamus; mino, minocycline; olf tub, olfactory tubercle; sept, septum; stria, stria; thal, thalamus; % WT, % wild-type levels.

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1 of huntingtin with a pathogenic stretch of CAG, develop intranuclear protein aggregates, striatal atrophy, changes in dopamine receptor levels and motor deficits (Davies *et al.,* 1997). These findings demonstrate that expression of an expanded polyQ stretch is sufficient to produce HD-like pathology. A number of transgenic models have been created since this original report, and all have confirmed that the expression of an expanded polyQ stretch leads to a HD-like pathology, regardless of protein context of the motif.

Two questions with profound implications for the development of a therapy could not be addressed with these classical transgenic models. First, is the cascade of pathological events initiated by the mutant protein dependent upon its continued expression? Second, are the behavioral or biochemical phenotypes irreversible, or might they improve if the toxic transgene were turned off? To explore these issues, we conditionally expressed exon 1 of the huntingtin gene with an expanded CAG repeat (HD94) using the tet regulatable system (Yamamoto *et al.,* 2000).

Expression of the transgene was restricted to the forebrain, by breeding BiTetO-HD94 mice with mice that express tTA under the control of the CamKIIα promoter. Expression of the mutant protein led to a progressive behavioral and pathological phenotype similar to HD. The mice developed cytoplasmic and nuclear aggregates, striatal atrophy, reactive astrocytosis, and a motor phenotype. After this phenotype was well established (18 weeks), mice were given a regimen of 2 mg/ml of oral dox in the drinking water. After 16 weeks without expression of the expanded polyQ there was a complete resolution of aggregates in the striatum, and a dramatic reduction of aggregates in the cortex. Furthermore, the motor phenotype reversed to levels approaching control. These results suggest that the progressive nature of HD is dependent upon continuous expression of the CAG expansion, and that abolishing expression of the mutant protein, can reverse HD-like behavioral and pathological phenotypes.

CONCLUSION

Transgenic mouse models have been invaluable in the effort to unravel the pathogenic processes underlying neurodegenerative disease. Inducible systems, although still at an early stage of development, offer a more powerful means to approach these complex questions by allowing investigators to express pathogenic proteins at specified times and discrete brain regions.

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