TNF-R2 induced NF-κB–dependent reporter gene activation in 293 cells that were not transfected with TNF-R2 (H. Hsu and D. V. Goeddel, unpublished results). This observation is consistent with results obtained from electrophoretic mobility–shift assays (Fig. 1).
21. M. Rothe and D. V. Goeddel, unpublished results.
22. M. Lewis *et al., Proc. Natl. Acad. Sci. U.S.A.* **88**, 2830 (1991).
23. Transient transfection of CT6 cells was done according to the DEAE-dextran method [F. M. Ausubel *et al., Current Protocols in Molecular Biology* (Green/Wiley, New York, 1994)]. CT6 cells (10^7) were transfected with a total of 10 μg of plasmid DNA in DEAE-dextran (250 μg/ml) for 90 min. Reporter gene activity was assayed 40 hours after transfection (*18*).
24. Similarly, TNF-R1 overexpression in the absence of exogenous TNF induces apoptosis and NF-κB activation in both HeLa cells and 293 cells [M. P. Boldin *et al., J. Biol. Chem.* **270**, 387 (1995); H. Hsu and D. V. Goeddel, unpublished results].
25. C. Laherty, H. Hu, A. Opipari, F. Wang, V. M. Dixit, *J. Biol. Chem.* **267**, 24157 (1992); M. Rowe *et al., J. Virol.* **68**, 5602 (1994).
26. S. Schütze *et al., Cell* **71**, 765 (1992).
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Inducible Gene Targeting in Mice

Ralf Kühn,* Frieder Schwenk, Michel Aguet,† Klaus Rajewsky

A method of gene targeting that allows the inducible inactivation of a target gene in mice is presented. The method uses an interferon-responsive promoter to control the expression of Cre recombinase. Here, Cre was used to delete a segment of the DNA polymerase β gene flanked by *loxP* recombinase recognition sites. Deletion was complete in liver and nearly complete in lymphocytes within a few days, whereas partial deletion was obtained in other tissues. This method can be used for the inducible inactivation of any other gene in vivo.

Studies of gene function in mice often involve the analysis of embryonic stem (ES) cell–derived gene-targeted mice. Such mice carry a predesigned mutation in their germ line and are devoid of a particular gene product throughout ontogeny (*1*). A strategy for conditional, cell type–specific gene targeting was recently developed (*2*) that uses the Cre/*loxP* recombination system of bacteriophage P1 (*3*). In this procedure, a target gene is flanked ("floxed") with recombinase recognition (*loxP*) sites introduced by homologous recombination in ES cells; the amount of expression of the floxed allele should be the same as that in the wild type unless it is inactivated by Cre-mediated deletion of the *loxP*-flanked gene segment. Gene inactivation can be restricted in vivo to a particular cell type by crossing a mouse strain harboring the floxed allele to a transgenic strain expressing Cre recombinase constitutively under the control of a cell type–specific promoter (*2*).

These approaches have a major drawback. The function of the gene product must be deduced from the phenotype of animals that are, either in all cells or in certain cell types from an early time point on, constantly deficient throughout ontogeny for the product of the disrupted gene. A mutant organism may compensate for the loss of a gene product so that no obvious deviation from the wild type is seen, or the organism may react to the mutation to give a complex, secondary phenotype. Moreover, if the complete loss of a gene product results in embryonic lethality (*4*), gene function at later stages of development cannot be analyzed. To overcome these limitations, we have developed a method to induce gene inactivation in mice at a given time during ontogeny. This method uses the inducible promoter of the mouse *Mx1* gene (*5*) to control the expression of a Cre recombinase transgene. *Mx1*, part of the defense to viral infections, is silent in healthy mice. The *Mx1* promoter can be transiently activated to high amounts of transcription in many tissues upon application of interferon α (IFN-α) or IFN-β or of synthetic double-stranded RNA [polyinosinic-polycytidylic acid (pI-pC), an IFN inducer (*6*, *7*)]. Hence, mice harboring a floxed target gene and a *Mx-cre* transgene acquire an inactivating mutation of the target gene upon treatment of the mice with IFN or pI-pC.

To assess the efficiency of induced Cre-mediated deletion of a target gene, we generated mice transgenic for a Mx-cre expression vector (*8*) and crossed them to a strain harboring an allele of the DNA polymerase β gene that contains two directly repeated *loxP* sites. This allele, *pol*β^{flox} (*2*), is inactivated upon excision of a *loxP*-flanked 1.5-kb segment containing the promoter region and first exon of the *pol*β gene. Cre-mediated deletion of the floxed gene segment in a given tissue can be quantified by Southern blot analysis of genomic DNA, which enables comparison of the intensities of the band from the *pol*β^{flox} allele and the fragment derived from the deleted (*pol*β^{Δ}) allele (Fig. 1).

Two of 42 Cre transgenic strains generated (Mx-cre31a and 31b) (*8*) exhibited low background recombination, but extensive deletion could be induced by treatment of adult mice with IFN or pI-pC. Groups of five mice (strain Mx-cre31a) received ei-

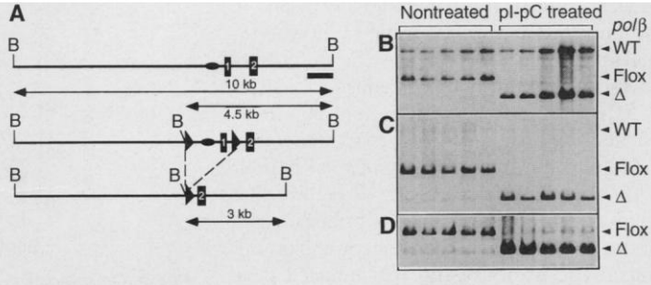

Fig. 1. Detection of Cre-mediated deletion. (**A**) Southern blot strategy to distinguish *pol*β wild-type (+) (upper line), *pol*β^{flox} (middle line), and *pol*β^{Δ} (lower line) alleles. Bam HI fragments (B) containing the promoter region (ellipses), the first two exons of the *pol*β gene (numbered rectangles), and an adjacent genomic probe used for hybridization (bar) are shown. In the *pol*β^{flox} allele a 1.5-kb gene segment has been flanked by two *loxP* sites (triangles) (*2*). Upon Bam HI digestion the wild-type, *pol*β^{flox}, and *pol*β^{Δ} alleles yield fragments of 10, 4.5, and 3 kb, respectively (*2*). (**B** through **D**) Southern blot analysis of DNA from liver (B) and spleen (D) of groups of *pol*β^{flox}/+ Mx-cre transgenic mice and from liver (C) of *pol*β^{flox}/*pol*β^{flox} Mx-cre transgenic mice (strain Mx-cre31a) that received three intraperitoneal (IP) injections of pI-pC (250 μg) at 2-day intervals or were left untreated. Genomic DNA was prepared 2 days after the last injection. The positions of the fragments derived from the *pol*β wild-type (WT), *pol*β^{flox} (Flox), and *pol*β^{Δ} (Δ) alleles are indicated. Each lane represents one mouse. In (C) the wild-type bands are absent but a weak background band appears at the same position. (**E**) Southern blot analysis of DNA from liver of mice (strain Mx-cre31a) that received three IP injections of human IFN-α_2/α_1 (10^6 units; lane 3) (*12*) or pI-pC (250 μg; lane 2) in comparison to a nontreated mouse (lane 1). Female littermates heterozygous for the *Mx-cre* transgene were used at an age of 8 weeks.

R. Kühn, F. Schwenk, K. Rajewsky, Institute for Genetics, University of Cologne, Weyertal 121, 50931 Cologne, Germany.
M. Aguet, Institute of Molecular Biology I, University of Zürich, Hönggerberg, 8093 Zürich, Switzerland.

*To whom correspondence should be addressed.
†Present address: Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA.

ther three injections of pI-pC at 2-day intervals or were left untreated. Genomic DNA was prepared from various organs of these mice 2 days after the last injection. Deletion of the floxed *pol*β gene segment was complete (100% deletion) in the liver of treated mice heterozygous or homozygous for the *pol*β^{flox} allele, whereas the background recombination was in the range of 1% in nontreated mice (1 ± 2%, mean ± SD; Figs. 1, B and C, and 2). In other organs the extent of induced deletion was variable, ranging from 94% in spleen (9) to 8% in brain (Figs. 1D and 2) (*10*), whereas no deletion was found in testis (*11*). This heterogeneity may reflect differing proportions of IFN-responsive cells or IFN availability in various tissues.

Similar amounts of deletion were obtained when recombinant human IFN-α_2/α_1 (*12*) was used for induction instead of pI-pC (Fig. 1E). The efficiency of Cre-mediated deletion was similar in organs from heterozygous and homozygous *pol*β^{flox} mice (Fig. 2) as well as hemizygous *pol*β^{flox}/*pol*β$^{\Delta}$ mice (*11*). Background recombination in nontreated mice was undetectable in some tissues but was as much as 10% in spleen (Fig. 2), possibly because of the endogenous production of IFN in these mice, which were maintained in a conventional facility. In Mx-cre mice kept under specific pathogen-free (SPF) conditions, endogenous IFN production will be lower and hence this background recombination may be reduced.

In addition to their antiviral activity, IFN-α/β and pI-pC exert transient antiproliferative and immunomodulatory effects in vitro and in vivo (*7*). When the liver and kidney of mice treated three times with pI-pC were examined histologically 2 days after the last injection, no gross abnormalities compared to nontreated controls were found (*13*). In spleen, the populations of B and T lymphocytes appeared normal by flow cytometric analysis (*14*) whereas the number of thymocytes, known to be reduced by stress (*15*), was diminished by 50% in pI-pC–treated mice and by 25% in IFN-treated mice. Although the treatment of mice with IFN-α/β or pI-pC has side effects, we believe that these effects are limited to the brief exposure of the mice to the inducing agent and do not impair the utility of the IFN-induced gene targeting system. However, to minimize potential side effects, we suggest the use of IFN-α/β as the inducer of choice because pI-pC can stimulate the production of cytokines other than IFN, such as tumor necrosis factor α (TNF-α) (*16*).

A single pI-pC injection was sufficient to induce the deletion of 97% of the *pol*β^{flox} allele within 2 days in the liver of Mx-cre transgenic mice (Fig. 3). A second injection after 2 days did not increase the amount of deletion, probably because the first stimulation elicits a state of IFN hyporesponsiveness that lasts longer than 48 hours (*7*). After a third dose of pI-pC, deletion was complete in liver and was increased in spleen and heart (Fig. 3). Thus, Cre-mediated deletion can occur rapidly and very efficiently in differentiated cells in vivo and is almost complete in liver after a single injection of pI-pC. These results demonstrate that gene inactivation can be induced with high efficiency in mice within a few days and that Cre-mediated deletion is achievable in organs composed mainly of resting cells. We have also shown that both alleles of a *loxP*-modified gene can be deleted in vivo with the same high efficiency as a single *loxP*-flanked allele in heterozygous mice; this result establishes the feasibility of the conditional gene targeting strategy because both alleles of a target gene must be inactivated to investigate gene function in a mutant.

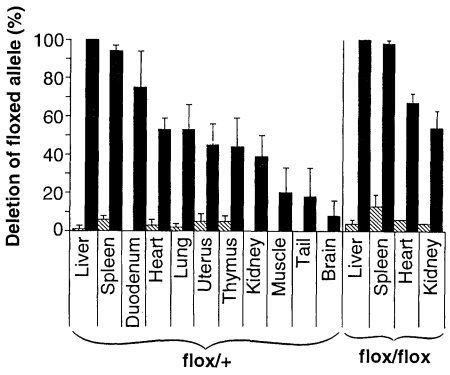

Fig. 2. Deletion of the *pol*β^{flox} allele in various organs of *pol*β^{flox}/+ Mx-cre transgenic mice (flox/+) and *pol*β^{flox}/*pol*β^{flox} Mx-cre transgenic mice (flox/flox). Groups of 5 mice (8-week-old females, strain Mx-cre31a) were treated with pI-pC as described in Fig. 1 (solid bars) or were left untreated (striped bars) and genomic DNA was prepared from the indicated organs 2 days after the last injection. Cre-mediated deletion of the *pol*β^{flox} allele was detected by Southern blot analysis and quantified with a Bio-Imaging analyzer (Fuji Bas 1000). Percentages of deletion (mean ± SD) were calculated by subtraction of the background radioactivity measured between the bands from the *pol*β^{flox} and *pol*β$^{\Delta}$ alleles from the activity measured in these bands; areas of the same size were used for all measurements. The remaining activity was added and set as 100%, and the proportion of the *pol*β$^{\Delta}$ allele was calculated. This method gave values of 46 to 54% for the ratio of *pol*β^{flox} to *pol*β$^{\Delta}$ alleles in the genome of *pol*β^{flox}/*pol*β$^{\Delta}$ mice in independent experiments (*11*).

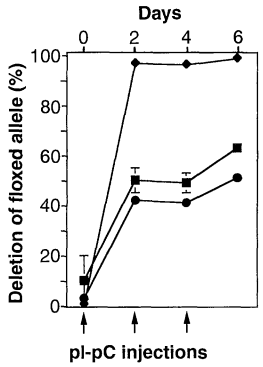

Fig. 3. Cre-mediated deletion of the *pol*β^{flox} allele in relation to the number of pI-pC injections. Groups of two *pol*β^{flox}/+ Mx-cre transgenic mice (8 weeks old, strain Mx-cre31b) received one, two, or three IP injections of pI-pC (250 μg) at 2-day intervals and genomic DNA was prepared from liver (◆), spleen (■), and heart (●) 2 days after the last injection (at day 2, 4, or 6). Deletion of the *pol*β^{flox} allele (mean ± SD) was measured as described in Fig. 2; for most data points the deviation was too small to be visualized. Values obtained from nontreated mice (day 0) are shown for comparison. Deletion in spleen of Mx-cre 31b mice is not as efficient as in Mx-cre31a mice (Fig. 2).

The ubiquitously expressed DNA polymerase β fills small single-stranded DNA gaps and is considered to be part of the mammalian DNA repair machinery (*17*). Polymerase β–deficient mice derived from gene-targeted ES cells are not viable (*2*). Thus, the IFN-inducible gene targeting system will be a valuable tool to investigate the possible roles of polymerase β in vivo, such as DNA repair in liver and, potentially, somatic hypermutation in B lymphocytes. The system should prove especially useful in studies of gene function in liver and the immune system because extensive deletion can be obtained in these tissues. The partial deletion obtained in other tissues of Mx-cre mice may be sufficient for gene inactivation experiments that result in the gain of a new, easily detectable phenotype and do not depend on the inactivation of a gene in all cells of a tissue. At present, Cre-mediated gene deletion in Mx-cre mice cannot be restricted to certain tissues. However, gene inactivation could be confined to a certain cell type if Mx-cre mice were crossed to IFN-α/β receptor–deficient mice (*18*) reconstituted with a murine or human IFN-α/β receptor gene controlled by a cell type–specific promoter.

REFERENCES AND NOTES

1. B. H. Koller and O. Smithies, *Annu. Rev. Immunol.* **10**, 705 (1992); D. W. Melton, *Bioessays* **16**, 633 (1994).
2. H. Gu, J. D. Marth, P. C. Orban, H. Mossmann, K. Rajewsky, *Science* **265**, 103 (1994).
3. N. Sternberg and D. Hamilton, *J. Mol. Biol.* **150**, 467 (1981); B. Sauer and N. Henderson, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5166 (1988).
4. A. J. Copp, *Trends Genet.* **11**, 87 (1995).
5. H. Hug, M. Costas, P. Staeheli, M. Aebi, C. Weissmann, *Mol. Cell. Biol.* **8**, 3065 (1988); H. Arnheiter, S. Skuntz, M. Noteborn, S. Chang, E. Meier, *Cell* **62**, 51 (1990).
6. P. Staeheli, P. Danielson, O. Haller, G. Sutcliffe, *Mol. Cell. Biol.* **6**, 4770 (1986); G. C. Sen and R. M. Ransohoff, *Adv. Virus Res.* **42**, 57 (1993); B. R. G. Wil-

liams, *Eur. J. Biochem.* **200**, 1 (1991); K.-C. Chang, G. Goldspink, J. Lida, *Arch. Virol.* **110**, 151 (1990).
7. E. de Clerq, *Antibiot. Chemother. (Basel)* **27**, 251 (1980); I. Gresser, *Cell. Immunol.* **34**, 406 (1977); F. D. Finkelman *et al., J. Exp. Med.* **174**, 1179 (1991).
8. To construct the Mx-cre expression vector, we ligated a 2.3-kb Eco RI–Bam HI fragment containing the *Mx1* gene promoter (*5*) to the modified coding region of Cre [H. Gu, Y. R. Zou, K. Rajewsky, *Cell* **73**, 1155 (1993)], which includes a nuclear localization signal, and to a 2.1-kb fragment of the human growth hormone gene, which provides splicing and polyadenylation signals [K. E. Chaffin *et al., EMBO J.* **9**, 3821 (1990)]. Transgenic mice were produced by pronucleus injection of fertilized (C57BL/6x CBA)F_2 eggs. Founder mice were first screened for IFN-inducible expression of Cre with the use of peripheral blood lymphocytes. Of 42 strains generated, 16 showed inducible Cre expression and were crossed to mice harboring the *pol*β^{flox} allele to test for Cre-mediated deletion in vivo. Strains Mx-cre31a and 31b, which contain ~10 transgene copies and originate from the same founder mouse but are distinguished by their transgene integration pattern, showed the most extensive deletion.
9. The extent of deletion was comparable in purified B cells, T cells, and non-B–non-T cells (*11*).
10. These results were reproducible in several independent experiments (R. Kühn, unpublished data).
11. R. Kühn, unpublished data.
12. H. Weber, D. Valenzuela, G. Luijber, M. Gubler, C. Weissmann, *EMBO J.* **6**, 591 (1987).
13. J. Löhler, unpublished data.
14. Stainings were performed with antibodies to B220, immunoglobulin M, CD3, CD4, and CD8 (R. Kühn, unpublished data).
15. T. F. Dougherty, *Physiol. Rev.* **32**, 379 (1952).
16. M. G. Whatelet, P. M. Berr, G. A. Huez, *Eur. J. Biochem.* **206**, 901 (1992).
17. R. K. Singhal and S. H. Wilson, *J. Biol. Chem.* **268**, 15906 (1993); T. S.-F. Wang, *Annu. Rev. Biochem.* **60**, 513 (1991); K. Wiebauer and J. Jiricny, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5842 (1990).
18. U. Müller *et al., Science* **264**, 1918 (1994).
19. We thank J. Löhler for the histological analysis, H. Gu for *pol*β gene-targeted mice, C. Weissmann for IFN, and U. Ringeisen for graphical work. Supported by Deutsche Forschungsgemeinschaft grant SFB 243, the Human Frontier Science Program, the Bundesministerium für Forschung und Technologie, and the Land Nordrhein-Westfalen. F.S. is supported by a stipend from the Boehringer Ingelheim Fonds.

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The Chromodomain Protein Swi6: A Key Component at Fission Yeast Centromeres

Karl Ekwall,* Jean-Paul Javerzat,*† Axel Lorentz, Henning Schmidt, Gwen Cranston, Robin Allshire‡

Centromeres attach chromosomes to the spindle during mitosis, thereby ensuring the equal distribution of chromosomes into daughter cells. Transcriptionally silent heterochromatin of unknown function is associated with centromeres in many organisms. In the fission yeast *Schizosaccharomyces pombe*, the silent mating-type loci, centromeres, and telomeres are assembled into silent heterochromatin-like domains. The Swi6 chromodomain protein affects this silencing, and now it is shown that Swi6p localizes with these three chromosomal regions. In cells lacking Swi6p, centromeres lag on the spindle during anaphase and chromosomes are lost at high rates. Thus, Swi6p is located at fission yeast centromeres and is required for their proper function.

Cytologically observable heterochromatin in *Drosophila* and mammals is, in general, incompatible with gene expression (*1*, *2*). The function of these transcriptionally silent regions remains enigmatic (*1*, *2*). Higher eukaryotic centromeres, with their associated kinetochore complex, are formed in the vicinity of centromeric heterochromatin, but whether heterochromatin itself contributes to centromere function is unknown. One possibility is that transcriptional repression at centromeres, and therefore heterochromatin, is a manifestation of normal centromere-kinetochore assembly. If this is true, then certain gene products should localize with centromeres and play a role in both the formation of silent heterochromatin and centromere function.

In the fruit fly *Drosophila melanogaster* and in mammals, proteins containing chromodomains, a motif first described as a region of similarity between HP-1 and *Polycomb* (*3*), are associated with heterochromatin or impose repression in a temporal manner on important developmental genes (*4*–*10*). Mutations in the *Drosophila* gene *Su(var)205*, which encodes the chromodomain protein HP-1, were originally identified as suppressors of variegating position effects, which suggested a role for HP-1 in the formation of heterochromatin (*11*). In the fission yeast *Schizosaccharomyces pombe*, the silent mating-type loci, centromeres, and telomeres share several properties with heterochromatic domains (*12*–*14*). The *swi6*$^+$ gene of fission yeast encodes a chromodomain protein and is required to maintain transcriptional repression at the silent mating-type loci and centromeres (*15*, *16*). Mutations in the *swi6* gene result in a highly increased rate of chromosome loss (*16*). The chromodomain of Swi6p is 46% identical to that of *Drosophila* HP-1 across this 50–amino acid domain (*15*). We now present evidence that the Swi6 protein is an important functional component at fission yeast centromeres.

A purified fusion protein was used to raise a polyclonal serum and to affinity-purify Swi6p-specific antibodies (anti-Swi6p). Protein immunoblotting with anti-Swi6p detected a single band migrating at ~50 kD in extracts from wild-type strains (Fig. 1A) (*17*). This band was absent in extracts from strains in which the *swi6*$^+$ gene is deleted (*swi6::his1*), and only a faint signal was seen in extracts from strains with the missense *swi6-115* allele (W269R).

Immunofluorescence analysis with affinity-purified anti-Swi6p produced a distinct pattern of staining in fixed cells (*17*). Several discrete spots were seen in interphase haploid and diploid cells (red, Fig. 1, B and C). Most haploid and diploid cells contained three and five spots of anti-Swi6p fluorescence, respectively (Fig. 1E). No staining with anti-Swi6p was detected in strains harboring a complete deletion of the *swi6* gene (Fig. 1D).

Mutations at *swi6* cause a high rate of chromosome loss during mitosis (Table 1) and affect silencing within the centromere (*16*). It therefore seemed plausible that some of the anti-Swi6p spots might repre-

Table 1. Frequency of chromosome lagging and loss in wild-type and *swi6* mutants at 18°C.

Background	Cells in late anaphase (%)*	Late anaphase cells with lagging centromeres (%)*	Rate of minichromosome loss (Ch16) per division†
Wild type	3.5	<1.0 (0/98)	<0.08 (0/1241)
swi6::his$^+$	3.7	48.8 (39/80)	4.6 (17/370)
swi6-115	2.7	24.5 (15/61)	1.3 (11/849)

*Late anaphase cells were defined as cells with a spindle length greater than 5 μm. Cells were classified as having lagging centromeres if a single centromere FISH signal was detected at least 1.5 μm away from an anaphase spindle pole (as in Fig. 4B). For all strains, the frequency of lagging centromeres and minichromosome loss were measured in cells from the same culture. Similar results were obtained in different cultures. †The rate of minichromosome loss was determined as described (*16*).

K. Ekwall, J.-P. Javerzat, G. Cranston, R. Allshire, Medical Research Council Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland, UK.
A. Lorentz and H. Schmidt, Institüt für Genetik, TU Braunschweig, Sperlmannstrasse 7, D-38106 Braunschweig, Germany.

*These authors contributed equally to this work.
†Present address: Laboratoire de Génétique, Université de Bordeaux II, Avenue de Facultes, 33405 Talence, France.
‡To whom correspondence should be addressed.