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Genetics

Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase

(gene targeting/retinoic acid receptor/retinoid X receptor/estrogen receptor/estradiol)

DANIEL METZGER, JOHN CLIFFORD, HIDEKI CHIBA, AND PIERRE CHAMBON

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université Louis Pasteur, Collège de France, 67404 Illkirch-Cedex, C.U. de Strasbourg, France

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ABSTRACT **We have developed a strategy to generate mutant genes in mammalian cells in a conditional manner by employing a fusion protein, Cre-ER, consisting of the *loxP* site-specific Cre recombinase linked to the ligand-binding domain of the human estrogen receptor. We have established homozygous retinoid X receptor α-negative (RXR$\alpha^{-/-}$) F9 embryonal carcinoma cells constitutively expressing Cre-ER and have shown that estradiol or the estrogen agonist/antagonist 4-hydroxytamoxifen efficiently induced the recombinase activity, whereas no activity was detected in the absence of ligand or in the presence of the antiestrogen ICI 164,384. Furthermore, using a targeting vector containing a selection marker flanked by *loxP* sites, we have inactivated one retinoic acid receptor α allele in such a line, demonstrating that the presence of the recombinase does not inhibit homologous recombination. Combining this conditional site-specific recombination system with tissue-specific expression of Cre-ER may allow modification of the mammalian genome *in vivo* in a spatiotemporally regulated manner.**

Gene targeting through homologous recombination provides a powerful tool for generating predetermined changes in animal genomes. Classical approaches to gene targeting usually involve the introduction of a null mutation into cell lines or embryonic stem cells (refs. 1–3; for review, see ref. 4). In some cases it is desirable that gene inactivation occur in a conditional manner—for instance, when null mutations are lethal or when it is necessary to analyze the effect of a mutation during cell differentiation.

The Cre recombinase, an integrase from bacteriophage P1, catalyzes site-specific recombination between 34-bp repeats termed *loxP* sites, in the absence of any additional cofactors. The intervening DNA between two *loxP* sites positioned head-to-tail is excised along with one *loxP* site in the presence of Cre. Alternatively, inversion of the intervening DNA occurs if the *loxP* sites are placed in head-to-head orientation. Prior placement of a *loxP* site in the chromosome, on the other hand, allows subsequent targeting of that site by Cre recombinase (ref. 5 and references therein).

The fusion of the ligand-binding domain (LBD) of steroid hormone receptors, notably that of the estrogen receptor (ER), to a variety of heterologous proteins has been shown to result in a ligand-dependent control of their activity. Such heterologous ligand-inducible proteins include oncoproteins, transcription factors, an RNA-binding protein, and serine/threonine and tyrosine kinases (for review, see ref. 6).

The nuclear receptor superfamily contains a number of ligand-dependent transcriptional regulators, including the steroid, thyroid hormone, and retinoic acid and retinoid X receptors (RARs and RXRs). These receptors play important roles during development and in homeostasis (refs. 7–12 and references therein). The mouse F9 embryonal carcinoma cell line expresses RARs and RXRs and, when treated with retinoic acid, differentiates into endodermal cells resembling those of the mouse blastocyst (13, 14). Thus, F9 cells present an attractive system for the study of cell differentiation and retinoid signaling. To better understand the role of the different RARs and RXRs in these cells, we have started to mutate the corresponding genes by homologous recombination, either individually (2, 3) or in combination (unpublished results). Multiple gene targeting in a cell line requires either the use of two selection markers for each gene or the selection for gene conversion of the second allele by increasing the drug concentration in the cell medium (2, 3). Alternatively, it is possible to remove the marker after integration using *loxP* site-flanked markers and either transient expression of the *cre* gene or introduction of the purified Cre protein into cells (refs. 15 and 16 and references therein). An inducible Cre recombinase would allow easy removal of the selection marker after gene targeting, as well as triggering mutations at any desired time.

We report here that a ligand-inducible Cre recombinase is obtained by fusing the *cre* gene with the cDNA encoding the LBD of human ER (hER). We have established an F9 murine teratocarcinoma cell line which expresses constitutively the Cre-ER fusion protein and is inactivated for RXRα expression. We show here that the chimeric protein mediates estradiol-dependent *loxP* site-specific recombination on *loxP*-flanked genes integrated by homologous recombination. We also show that 4-hydroxytamoxifen (OHT), which has a mixed agonist/antagonist activity on ER, but not ICI 164,384 (ICI), which is a pure antiestrogen (ref. 17 and references therein), induces the recombinase activity of the chimera.

MATERIALS AND METHODS

Gene Targeting of F9 Cells. F9 cells were cultured under standard conditions (2). Cells (5×10^6) suspended in 500 μl of phosphate-buffered saline were electroporated with 5 μg of purified DNA by a Bio-Rad Gene Pulser set at 200 V and 960 μF. Cells were plated at a density of 6×10^5 per 100-mm culture plate, incubated for 24 hr, and then subjected to neomycin selection [Geneticin (G418 sulfate), 330 μg/ml] for 14 days. Individual colonies were isolated, propagated, and frozen, and aliquots were used for Southern blot analysis.

Cells plated at a density of 10^4 per 100-mm culture plate were treated with hormone [0.1 μM estradiol (E_2), 1 μM OHT, or 1 μM ICI] for 48 hr and then diluted to 100 cells per plate. After 10 days, individual colonies were propagated and analyzed. The sensitivity to G418 was determined for cells plated

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Abbreviations: ER, estrogen receptor; hER, human ER; LBD, ligand-binding domain; Cre-ER, fusion protein between the Cre recombinase and the LBD of the hER; E_2, estradiol; OHT, 4-hydroxytamoxifen; ICI, ICI 164,384; RAR, retinoic acid receptor; RXR, retinoid X receptor; TKneo, thymidine kinase/neomycin-resistance fusion gene; PGK, phosphoglycerate kinase.

at a density of 100 cells per well of a 24-well plate, grown in medium with or without G418 sulfate (330 μg/ml) for 7 days.

Genomic DNA extraction, Southern blotting, and probe preparation were as described (18).

Generation of Cre-ER-Expressing Cell Lines. The expression vector pCre-ER was constructed by inserting the Cre coding region [cloned by PCR from bacteriophage P1 (19)] into the T4 DNA polymerase-treated *Bam*HI site of plasmid pSG6-HE14. pSG6-HE14 was obtained by cloning the 1-kb *Eco*RI fragment of HE14 (20) into the *Eco*RI site of pSG6 [pSG5 (21) lacking the *Bam*HI and *Bgl* II sites]. A Kozak consensus sequence for translation initiation was created at the ATG codon of the fusion gene by site-directed mutagenesis, which was also used to link the two coding sequences and to introduce an *Xho* I site and three additional codons (Leu-Glu-Pro) at the junction.

Integration of pCre-ER in F9 cells was performed by coelectroporation (as above) of 20 μg pCre-ER and 1 μg pPGK-hyg (22) that had been digested with *Nde* I and *Pvu* II, respectively. Hygromycin selection (500 μg/ml) was begun 24 hr after plating and continued for 10 days, and individual colonies were propagated and analyzed.

COS-1 cell transfection and Western immunoblot analysis were performed as described (23).

RESULTS

To express a hormone-inducible Cre derivative, we constructed an expression vector, pCre-ER, encoding the Cre-ER fusion protein, which consists of Cre fused to the hER LBD (HE14; Fig. 1*A*). Western blot analysis of extracts of pCre-ER-transfected COS-1 cells revealed that the chimeric protein was synthesized (Fig. 1*B*, compare lane 3 with lanes 1 and 2). The estrogen-inducible recombinase activity of this chimera was tested in the genetically modified F9 cell line RXR$\alpha^{-(L)/-(LNL)}$, which carries a *loxP* site-flanked thymidine kinase/neomycin-resistance fusion gene (TKneo) inserted in exon 4 of one RXRα allele [$-$(LNL) and $-$(L) signify the targeted locus before and after Cre-mediated excision, respectively]. This cell line, which was generated as outlined in Fig. 2*A*, was then electroporated with the expression vector pCre-ER, together with a vector expressing a hygromycin-resistance gene. Southern blot analysis of hygromycin-resistant clones revealed that 80% of the clones had cointegrated Cre-ER (data not shown). Three of these clones (C1RXR$\alpha^{-(LNL)/-(L)}$, C2RXR$\alpha^{-(LNL)/-(L)}$, and C3RXR$\alpha^{-(LNL)/-(L)}$) were selected for further analysis. The structure of the targeted RXRα loci in these lines was identical to that of the parental line RXR$\alpha^{-(LNL)/-(L)}$, as determined by genomic mapping [Fig. 2 *A* and *B* (compare lanes 4 and 5) and data not shown]. Furthermore, analysis of the growth and differentiation pattern did not reveal any difference between these lines and the parental line (data not shown).

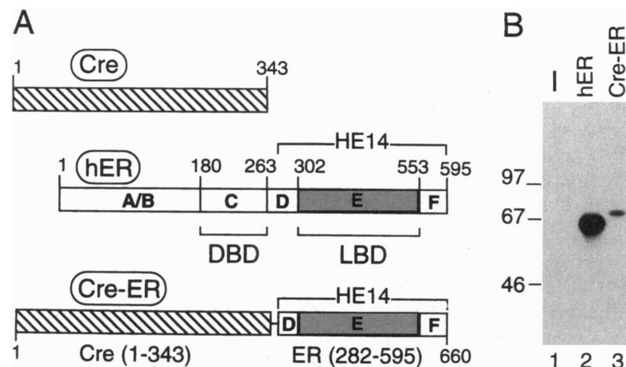

FIG. 1. Schematic representation and Western blot analysis of Cre recombinase, hER, and Cre-ER fusion protein. (*A*) Amino acid sequences of Cre and of the LBD of the hER are represented by hatched and gray boxes, respectively. Numbers refer to amino acid positions. The hER A/B, C, D, E, and F regions (24), HE14 (20), the DNA-binding domain (DBD), and LBD are indicated. (*B*) Western blot analysis of transfected COS-1 cells. Lanes 1–3 contained 10 μg of protein from extracts of cells transfected with 1 μg of either pSG6 expression vector, HE0 (25), or pCre-ER, respectively. The extracts were immunoprobed with the F3 monoclonal antibody, which is specific for hER region F (23). Positions of molecular size (kDa) standards are indicated at left.

The recombinase activity of Cre-ER was tested in the three lines in the presence or absence of 0.1 μM E_2 for 48 hr. The E_2 treatment did not result in any morphological change in the cells (data not shown). Individual clones were isolated and expanded, and the loss of the neomycin-resistance gene was determined by sensitivity to G418 and by Southern blotting. In the absence of E_2 treatment, none of the 18 cell lines analyzed was sensitive to G418, whereas 5, 10, and 9 of the 12 lines derived from C1RXR$\alpha^{-(LNL)/-(L)}$, C2RXR$\alpha^{-(LNL)/-(L)}$, and C3RXR$\alpha^{-(LNL)/-(L)}$, respectively, were sensitive to G418 after E_2 treatment (Table 1). Southern blot analysis confirmed that the neomycin-resistance gene was eliminated in these G418-sensitive CRXR$\alpha^{-(L)/-(L)}$ lines (Fig. 2 *A* and *B*, lane 6, and data not shown). Further Southern blot analysis of DNA from several CRXR$\alpha^{-(L)/-(L)}$ cell lines, digested with *Xba* I, *Spe* I, *Xho* I or *Bam*HI and hybridized with X1, X2, X3, X4, or *neo* probe (Fig. 2*A*), demonstrated that the restriction sites located at the junction between the genomic DNA and the *loxP* sites were present after E_2 treatment and that the marker gene sequences were absent [Fig. 2*B* (compare lane 6 with lanes 1–5) and data not shown]. Thus, removal of the marker gene had occurred by excision of the sequences located between the *loxP* sites, and we conclude that the recombinase activity of Cre-ER is *loxP* site-specific and E_2-inducible.

To investigate whether a gene could be inactivated with a targeting vector containing a *loxP* site-flanked marker in a cell line expressing Cre-ER, we electroporated C2RXR$\alpha^{-(L)/-(L)}$ cells with a RARα gene targeting construct containing a *loxP* site-flanked, TKneo marker gene (Fig. 3*A*). Neomycin-resistant clones were expanded, and Southern blot analysis using the a1 probe located immediately 3′ to the targeting construct (Fig. 3*A*) revealed that 5 of 96 clones had integrated the construct by homologous recombination (Fig. 3*B* and data not shown). One positive clone, C2#9RXR$\alpha^{-(L)/-(L)}$/RAR$\alpha^{+/-(LNL)}$, was expanded and treated with E_2, and subclones were analyzed for Cre-mediated excision. Southern blot analysis revealed that 2 of 6 such subclones treated with E_2 had lost the neomycin-resistance marker gene, presumably yielding RXR$\alpha^{-/-(L)}$/RAR$\alpha^{+/-(L)}$ cells (Fig. 3*C*). Further analysis of one of these lines was performed. DNA was digested with *Xba* I, *Bgl* II, or *Kpn* I and hybridized with the a1 probe (Fig. 3 *A* and *D*). The pattern obtained clearly indicated that the excision of the marker had occurred at the RARα targeted locus [Fig. 3*D* (compare lanes 3, 6, and 9 with lanes 1 and 2, 4 and 5, and 7 and 8, respectively) and data not shown], and confirmed the induction of Cre activity by E_2 in this cell line. We also verified that the *loxP* site-containing RXRα loci were not rearranged during excision of the marker genes at the RARα locus (Fig. 2*B*, compare lane 8 with lane 7).

To determine whether antiestrogens could induce the Cre recombinase activity, C1RXR$\alpha^{-(LNL)/-(L)}$ cells were treated with E_2, OHT, or ICI, and excision of the neomycin-resistance marker gene was monitored by Southern blot analysis (data not shown) for six treated subclones. Both E_2 and OHT treatment resulted in the excision of the marker gene in three out of six clones, whereas ICI treatment did not result in marker excision.

DISCUSSION

We demonstrate here that the activity of the Cre recombinase protein can be controlled by E_2 when it is fused to the LBD of

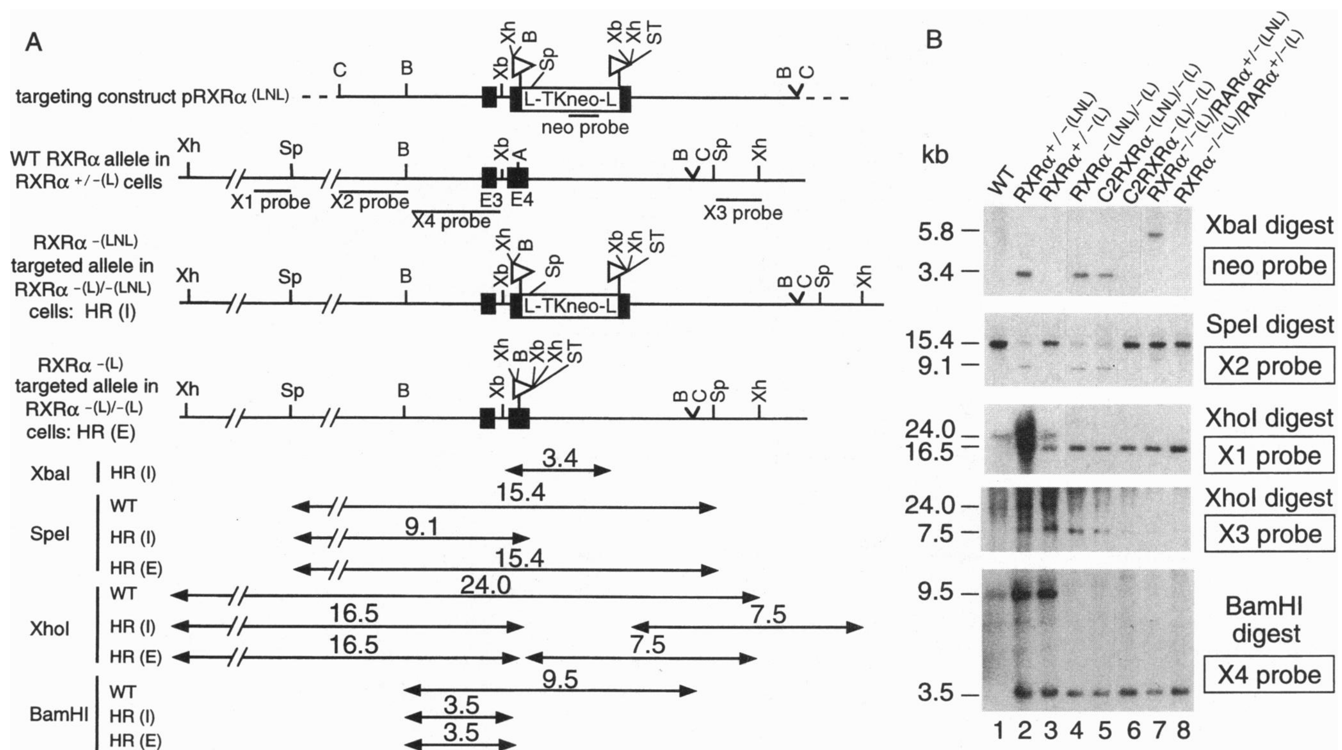

FIG. 2. Targeted disruption of the RXRα gene by homologous recombination using the Cre/*loxP* system. (*A*) Diagram showing the wild-type (WT) RXRα locus, the targeting construct, and the mutated locus after homologous recombination resulting in integration [HR (I)] and subsequent excision [HR (E)] of the *loxP*-flanked TKneo cassette. Black and open boxes indicate exons and the TKneo cassette, respectively. Open arrowheads signify *loxP* sites. Restriction sites (A, *Acc* I; B, *Bam*HI; C, *Cla* I; Sp, *Spe* I; Xb, *Xba* I; Xh, *Xho* I), stop codons (ST), and the location of the X1–X4 and neo probes are as indicated. The neo probe corresponds to a 0.8-kb *Pst* I fragment (from pKJ1; ref. 26). Probes X1, X2, X3, and X4 correspond to 0.6-kb *Sca* I–*Spe* I, 2.1-kb *Sal* I–*Bam*HI, 1.2-kb *Spe* I–*Xho* I, and 3.1-kb *Bam*HI–*Xba* I fragments, respectively. The RXRα disruption vector, pRXRα$^{(LNL)}$, as well as the probes will be described in detail elsewhere (J.C., D.M., and P.C., unpublished work). Briefly, pRXRα$^{(LNL)}$ contains an 11.8-kb genomic DNA fragment encompassing exon 4 of the RXRα gene into which a phosphoglycerate kinase (PGK) promoter-driven TKneo fusion gene, flanked by *loxP* sites, has been inserted. The 11.4-kb *Cla* I fragment was gel-purified and electroporated. The cell line RXRα$^{-(L)/(LNL)}$ was generated by initially targeting the first RXRα allele with the construct pRXRα$^{(LNL)}$ (to be described elsewhere). The selection markers located between the *loxP* sites of the resulting RXRα$^{+/-(LNL)}$ cell line were then excised by transiently expressing the Cre protein encoded by pPGK-Cre, in the presence of ganciclovir (thymidine kinase selection), yielding the RXRα$^{+/-(L)}$ cell line. After Southern blotting verified that the pPGK-Cre vector was no longer present in the RXRα$^{+/-(L)}$ cell line, the second RXRα allele was inactivated by using the same pRXRα$^{(LNL)}$ targeting vector as shown here. (*B*) Detection by Southern blot analysis of WT and mutant RXRα alleles obtained after integration of the targeting construct and excision of the *loxP*-flanked marker. Genomic DNA isolated from the indicated cells was digested with *Xba* I, *Spe* I, *Xho* I, or *Bam*HI and analyzed by Southern blotting with X1–X4 and neo probes as indicated. Positions and sizes (kb) of WT and targeted genomic fragments are indicated (and described by double-headed arrows in *A*).

the hER. Constitutive expression of this Cre-ER fusion protein in F9 cells mediates conditional recombination between *loxP* sites within a targeted gene (encoding RXRα). Treatment with E_2 for 48 hr induces recombination between the *loxP* sites flanking a *TKneo* marker gene inserted in the genome, at an efficiency varying between 30% and 85% (see Table 1 and Fig. 3*B*). The partial agonist OHT is as efficient as E_2, whereas the pure antiestrogen ICI is inactive. Furthermore, targeting of one allele of another gene (RARα) by homologous recombination with a construct containing *loxP* sites has been achieved

Table 1. E_2 treatment-induced excision of the *loxP*-TKneo-*loxP* cassette in cell lines constitutively expressing Cre-ER recombinase

Cell line	Neomycin-sensitive clones $-E_2$	Neomycin-sensitive clones $+E_2$
C1RXRα$^{-(LNL)/-(L)}$	0/6	5/12
C2RXRα$^{-(LNL)/-(L)}$	0/6	10/12
C3RXRα$^{-(LNL)/-(L)}$	0/6	9/12

The viability of untreated and E_2-treated C1RXRα$^{-(LNL)/-(L)}$, C2RXRα$^{-(LNL)/-(L)}$, and C3RXRα$^{-(LNL)/-(L)}$ cells cultured in medium containing G418 sulfate (330 μg/ml) was determined. The frequency of clones which lost neomycin resistance is indicated.

in these Cre-ER-expressing F9 cells. The continued application of this system will allow the sequential inactivation of multiple genes in this cell line.

In addition to the obvious potential that such cell lines present for the study of gene function, the efficient conditional control of Cre-ER activity makes a number of other applications possible. For example, the placement of *loxP* sites flanking a gene would allow its conditional inactivation. This would be particularly useful for the study of genes whose inactivation results in cell lethality. Conversely, the conditional activation of genes by excision of a *loxP*-flanked terminator located between a gene and its promoter should also be feasible.

A conditional Cre activity has also great potential for the study of gene function in mice. Cell type-specific gene inactivation has already been achieved with the Cre/*loxP* system by crossing transgenic mice, one expressing Cre in a tissue-specific manner and the other containing a *loxP*-flanked target gene (15). Combining the cell type-specific expression of the Cre recombinase with the conditional control of its activity would allow both spatial and temporal control of gene expression in either the mouse embryo or the adult animal. However, the ER LBD may not be the best ligand-inducible domain to use in mice, due to the presence of endogenous estrogens

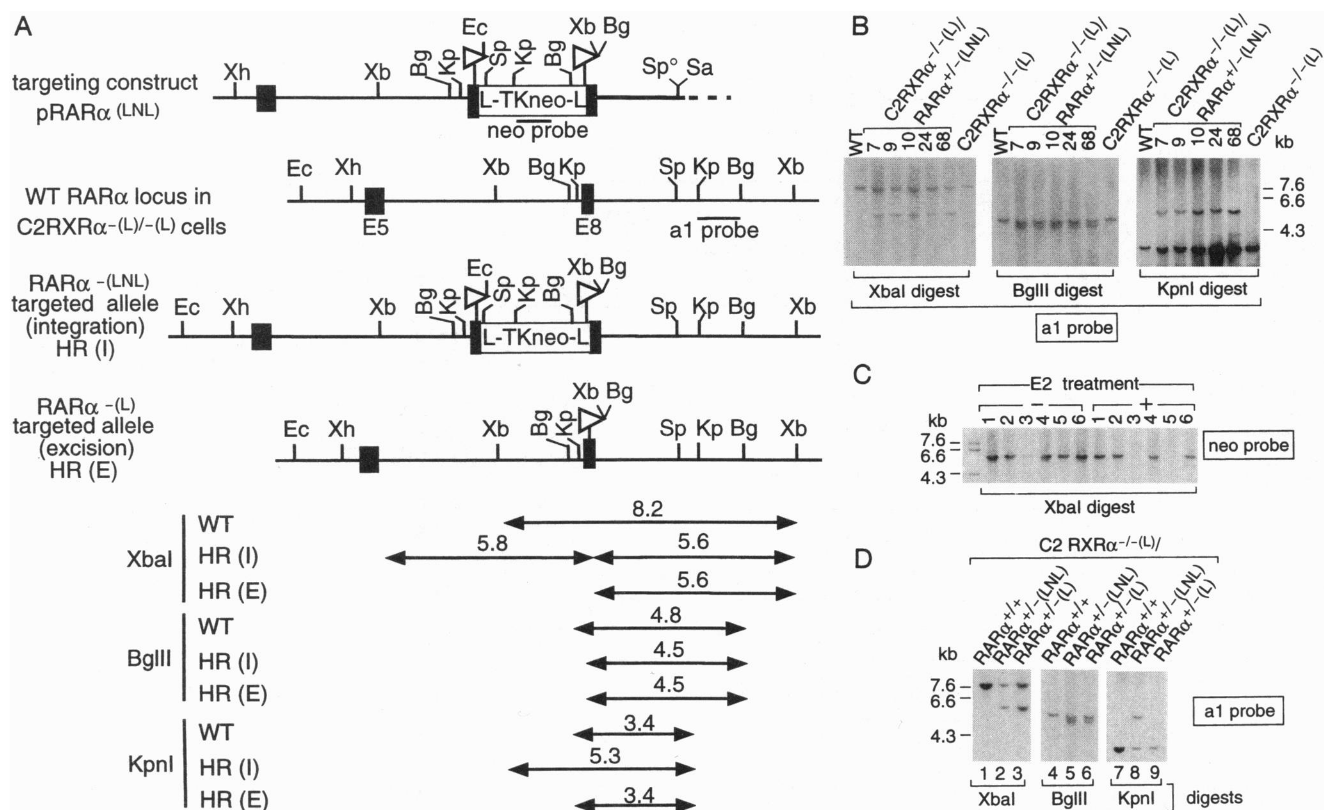

FIG. 3. Targeted disruption of one RARα allele by homologous recombination in a $RXR\alpha^{-(L)/-(L)}$ cell line expressing Cre-ER. (*A*) Diagram showing the wild-type (WT) RARα locus, the targeting construct, and the mutated locus after integration [HR (I)] and excision [HR (E)] of the *loxP*-flanked TKneo cassette. Black and open boxes indicate exons and the TKneo marker, respectively. Open arrowheads signify *loxP* sites. Restriction sites (Bg, *Bgl* II; Ec, *Eco*RI; Kp, *Kpn* I; Sa, *Sal* I; Sp, *Spe* I, Xb, *Xba* I; Xh, *Xho* I) and the a1 and neo probes are indicated; Sp° signifies that this restriction site has been destroyed in construction of the vector. The a1 probe is a 0.8-kb *Spe* I–*Kpn* I fragment isolated from λG2mRARα (27). The neo probe is described in the legend to Fig. 2. The RARα disruption vector $pRAR\alpha^{(LNL)}$ is derived from p826B1 (27). p826B1 was digested with *Not* I and blunt-ended with T4 DNA polymerase, thereby removing the *Not* I fragment containing the GTI-II enhancer-driven neomycin-resistance gene. This construct was then ligated to the 3.2-kb *Sma* I fragment containing the PGK promoter-driven TKneo fusion gene residing between two direct repeats of *loxP* sites (sense orientation), isolated from pHR56S. pHR56S was constructed by converting the *Bam*HI site of pHR56 into a *Sma* I site. pHR56 contains a *loxP* site-flanked PGK-TKneoA+ cassette obtained by replacement of the *Pst* I fragment (containing the neomycin-resistance marker) from pKJ-1 (26) with the 2.1-kb *Bgl* II–*Sma* I fragment from TNFUS69 (28). The restriction sites for *Not* I, *Sal* I, *Xho* I, *Kpn* I, and *Bam*HI, and for *Xba* I, *Bgl* II, *Sma* I, *Xho* I, *Sal* I, and *Not* I are flanking the *loxP* sites located at the 5′ and 3′ ends of the cassette, respectively. The 13-kb *Xho* I–*Sal* I fragment from $pRAR\alpha^{(LNL)}$, used for electroporation, was isolated from an agarose gel, electroeluted, and purified with Elutip columns (Schleicher & Schüll), according to the manufacturer's instructions. (*B–D*) Detection by Southern blot analysis of WT and mutant RARα alleles obtained after integration of the targeting construct (*B*) and excision of the *loxP*-flanked marker (*C* and *D*). Genomic DNA isolated from the indicated cells (*B* and *D*) and/or from $C2\#9RXR\alpha^{-(L)/-(L)}/RAR\alpha^{+/-(LNL)}$ subclones (*C*) was digested with *Xba* I (*B–D*) or *Bgl* II and *Kpn* I (*B* and *D*) and analyzed by Southern blotting with the a1 probe (*B* and *D*) or neo probe (*C*). The cell line designated $C2RXR\alpha^{-/-(L)}/RAR\alpha^{+/+}$ in lanes 1, 4, and 7 of *D* is the same as $C2RXR\alpha^{-/-(L)}$. Positions and sizes (kb) of DNA fragment markers are indicated.

during the life cycle. This problem may possibly be circumvented by the use of mutated LBDs that would respond to synthetic ligands (e.g., OHT) but not to endogenous ones. The LBDs of other nuclear receptors, such as the ecdysone receptor, might also provide a suitable alternative. We expect that such ligand-inducible Cre/*loxP* systems will provide a powerful tool for the study of gene function.

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1. Thomas, K. R. & Capecchi, M. R. (1987) *Cell* **51,** 503–512.
2. Boylan, J. F., Lohnes, D., Taneja, R., Chambon, P. & Gudas, L. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9601–9605.
3. Boylan, J. F., Lufkin, T., Achkar, C. C., Taneja, R., Chambon, P. & Gudas, L. J. (1995) *Mol. Cell. Biol.* **15,** 843–851.
4. Bronson, S. K. & Smithies, O. (1994) *J. Biol. Chem.* **269,** 27155–27158.
5. Sauer, B. & Henderson, N. (1990) *New Biol.* **2,** 441–449.
6. Picard, D. (1994) *Curr. Biol.* **5,** 511–515.
7. Evans, R. M. (1988) *Science* **240,** 889–895.
8. Beato, M. (1991) *FASEB J.* **5,** 2044–2051.
9. Laudet, V., Hanni, C., Coll, J., Catzeflis, F. & Stehelin, D. (1992) *EMBO J.* **11,** 1003–1013.
10. Blomhoff, R. B. (1994) *Vitamin A in Health and Disease* (Dekker, New York).
11. Chambon, P. (1994) *Semin. Cell Biol.* **5,** 115–125.

12. Sporn, M. B., Roberts, A. B. & Goodman, D. S. (1994) *The Retinoids: Biology, Chemistry and Medicine* (Raven, New York).
13. Strickland, S. (1981) *Cell* **24,** 277–278.
14. Gudas, L. J., Sporn, M. B. & Roberts, A. B. (1994) in *The Retinoids: Biology, Chemistry and Medicine*, eds. Sporn, M. B., Roberts, A. B. & Goodman, D. S. (Raven, New York), pp. 443–520.
15. Gu, H., Marth, J. D., Orban, P. C., Mossmann, H. & Rajewsky, K. (1994) *Science* **265,** 103–106.
16. Baubonis, W. & Sauer, B. (1993) *Nucleic Acids Res.* **21,** 2025–2029.
17. Metzger, D., Berry, M., Ali, S. & Chambon, P. (1995) *Mol. Endocrinol.* **9,** 575–591.
18. Lufkin, T., Dierich, A., Le Meur, M., Mark, M. & Chambon, P. (1991) *Cell* **66,** 1105–1119.
19. Sternberg, N., Sauer, B., Hoess, R. & Abremski, K. (1986) *J. Mol. Biol.* **187,** 197–212.
20. Kumar, V., Green, S., Staub, A. & Chambon, P. (1986) *EMBO J.* **5,** 2231–2236.
21. Green, S., Issemann, I. & Sheer, E. (1988) *Nucleic Acids Res.* **16,** 369.
22. te Riele, H., Maandag, E. R., Clarke, A., Hooper, M. & Berns, A. (1990) *Nature (London)* **348,** 649–651.
23. Ali, S., Lutz, Y., Bellocq, J. P., Chenard-Neu, M. P., Rouyer, N. & Metzger, D. (1993) *Hybridoma* **12,** 391–405.
24. Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornert, J. M. & Chambon, P. (1986) *EMBO J.* **5,** 891–897.
25. Tora, L., Mullick, A., Metzger, D., Ponglikitmongkol, M., Park, I. & Chambon, P. (1989) *EMBO J.* **8,** 1981–1986.
26. Adra, C. N., Boer, P. H. & McBurney, M. W. (1987) *Gene* **60,** 65–74.
27. Lufkin, T., Lohnes, D., Mark, M., Dierich, A., Gorry, P., Gaub, M. P., Le Meur, M. & Chambon, P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7225–7229.
28. Schwartz, F., Maeda, N., Smithies, O., Hickey, R., Edelmann, W., Skoultchi, A. & Kucherlapati, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10416–10420.