

- were then fed for 10 min on the bead mixture suspended in 18 ml of 0.45- μ m filtered seawater. The short period ensured that particles were not defecated. The acclimation and feeding containers were rinsed, 20-ml borosilicate vials. Temperatures were maintained by recirculating water baths, and vials were turned gently every 3 min to keep larvae suspended. Larvae were fixed and inspected at a magnification of $\times 400$ to count particles in the stomach and intestine. The measurement recorded was the average number of particles ingested by all feeding larvae within a vial. I inspected on average 9.0 (SD = 1.8) larvae per vial ($\times 6$ treatments $\times 16$ cohorts $\times 2$ rearing temperatures).
15. Concentrations of dextran needed to alter seawater viscosity from 1.02 to 1.30 cP and to 1.60 cP were 0.75 and 1.4%, respectively, as determined with a falling-ball viscometer (GV-2100, Gilmont Instruments, Barrington, IL).
 16. For populations in Puget Sound, larval development is normal in this temperature range (12), and individuals may be exposed to this range during development near the ocean surface [R. B. Emlet, *Mar. Ecol. Prog. Ser.* **31**, 245 (1986)]. In addition, *D. excentricus* experiences a wide temperature range over its distribution from Baja California to Alaska. All larvae used in experiments were at the four-arm to early six-arm stage of development (12).
 17. The six treatments were run in parallel for each cohort of larvae. The relevant predictions were (i) $A > B > C$, (ii) $A > B > D$, (iii) $E > B$, (iv) $A > F$, and (v) cold-reared $>$ warm-reared. I performed a two-way nested analysis of variance (ANOVA) on particles ingested to test for effects of treatment and rearing temperature, with cohorts nested within temperature. The treatment predictions reduce to five a priori pair-wise comparisons ($A > B$ and $F > C$ and D ; $E > B$), which I tested using planned contrasts and a Bonferroni adjustment to α for five comparisons ($\alpha = 0.01$).
 18. R. R. Strathmann, *J. Exp. Mar. Biol. Ecol.* **62**, 225 (1982). High viscosity increases the distance at which objects disturb flow fields around each other and could potentially alter particle detection or capture [S. M. Gallagher, *J. Plankton Res.* **15**, 1277 (1993)].
 19. J. S. Pearse, I. Bosch, V. B. Pearse, L. V. Basch, *Antarct. J. U.S.* **26**, 170 (1991).
 20. The standard ratio of rates over 10°C change (Q_{10}) for ingestion was 3.05. A "viscosity-free" Q_{10} (with the effects of viscosity removed) was 1.81. The ratio of rates per 1-cP viscosity change (V_1) was 5.9 over the 17°C (0.58-cP) interval examined.
 21. A. Clarke, *Am. Zool.* **31**, 81 (1991).
 22. This pattern could occur if small particles are caught by a method other than ciliary reversal (10), such as diffusional deposition [J. Shimeta, *Limnol. Oceanogr.* **38**, 456 (1993)]. Other changes in the fluid (for example, electrostatic charge) could have differentially affected particles by size.
 23. B. W. Frost, *ibid.* **22**, 472 (1977); T. Fenchel, *Microb. Ecol.* **6**, 1 (1980); M. A. R. Koehl, in (6), p. 135; B. C. Monger and M. R. Landry, *Mar. Ecol. Prog. Ser.* **74**, 239 (1991).
 24. L. G. Williams, *Mar. Biol.* **66**, 171 (1982). Direct interception theory suggests a relative increase in contact of large particles at low viscosity due to streamline compression around the collecting device [J. Shimeta, thesis, University of Washington, Seattle (1993)] and to interactions of hydrodynamic and cell surface forces [B. C. Monger and M. R. Landry, *Mar. Ecol. Prog. Ser.* **65**, 123 (1990)].
 25. G. A. Jackson, *Limnol. Oceanogr.* **32**, 1253 (1987).
 26. R. B. Emlet, L. R. McEdward, R. R. Strathmann, in *Echinoderm Studies*, M. Jangoux and J. M. Lawrence, Eds. (Balkema, Rotterdam, Netherlands, 1987). Larvae increase eightfold in protein content and likely experience high mortality during 1 to 2 months spent in the plankton [L. R. McEdward, *J. Exp. Mar. Biol. Ecol.* **82**, 259 (1984); R. B. Emlet, *Mar. Ecol. Prog. Ser.* **31**, 245 (1986)].
 27. G. Paulay, L. Boring, R. R. Strathmann, *J. Exp. Mar. Biol. Ecol.* **93**, 1 (1985); R. R. Olson and M. H. Olson, *Annu. Rev. Ecol. Syst.* **20**, 225 (1989).
 28. V. B. Pearse and J. S. Pearse, *Antarct. J. U.S.* **27**, 132 (1992). In addition, clearance rates for temperate larvae declined to polar levels when viscosity was increased; however, the polymers used and the lack of polymer and temperature controls make these results difficult to interpret (9). Feeding rate changes per centipoise were 10 to 59 times greater than those measured here and were well beyond what would result from the effects of temperature and viscosity combined, suggesting an artifact of the polymers.
 29. J. S. Pearse, in *Reproduction, Larval Biology, and Recruitment in the Deep-Sea Benthos*, K. J. Eckelbarger and C. M. Young, Eds. (Columbia Univ. Press, New York, in press). Many factors likely contribute to this pattern, including thermal effects on metabolic and development rates and the abundance, seasonality, and quality of larval food.
 30. Responses could involve acclimatory or genetic changes in feeding structures, body form, or behavior [I. C. Boidron-Metairon, *J. Exp. Mar. Biol. Ecol.* **119**, 31 (1988); R. B. Emlet, *Am. Zool.* **31**, 707 (1991); M. A. Sleight, *Comp. Biochem. Physiol.* **94A**, 359 (1989); S. Vogel, *Life in Moving Fluids* (Princeton Univ. Press, Princeton, NJ, 1981)].
 31. Water movement is ultimately limited by the mechanical properties of cilia and by hydrostatic pressures [(9); T. J. Sleight and J. R. Blake, in *Scale Effects in Animal Locomotion*, T. J. Pedley, Ed. (Academic Press, London, 1977), pp. 243–256; T. Fenchel, *Limnol. Oceanogr.* **25**, 733 (1980)]. Recent work has shown a correspondence between ambient viscosity and filtering rate in mussels, polychaetes, ascidians, and sponges, although temperature and viscosity were not controlled independently [C. B. Jørgensen, P. S. Larsen, H. U. Riisgård, *Mar. Ecol. Prog. Ser.* **64**, 89 (1990); H. U. Riisgård and N. M. Ivarsson, *ibid.* **62**, 249 (1990); J. K. Petersen and H. U. Riisgård, *ibid.* **88**, 9 (1992); H. U. Riisgård, S. Thomassen, H. Jakobsen, J. M. Weeks, P. S. Larsen, *ibid.* **96**, 177 (1993)].
 32. Alternatively, partial feeding compensation may be adequate to match reduced energetic demands of lower temperature. For larvae feeding at their respective rearing temperatures, mean ingestion rate at 22°C was greater than at 12°C by a factor of 2.1 (Fig. 2, comparison 1), whereas potential metabolic rate increased by a factor of 3.1 [L. R. McEdward, *J. Exp. Mar. Biol. Ecol.* **93**, 169 (1985)]. Comparisons of polar and temperate larvae show a similar correspondence between feeding and metabolic rates [O. Hoegh-Guldberg, J. R. Welborn, D. T. Manahan, *Antarct. J. U.S.* **26**, 163 (1991)].
 33. S. H. Wright and D. T. Manahan, *Annu. Rev. Physiol.* **51**, 585 (1989).
 34. R. D. Podolsky, unpublished data.
 35. A. G. Murray and G. A. Jackson, *Mar. Ecol. Prog. Ser.* **89**, 103 (1992).
 36. Future work must incorporate the role of turbulence in assessing viscosity effects (21) [P. S. Hill, A. R. M. Nowell, P. A. Jumars, *J. Mar. Res.* **50**, 643 (1992)].
 37. I thank R. Strathmann, J. Pearse, the Rohwer manuscript group, and two anonymous reviewers for helpful comments on the manuscript and R. Emlet for encouraging my interest in this work. Supported by an NSF graduate fellowship, a University of Washington Graduate School Fund grant, and NSF grant OCE-9301665 to R. Strathmann.

7 January 1994; accepted 13 May 1994

Deletion of a DNA Polymerase β Gene Segment in T Cells Using Cell Type-Specific Gene Targeting

Hua Gu,* Jamey D. Marth, Paul C. Orban, Horst Mossmann, Klaus Rajewsky

Deletion of the promoter and the first exon of the DNA polymerase β gene (*pol β*) in the mouse germ line results in a lethal phenotype. With the use of the bacteriophage-derived, site-specific recombinase Cre in a transgenic approach, the same mutation can be selectively introduced into a particular cellular compartment—in this case, T cells. The impact of the mutation on those cells can then be analyzed because the mutant animals are viable.

Gene targeting in embryonic stem (ES) cells provides a powerful tool for generating mice carrying predesigned mutations in the germ line (1). Current approaches to gene inactivation usually involve the introduction of a null mutation directly into ES cells from which homozygous mutant mice can be generated. Because the null mutation is carried in the germ line of the mutant animals, it will exert its effects from the

onset of animal development. Although this approach to gene inactivation is valuable, for many applications it is important that the inactivation of a particular gene occurs in a conditional manner—for instance, in a predefined cell lineage or at a certain stage of development. Such conditional gene targeting would not only overcome problems posed by the fact that null mutations in the germ line are often lethal, but would also allow a more precise analysis of the impact of a mutation on individual cell lineages.

Somatic gene rearrangement and hypermutation at lymphocyte antigen receptor gene loci are unique events that require DNA repair (2, 3). The *pol β* gene has been shown to be one of various enzymes involved in the DNA repair machinery (4).

H. Gu and K. Rajewsky, Institute for Genetics, University of Cologne, 50931 Cologne, Germany.
J. D. Marth, Biomedical Research Center and Department of Medical Genetics, University of British Columbia, Vancouver, Canada V6T 1Z3.
P. C. Orban, Biomedical Research Center, University of British Columbia, Vancouver, Canada V6T 1Z3.
H. Mossmann, Max-Planck Institute for Immunobiology, 79011 Freiburg, Germany.

*To whom correspondence should be addressed.

However, despite its ubiquitous expression (5) the importance of this enzyme for the generation of cell lineages, the survival of cells, and animal development in general remains elusive. To explore the function of the *polβ* gene in mice, particularly in the development of lymphocytes, one attractive approach is to generate *polβ*-deficient mice with the use of gene targeting. Because of the potential problem of embryonic lethality caused by a null mutation of the *polβ* gene, we developed a general method for conditional gene inactivation with the use of the *polβ* gene as a model. This method includes the concomitant production of a conventional (nonconditional) deletion mutant.

Our approach is based on the Cre-*loxP* recombination system of bacteriophage P1 (6). We and others have previously shown that this system is capable of mediating *loxP* site-specific recombination in both ES cells (7) and transgenic mice (8, 9). The strategy for conditional gene targeting is shown schematically in Fig. 1A. Two mouse strains are required: One is a conventional transgenic strain in which a *cre* transgene is

expressed in a cell type-specific or developmentally stage-specific manner. The second strain carries the target gene flanked by two *loxP* sites. In offspring derived from an intercross between these strains carrying the *cre* transgene and a *loxP*-flanked ("floxed") target gene, Cre-*loxP* site-dependent recombination will occur in cells where the *cre* gene is expressed, thereby deleting the target gene. In contrast, the target gene should remain functional in cells of all the other tissues, where the *cre* transgene is not expressed.

Depicted in Fig. 1B is a two-step strategy for generating in parallel a floxed gene or gene segment and a deletion of the same piece of DNA in ES cells in vitro. In the first step, three *loxP* sites, in addition to the selection marker genes for neomycin resistance (*neo^r*) and herpes simplex virus-thymidine kinase (*HSV-tk*), are introduced into the flanking regions of the target gene

through homologous recombination. In the second step, the Cre enzyme is expressed in the genetically modified ES cells. If the expression of the Cre enzyme is transient, we expect that in some ES cells the recombination event will occur only once between any two of the three *loxP* sites, and different types of deletion should be generated. Type I deletion results in the deletion of the target gene from the genome of the ES cells, and animals derived from the corresponding ES cells will carry the deletion in the germ line. In contrast, type II deletion results in a floxed gene or gene segment at the targeted locus. The third possible type of deletion, which deletes the target gene but leaves the *neo^r* and *HSV-tk* genes in the genome, should not be observed in the mutant progeny, because ES cells carrying such a deletion should die after ganciclovir treatment during selection of type I and II deletion mutants (1).

To generate *polβ* mutant mice, we trans-

Table 1. Deletion of the *polβ* gene in ES cells. In clones not carrying a type I or type II deletion, only wild-type *polβ* loci could be detected.

Experiment	Clones analyzed (n)	Clones (n) with deletion of	
		Type I	Type II
1	24	2	4
2	24	1	3

Table 2. Genotypic analysis of the offspring of *polβ^A/+* mice.

Age	Mice (n) with genotype		
	<i>polβ^A/polβ^A</i>	<i>polβ^A/+</i>	<i>+/+</i>
10.5 days (embryos)	5	19	8
4 weeks (mice)	0	22	7

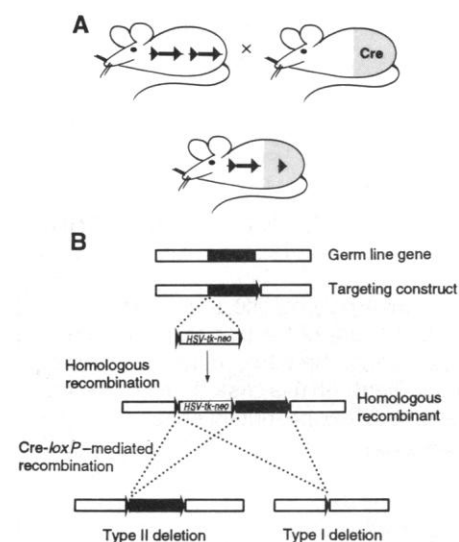
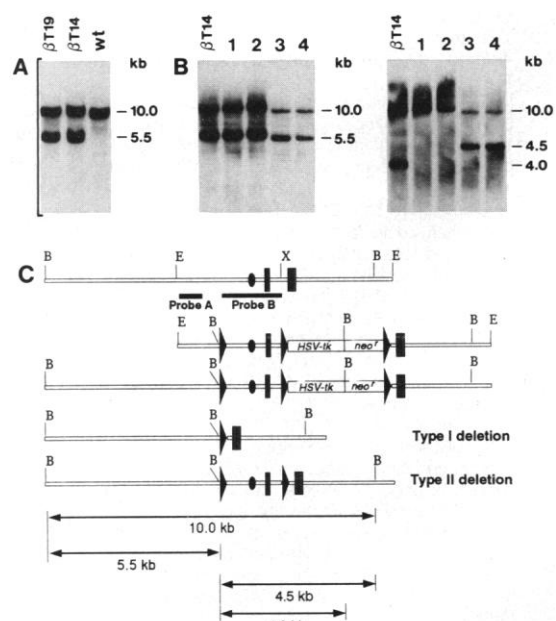


Fig. 1. (A) Scheme for cell type-specific gene targeting. (Upper left) A mouse strain in which a gene in the germ line (black bar) is flanked by two *loxP* sequences (triangles). (Upper right) A *cre* transgenic mouse strain. The gray area represents the tissue in which the Cre enzyme is expressed. (Bottom) *F₁* mouse derived from the two mouse strains above. The target gene is deleted in the tissue in which the Cre enzyme is expressed. (B) Strategy for generating a gene flanked by *loxP* sites in ES cells. Shown are a genetic locus, a corresponding targeting construct, the resulting homologous recombinant, and the two types of Cre-mediated deletions. The gene segment to be excised is depicted as a black bar. The *loxP* sequences are represented by triangles. The position of the selection marker cassette containing the *neo^r* and *HSV-tk* genes is indicated.

Fig. 2. Southern blot analysis of homologous recombination and Cre-*loxP*-mediated recombination at the mouse *polβ* locus. The targeting and deletion experiments were performed as described (10) (Fig. 1). DNA was digested with Bam HI. (A) Targeting of *loxP* sites into the *polβ* locus of ES cells. Shown are DNA samples from two candidate mutant ES cell clones (βT14 and βT19). The DNA from wild-type (wt) ES cells is shown as a control. The 5.5-kb band represents the targeted *polβ* allele. (B) Cre-*loxP*-mediated deletion of the promoter and first exon of the *polβ* gene in ES cells. Genomic DNA was obtained from the parental mutant ES cell (βT14) and four sub-clones (1 to 4) carrying deletions at the *polβ* locus. Clones 1 and 2 carry a type I deletion, as shown by a 5.5-kb band when they were hybridized with probe A (left panel) and the absence of a 4.5-kb band when they were hybridized with probe B (right panel) (C). Clones 3 and 4 have a type II deletion, as shown by a 4.5-kb band when they were probed with probe B (C). (C) Restriction maps for (from top to bottom) the 5' portion of the mouse *polβ* locus, the targeting construct, the homologous recombinant, and the type I and type II deletion mutants. The dark rectangles represent the first and second exons of the *polβ* gene; the ovals represent the *polβ* promoter; the *loxP* sites are represented by triangles; and the black bars represent the probes used for hybridization. Restriction sites of Bam HI (B), Eco RI (E), and Xho I (X) are indicated. Numbers on the right side of the blots indicate the sizes of the bands.



fecting the linearized targeting vector pMGB9 (Fig. 2C) into ES cells, and homologous recombinants were identified by Southern (DNA) blot analysis (10). Out of 288 G418-resistant clones analyzed, 16 were homologous recombinants, representing a frequency of 1 in 18. All these recombinants also carried a co-integrated loxP site approximately 1.5 kb upstream from the *polβ* gene promoter (Fig. 2A). To generate type I and type II deletions, we transfected two mutant ES cell clones transiently with Cre-encoding plasmid DNA. Subclones carrying desired deletions at the *polβ* locus were identified by Southern blot hybridization (Fig. 2B). In two independent experiments, both type I and type II deletions were consistently obtained (Table 1). For convenience, we refer to the type I deletion at the *polβ* locus as *polβ^Δ* and the type II deletion as *polβ^{lox}*.

Mice carrying the *polβ^Δ* and *polβ^{lox}* mutations in the germ line were generated by the standard protocol (11). The impact of the *polβ^Δ* mutation was examined in offspring derived from an intercross between *polβ^Δ* heterozygous mice. No lethality was

observed among the offspring from the time of birth to the age of 4 months. Genotypic examination of 4-week-old offspring revealed the absence of homozygous mutant animals (Table 2). However, at day 10.5 of fetal life, embryos homozygous for the *polβ^Δ* mutation were present at the frequency predicted by Mendelian laws (Table 2). On the basis of these results, we conclude that the homozygous mutant animals die in the course of fetal development.

As expected, animals homozygous for the *polβ^{lox}* mutation are viable. The overall development of these mutant mice also appears normal. These results indicate that the *polβ^{lox}* mutation does not severely hamper *polβ* expression in vivo.

Cell type-specific deletion of the *polβ* gene was investigated in *polβ^{lox}/+* mice carrying a *cre^{lck}* transgene. The *cre* transgene in these mice is driven by the *lck* proximal promoter and is, therefore, selectively expressed in T lineage cells (9). The extent of *polβ* gene deletion was assessed in various tissues of the animals by Southern hybridization (12). As expected, deletion occurred selectively in T cells. Quantitative

analysis indicated that 63 to 84% of splenic T cells carried the deletion (Table 3). In contrast, no detectable deletion of the *polβ* gene was observed in either kidney, liver, or B lymphocytes (Fig. 3A). These results demonstrate that cell type-specific gene inactivation can be achieved through our approach.

To obtain T cells homozygous for the *polβ^Δ* mutation, we mated mice carrying the *polβ^{lox}* mutation and the *cre^{lck}* transgene to heterozygous *polβ^Δ* mice. We chose for further analysis offspring of genotype *polβ^{lox}/polβ^Δ* that carried the *cre^{lck}* transgene, because in such animals every single cell has only a single functional *polβ* (namely, *polβ^{lox}*) gene, the deletion of which will result in homozygosity for the *polβ^Δ* mutation.

The overall development of *polβ^Δ/polβ^{lox}; cre^{lck}* transgenic mice appeared normal. Of these mice, both males and females were able to generate offspring when mated to normal mice, which suggests that germ cells developed normally in these animals. Flow cytometric analysis of T lineage cells in the thymus revealed no difference between the mutant mice and the wild-type controls in terms of total number of thymocytes and the distribution of CD4 and CD8 expression (13) on the surface of these cells (Fig. 4). In the blood of the mutants, essentially all T cells [as identified by the Thy-1 surface marker (13)] express the $\alpha\beta$ T cell receptor (TCR), and the number of splenic Thy-1⁺ cells is also normal compared to that in wild-type mice (14).

To estimate the fraction of T cells homozygous for the *polβ^Δ* mutation in these mice, we performed Southern blot analysis using DNA from purified T and B cells (Fig. 3B) (12). We found that approximately 40% of CD4⁺CD8⁺ thymocytes were homozygous for the *polβ^Δ* mutation (Table 3). The percentage of such cells was not increased in the peripheral T cells, which suggests that no further deletion of the *polβ* gene occurred in T cells after the CD4⁺CD8⁺ stage of T cell development in the thymus. This is consistent with the

Table 3. Efficiency of *polβ* gene deletion in T lineage cells from *polβ^{lox}/+*; *cre^{lck}* (control) and *polβ^{lox}/polβ^Δ*; *cre^{lck}* (experimental) mice. The total mutant alleles present (*polβ^{lox}* + *polβ^Δ*) are defined as 100%. The percentage of cells of genotype *polβ^Δ/polβ^Δ* was calculated with the assumption that B cells carry equal proportions of *polβ^Δ* and *polβ^{lox}* alleles, because no *polβ* deletion was detectable in B cells from *polβ^{lox}/+*; *cre^{lck}* transgenic mice (Fig. 3A).

Cells	<i>polβ^{lox}</i> allele (%)	<i>polβ^Δ</i> allele (%)	Cells of genotype <i>polβ^Δ/polβ^Δ</i> (%)
<i>Control mice</i>			
CD4 ⁺ CD8 ⁺ thymocytes	36	64	—
Splenic T*	37 or 16†	63 or 84†	—
<i>Experimental mice</i>			
CD4 ⁺ CD8 ⁺ thymocytes	31	69	38
Splenic T	31	69	38
Splenic B	51	49	0

*Defined as Thy-1⁺ cells (14). †These different percentages are values obtained in an independent experiment with mice from a different litter.

Fig. 3. Southern blot analysis of cell type-specific deletion of the *polβ* gene. DNA was obtained from various tissues of mutant mice. T lineage and B cells were purified with a FACS sorter (12). Symbols are as in Fig. 2. (A) Cell type specificity of *polβ* gene deletion. Shown are Southern blot analyses of various tissues from *polβ^{lox}/+*; *cre^{lck}* transgenic mice. Genomic DNA was obtained from liver (L), kidney (K), splenic B lymphocytes (B), and splenic Thy-1⁺ (T) cells and digested with Bam HI. The probe used for hybridization is indicated in (C) as a black bar. (B) Efficiency of *polβ* gene deletion in T lineage cells. Samples were obtained from mice of the following genotypes: *polβ^{lox}/+*; *cre^{lck}* (lanes 1 and 2), *polβ^{lox}/polβ^Δ*; *cre^{lck}* (lane 3, 4, and 5), and *polβ^{lox}/polβ^{lox}*; *cre^{lck}* (lane 6). Lanes 1 and 3 represent DNA from thymic CD4⁺CD8⁺ cells; lanes 2, 4, and 6 from splenic Thy-1⁺ cells; and lane 5 from splenic B cells. (C) Restriction maps of the 5' portion of the *polβ* gene. Symbols are as in Fig. 2.

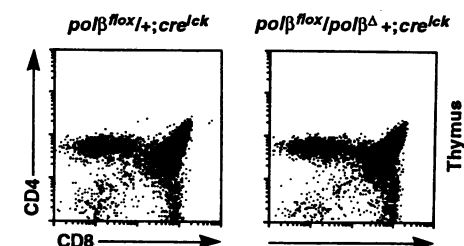
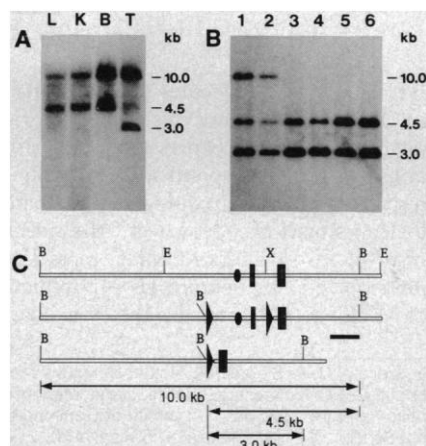


Fig. 4. Flow cytometric analysis of the thymocytes from mutant mice. The cells were stained with CD4 and CD8 antibodies and analyzed with a FACStar (12). The genotypes of the mice are indicated on top of each profile.

observation that the *cre^{lck}* transgene is not expressed in mature T cells (9).

There may be two main reasons for the incompleteness of *polβ* gene deletion in our experimental system. First, the *lck* proximal promoter is active only at early stages of T cell development (15). Earlier data also indicate that the *cre^{lck}* transgene is expressed only transiently in the thymus (9). It is therefore conceivable that in the transgenic T cells the *polβ^{lox}* alleles have only a brief period of time to accomplish Cre-*loxP*-mediated recombination. Second, the *cre* gene that we have used corresponds to the wild-type *cre* gene of P1 phage (6). It is known that the expression of this gene in eukaryotic cells is suboptimal, but it can be improved by appropriate genetic manipulation (7, 16). Thus, there are straightforward ways in which our experimental system can be improved to obtain a more efficient deletion of the target gene.

Our data provide no direct evidence at this stage about a possible involvement of *polβ* in the control of TCR gene rearrangements. However, we might interpret the lesser extent of *polβ* deletion in *polβ^Δ/polβ^{lox}*; *cre^{lck}* mice as compared to that in *polβ^{lox}/+*; *cre^{lck}* transgenic mice (Table 3) to mean that in the former case Cre-*loxP*-mediated *polβ* inactivation results in cell death if it happens to occur before the completion of TCR gene rearrangement.

In principle, Cre-*loxP*-mediated gene targeting should allow the inactivation of any gene in any tissue at any stage of development. It can also be adapted to conditional reconstitution of gene function. Furthermore, through lineage-specific inactivation of genes critical for cell survival, this approach can potentially be used for the ablation of cell lineages in vivo.

REFERENCES AND NOTES

1. K. R. Thomas and M. R. Capecchi, *Cell* 51, 503 (1987).
2. F. W. Alt, T. K. Blackwell, G. D. Yancopoulos, *Science* 238, 1079 (1987).
3. S. Brenner and C. Milstein, *Nature* 211, 242 (1966); C. Kocks and K. Rajewsky, *Annu. Rev. Immunol.* 7, 537 (1989).
4. M. Fry, in *Enzymes of Nucleic Acid Synthesis and Modification*, S. T. Jacob, Ed. (CRC Press, Boca Raton, FL, 1983), pp. 39–92.
5. F. Hirose et al., *Exp. Cell Res.* 181, 169 (1989).
6. 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).
7. H. Gu, Y.-R. Zou, K. Rajewsky, *Cell* 73, 1155 (1993).
8. M. Lakso et al., *Proc. Natl. Acad. Sci. U.S.A.* 89, 6232 (1992).
9. P. C. Orban, D. Chui, J. D. Marth, *ibid.*, p. 6861.
10. A 7.1-kb mouse genomic DNA (Eco RI–Eco RI) fragment containing the promoter and the first and second exon of the DNA *polβ* gene (17) was used to produce the targeting construct pMGβ9. A gene cassette containing the *neo^r* and *HSV-tk* genes flanked by two *loxP* sites was inserted into the Xho I site between the first and second exon. A third *loxP* site was introduced into a Sac I site approximately 2 kb upstream from the first exon. The final targeting construct contains 1 kb of flanking genomic se-

quences further upstream from the third *loxP* site and a 3-kb fragment including the second exon of the *polβ* gene downstream from the Xho I site (Fig. 2C). To generate homologous recombinants, we transfected E14-1 ES cells [R. Kühn, K. Rajewsky, W. Müller, *Science* 254, 707 (1991)] with 25 μg of DNA (of the linearized targeting construct) by electroporation. The transfected ES cells were grown on a single layer of mitomycin C-treated embryonic fibroblasts. After 1 week of selection in G418-containing medium, homologous recombinants were identified by Southern blot hybridization based on the strategy depicted in Fig. 2C. A targeted clone should yield a 5.5-kb band in addition to an equally intense 10-kb wild-type band upon hybridization to probe A (Fig. 2). To generate type I and type II deletions, 1 to 3 μg of supercoiled Cre-encoding plasmid, pIC-Cre (6), was introduced into the targeted ES cells by electroporation. After selection in ganciclovir-containing medium (1 × 10⁻⁶ M) for 5 days, surviving clones were picked and expanded. Genomic DNA was then prepared from the expanded cells for Southern blot analysis. Probe A is a 1-kb genomic DNA fragment (Hind III–Sac I) of the *polβ* gene, and probe B is a 900-bp fragment (Bam HI–Hind III) (17).

11. A. Bradley, in *Teratocarcinomas and Embryonic Stem Cell: A Practical Approach*, E. J. Robertson Ed. (IRL Press, Oxford, 1987), pp. 113–157.
12. On the basis of the expression of specific cell surface markers, we purified T and B cells by fluorescence-activated cell sorting using a FACStar (Becton Dickinson). T cells were sorted as Thy-1⁺ cells from the spleen. The CD4⁺CD8⁺ cells were from the thymus. B cells were sorted from the spleen as surface CD45R/B220⁺ cells.

Usually, 5 × 10⁶ to 1 × 10⁷ sorted cells were used for DNA preparation. The Southern hybridization was performed according to the standard protocol [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989)] with DNA from 2 × 10⁶ sorted cells. The probe was a 900-bp Bam HI–Hind III fragment of the *polβ* gene (17). Densitometric analysis was performed with a Bio-Imaging analyzer (Fuji) or a densitometric scanner (Pharmacia).

13. For a detailed description of thymic T cell development, see J. Sprent [in *Fundamental Immunology*, W. E. Paul Ed. (Raven Press, New York, 1993), pp. 75–109].
14. H. Gu and K. Rajewsky, unpublished data.
15. O. Sartor et al., *Mol. Cell. Biol.* 9, 2983 (1989); A. M. Garvin et al., *Int. Immunol.* 2, 1973 (1990); K. E. Chaffin et al., *EMBO J.* 9, 3821 (1990).
16. B. Sauer and N. Henderson, *New Biol.* 2, 441 (1990).
17. M. Yamaguchi et al., *Mol. Cell. Biol.* 7, 2012 (1987).
18. We thank A. Matsukage for providing the plasmid containing the genomic *polβ* gene and R. Kühn for the E14-1 ES cell line. We are grateful to R. Torres, F. Huetz, and Y.-R. Zou for critical reading of the manuscript; to U. Ringeisen for graphical work; to W. Müller for help at many levels; and to all our colleagues for helpful discussion. Supported by the Deutsche Forschungsgemeinschaft through SFB 243, the Fazit Foundation, the Bundesministerium für Forschung und Technologie, and the Human Frontier Science Program.

7 April 1994; accepted 13 May 1994

Regulation of MHC Class II Expression by Interferon-γ Mediated by the Transactivator Gene CIITA

Viktor Steimle, Claire-Anne Siegrist, Annick Mottet, Barbara Lisowska-Grospierre, Bernard Mach*

Major histocompatibility complex (MHC) class II genes are expressed constitutively in only a few cell types, but they can be induced in the majority of them, in particular by interferon-γ (IFN-γ). The MHC class II transactivator gene CIITA is defective in a form of primary MHC class II deficiency. Here it is shown that CIITA expression is controlled and induced by IFN-γ. A functional CIITA gene is necessary for class II induction, and transfection of CIITA is sufficient to activate expression of MHC class II genes in class II-negative cells in the absence of IFN-γ. CIITA is therefore a general regulator of both inducible and constitutive MHC class II expression.

MHC class II molecules present antigens to T helper lymphocytes, and the tight regulation of their expression is of critical importance for the control of the immune response both in physiological and pathological situations. However, the mechanism of IFN-γ-induced MHC class II expression remains obscure (1–4). Induction of MHC class II genes by IFN-γ is charac-

terized by an unusually long lag period and total dependence on de novo protein synthesis (5–7). These genes thus exhibit a “secondary” response to IFN-γ (8, 9), which has led to the postulation of an intermediary activator, itself induced by IFN-γ (5–7). Despite considerable effort it has not been possible to define “interferon response elements” convincingly in MHC class II genes (2). The same DNA sequence elements seem to be required for IFN-γ-induced transcription and constitutive expression in B cells (2, 3). The factors implicated thus far in the regulation of MHC class II genes are expressed constitutively, and their binding to MHC class II

V. Steimle, C.-A. Siegrist, A. Mottet, B. Mach, Jeantet Laboratory of Molecular Genetics, Department of Genetics and Microbiology, University of Geneva Medical School, CH-1211 Geneva 4, Switzerland.
B. Lisowska-Grospierre, INSERM U132, Hôpital des Enfants Malades, 15015 Paris, France.

*To whom correspondence should be addressed.