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 (polβ) 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 predefined 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 polβ gene has been shown to be one of various enzymes involved in the DNA repair machinery (4).

References


2. T. J. Sleigh and J. R. Blake, in (6), pp. 243–256; T. Fenchel, Limnol. Oceanogr. 25, 733 (1980). Recent work has shown a correspondence between temperature and deposition rates, which suggests that the effects on larval food.


5. The standard ratio of rates over 10°C change (Q10) for ingestion was 3.05. A “viscosity-free” Q10 (with the effects of viscosity removed) was 1.81, the ratio of rates per 1°C viscosity change (V) was 5.9 over the 17°C (0.58-cP) interval examined.


7. This pattern could occur if small particles are caught by a method other than cryptic reversal (10), such as diffusional depredation (J. Shimeta, Limnl. Oceanogr. 38, 456 (1993)). Other changes in the fluid (for example, electrostatic charge) could have differentially affected particles by size.


13. V. B. Pearse and J. S. Pearse, Antarct. J. U.S. 27, 132 (1990). 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 centipede 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.

14. J. S. Pearse, in Reproduction, Larval Biology, and Recruitment in the Deep-Sea Beveroids, K. J. Essed, 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.


17. Alternatively, partial feeding compensation may be inadequate 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. 89, 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)).


21. Future work must incorporate the role of turbulence in assessing viscosity effects (21) [P. S. Hill, in (11)].

22. 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.

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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 polp gene in mice, particularly in the development of lymphocytes, one attractive approach is to generate polp-deficient mice with the use of gene targeting. Because of the potential problem of embryonic lethality caused by a null mutation of the polp gene, we developed a general method for conditional gene inactivation with the use of the polp 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 recombinations 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 recombinat 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') 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' 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 polp mutant mice, we trans-

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

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Clones analyzed (r)</th>
<th>Clones (r) with deletion of polp</th>
<th>Polp/Polp</th>
<th>Polp/Polp</th>
<th>Polp/+</th>
<th>Polp/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>2</td>
<td>4</td>
<td>10.5 days (embryos)</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>1</td>
<td>3</td>
<td>4 weeks (mice)</td>
<td>0</td>
<td>22</td>
</tr>
</tbody>
</table>

**Table 2. Genotypic analysis of the offspring of polp+/+ mice.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Mice (r) with genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>polp/+ polp/Polp/Polp/</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>10.5 days (embryos)</td>
<td>5 19 8</td>
</tr>
<tr>
<td>4 weeks (mice)</td>
<td>0 22 7</td>
</tr>
</tbody>
</table>

**Fig. 2. Southern blot analysis of homologous recombination and Cre-loxP-mediated recombination at the mouse polp 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 polp locus of ES cells. Shown are DNA samples from two candidate mutant ES cell clones (BT14 and BT19). The DNA from wild-type (wt) ES cells is shown as a control. The 5.5-kb band represents the targeted polp allele. (B) Cre-loxP-mediated deletion of the promoter and first exon of the polp gene in ES cells. Genomic DNA was obtained from the parental mutant ES cell clone (BT14) and four sub-clones (1 to 4) carrying deletions at the polp 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). 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 polp 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 polp gene; the ovals represent the polp 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.
fect the linearized targeting vector pMGB9 (Fig. 2C) into ES cells, and homologous recombinants were identified by Southern (DNA) blot analysis \( \text{(10)} \). 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 \( \text{(2A)} \). 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 \( \text{(Fig. 2B)} \). In two independent experiments, both type I and type II deletions were consistently obtained \( \text{(Table 1)} \). For convenience, we refer to the type I deletion at the polβ locus as polβ\( ^{A} \) and the type II deletion as polβ\( ^{fox} \).

Mice carrying the polβ\( ^{A} \) and polβ\( ^{fox} \) mutations in the germ line were generated by the standard protocol \( \text{(11)} \). The impact of the polβ\( ^{A} \) mutation was examined in offspring derived from an intercross between polβ\( ^{A} \) 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 \( \text{(Table 2)} \). However, at day 10.5 of fetal life, embryos homozygous for the polβ\( ^{A} \) mutation were present at the frequency predicted by Mendelian laws \( \text{(Table 2)} \). 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\( ^{A} \) mutation are viable. The overall development of these mutant mice also appears normal. These results indicate that the polβ\( ^{A} \) mutation does not severely hamper polβ expression in vivo.

Cell type-specific deletion of the polβ gene was investigated in polβ\( ^{fox} \)/+ mice carrying a cre\( ^{ck} \) transgene. The cre\( ^{ck} \) transgene in these mice is driven by the IκB proximal promoter and is, therefore, selectively expressed in T lineage cells \( \text{(9)} \). The extent of polβ gene deletion was assessed in various tissues of the animals by Southern hybridization \( \text{(12)} \). As expected, deletion occurred selectively in T cells. Quantitative analysis indicated that 63 to 84% of splenic T cells carried the deletion \( \text{(Table 3)} \). In contrast, no detectable deletion of the polβ gene was observed in either kidney, liver, or B lymphocytes \( \text{(Fig. 3A)} \). These results demonstrate that cell type-specific gene inactivation can be achieved through our approach.

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

The overall development of polβ\( ^{A} \)/polβ\( ^{fox} \), cre\( ^{ck} \) 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 \( \text{(13)} \) on the surface of these cells \( \text{(Fig. 4)} \). In the blood of the mutants, essentially all T cells \( \text{as identified by the Thy-1 surface marker (13)} \) express the αβ T cell receptor (TCR), and the number of splenic Thy-1\( ^{+} \) cells is also normal compared to that in wild-type mice \( \text{(14)} \).

To estimate the fraction of T cells homozygous for the polβ\( ^{A} \) mutation in these mice, we performed Southern blot analysis using DNA from purified T and B cells \( \text{(Fig. 3B)} \). We found that approximately 40% of CD4\( ^{+} \)CD8\( ^{-} \) thymocytes were homozygous for the polβ\( ^{A} \) mutation \( \text{(Table 3)} \).

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

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

<table>
<thead>
<tr>
<th>Cells</th>
<th>polβ( ^{fox} ) allele (%)</th>
<th>polβ( ^{A} ) allele (%)</th>
<th>Cells of genotype polβ( ^{A} )/polβ( ^{A} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4( ^{+} )CD8( ^{+} ) thymocytes</td>
<td>36</td>
<td>64</td>
<td>—</td>
</tr>
<tr>
<td>Splenic T*</td>
<td>37 or 16†</td>
<td>63 or 84†</td>
<td>—</td>
</tr>
<tr>
<td>CD4( ^{+} )CD8( ^{+} ) thymocytes</td>
<td>31</td>
<td>69</td>
<td>38</td>
</tr>
<tr>
<td>Splenic T</td>
<td>31</td>
<td>69</td>
<td>38</td>
</tr>
<tr>
<td>Splenic B</td>
<td>51</td>
<td>49</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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**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 \( \text{(12)} \). Symbols are as in Fig. 2. **(A)** Cell type specificity of polβ gene deletion. Shown are Southern blot analyses of various tissues from polβ\( ^{fox} \)/+; cre\( ^{ck} \) 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\( ^{fox} \)/+; cre\( ^{ck} \) (lanes 1 and 2), polβ\( ^{fox} \)/polβ\( ^{A} \); cre\( ^{ck} \) (lane 3, 4, and 5), and polβ\( ^{fox} \)/polβ\( ^{fox} \); cre\( ^{ck} \) (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 \( \text{(12)} \). The genotypes of the mice are indicated on top of each profile.
observation that the cre<sup>tk</sup> 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 I<sub>x</sub>p proximal promoter is active only at early stages of T cell development (15). Earlier data also indicates that the cre<sup>tk</sup> transgene is expressed only transiently in the thymus (9). It is therefore conceivable that in the transgenic T cells the polβ<sup>tsko</sup> 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<sup>−/−</sup> polβ<sup>tsko</sup>; cre<sup>tk</sup> mice as compared to that in polβ<sup>tsko</sup>/+; cre<sup>tk</sup> 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

11. 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 pMQ39. A gene cassette containing the neo<sup>R</sup> and HSV-1 genes flanked by two lox<sup>P</sup> sites was inserted into the Xho I site between the first and second exon. A third lox<sup>P</sup> 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 sequence further upstream from the third lox<sup>P</sup> 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-T 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 DNA were transfected into 293 cells (Fig. 6, C and D). One targeted clone was identified from each transfection. Cre expression was induced by a tetracycline-responsive promoter, pIC-Cre (6), was introduced into the targeted ES cells by electroporation. After selection in ganciclovir-containing medium (1 × 10<sup>6</sup> M) for 5 days, surviving clones were picked and expanded. Genomic DNA was then prepared from the expanded cells for Southern blot analysis. Probe B is a 1-kb genomic DNA fragment (Hind III–Sac I) of the polβ gene, and probe B is a 650-bp fragment (Sam II–Hind III) (17).
13. 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 identified as Thy-1<sup>+</sup> cells from the spleen. The CD4<sup>+</sup>CD8<sup>+</sup> cells were from the thymus. B cells were sorted from the spleen as surface CD45R/B220<sup>+</sup> cells.

14. To our knowledge, there are no mice available with a deficiency of polβ. Therefore, the ablation of polβ was approached by using a knock-in strategy. Using this approach, Mottet, C.-A.; Steinle, C.; Mottet, B. [Proc. Natl. Acad. Sci. U.S.A. 90, 4326 (1993)] showed that polβ gene ablation can lead to cell death.

15. We thank A. Matsukage for providing the plasmid containing the genomic polβ gene and R. Kühn for the E14-T ES cell line. We are grateful to R. Torres, F. Huetz, and Y.-R. Zou for critical reading of the manuscript; to U. Ringelsen 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 FZT Foundation, the Bundesministerium für Bildung und Technologie, and the Human Frontier Science Program.

4. The probewasa 900-bp Bam H1-Hind III fragment of the polβ gene (17). Densitometric analysis was performed with a Bio-Imaging analyzer (Fuji) or a densitometric scanner (Pharmacia).

5. 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].


7. We thank A. Matsukage for providing the plasmid containing the genomic polβ gene and R. Kühn for the E14-T ES cell line. We are grateful to R. Torres, F. Huetz, and Y.-R. Zou for critical reading of the manuscript; to U. Ringelsen 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 FZT Foundation, the Bundesministerium für Bildung und Technologie, and the Human Frontier Science Program.

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

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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.

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