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11. Abbreviations for the amino acid residues are as follows: D, Asp; K, Lys; N, Asn; and X, any amino acid.
12. An ~825–base pair Sac II–Eco RI fragment encompassing the GTP-binding domain of FtsY was subcloned into plasmid Blue Script SK+ (Stratagene). Site-directed mutagenesis [T. A. Kunkel, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 488 (1985); L. B. Evnin and C. S. Craik, *Ann. N.Y. Acad. Sci.* **542**, 61 (1988)] was used to introduce the Asp → Asn mutation corresponding to position 441 in the coding region of FtsY by means of the 20-mer oligonucleotide 5′-CGAAACTGAACGGCACGGCG-3′. This fragment was then introduced into a pGEX1 vector that contained FtsY fused to the COOH-terminus of GST, as described (*7*). The resulting mutant FtsY-GST fusion protein was then expressed and purified (*7*).
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14. We cannot, however, rule out more complicated alternative mechanisms. For example, the hydrolysis of only one GTP bound to either Ffh or FtsY might be sufficient to dissociate Ffh-4.5*S* RNP and FtsY during an interaction cycle, or FtsY may function as a GAP for Ffh while Ffh functions as a GNRF for FtsY (for which the affinity of GDP is unknown). The reverse of the latter scenario is very unlikely because of the low affinity of Ffh for nucleotide (Fig. 1).
15. This scenario additionally requires that after divergence, these GTPases no longer stimulate their own GTPase. In this regard, we note that the basal, FtsY-independent GTP hydrolysis rate of Ffh-4.5*S* RNP is strictly linear with enzyme concentration, with no detectable cooperativity (H. Wilhelm and P. Walter, unpublished data). Thus, this basal reaction does not seem to involve the cooperation of two Ffh molecules, but rather results from an intrinsic property of monomeric Ffh itself.
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TRAF2-Mediated Activation of NF-κB by TNF Receptor 2 and CD40

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TNF receptor–associated factor (TRAF) proteins are candidate signal transducers that associate with the cytoplasmic domains of members of the tumor necrosis factor (TNF) receptor superfamily. The role of TRAFs in the TNF-R2 and CD40 signal transduction pathways, which result in the activation of transcription factor NF-κB, was investigated. Overexpression of TRAF2, but not TRAF1 or TRAF3, was sufficient to induce NF-κB activation. A truncated derivative of TRAF2 lacking an amino-terminal RING finger domain was a dominant-negative inhibitor of NF-κB activation mediated by TNF-R2 and CD40. Thus, TRAF2 is a common mediator of TNF-R2 and CD40 signaling.

Cytokines trigger changes in gene expression by modifying the activity of otherwise latent transcription factors (*1*). TNF is an inducer of nuclear factor κB (NF-κB) (*2*), a homo- or heterodimer of members of the Rel family of transcriptional activators that control the expression of a variety of important cellular and viral genes (*3*, *4*). TNF initiates pleiotropic inflammatory and immunoregulatory responses by binding to two distinct cell surface receptors of ~55 kD (TNF-R1) and 75 kD (TNF-R2) (*5*). Both TNF receptors independently mediate NF-κB activation by TNF (*6*–*8*). Two putative effectors of TNF-R2 signaling have been identified—TRAF1 and TRAF2—that share a COOH-terminal homology region, the TRAF domain (*7*). TRAF2 homodimers as well as TRAF1:TRAF2 heterodimers can associate with a COOH-terminal region in the cytoplasmic domain of TNF-R2 that is required for signaling growth proliferation and NF-κB activation. A third TRAF domain protein, TRAF3 [also known as CD40bp, CRAF1, or LAP1 (*9*–*11*)], interacts with the cytoplasmic domain of CD40, another member of the TNF receptor superfamily (*12*). Because CD40 can also signal NF-κB activation (*13*), we investigated the potential role of TRAF proteins in this process.

In the yeast two-hybrid assay (*14*), TRAF3 self-associated, but it did not form heterotypic complexes with either TRAF1 or TRAF2 (Table 1). None of the three TRAFs associated with the cytoplasmic domain of either TNF-R1 or the Fas antigen (Table 1). Also, TRAF1 did not interact with CD40, nor did TRAF3 interact with TNF-R2. However, TRAF2 associated strongly with the cytoplasmic domains of both CD40 and TNF-R2 (Table 1) (*15*).

Induction of NF-κB activity by TNF has been demonstrated in numerous cell types, including human embryonic kidney 293 cells (*8*) (Fig. 1). In 293 cells, both TNF receptors are capable of signaling NF-κB activation. Because of a low level of endogenous TNF-R2 (*16*), only TNF-R1 mediates NF-κB activation in non-transfected 293 cells as indicated by stimulation with receptor-specific agonistic antibodies (Fig. 1). However, upon transfection TNF-R2 also signals NF-κB activation (below). To examine a functional role for TRAFs in NF-κB activation, we transiently transfected 293 cells with TRAF expression vectors (*17*) and performed electrophoretic mobility–shift assays (*18*). Expression of TRAF1 or TRAF3 did not result in induction of NF-κB DNA-binding activity. In contrast, TRAF2-expressing 293 cells contained activated NF-κB (Fig. 1). The major component of the active NF-κB complex was the p65:p50 heterodimer as indicated by supershift experiments (Fig. 1) (*18*).

To determine whether TRAF2 expression might activate an NF-κB–dependent reporter gene, we cotransfected an E-selectin–luciferase reporter construct (*19*) with the various TRAF expression vectors into 293 cells (*18*). TRAF2 expression potently activated the reporter gene, whereas expression of TRAF1 or TRAF3 had no effect (Fig. 2A). In all cases, reporter gene activity could be coinduced through the TNF-R1 pathway by the addition of TNF (Fig. 2A) (*20*). The observed reporter gene induction was dependent on NF-κB activation because TRAF2 expression failed to activate a control reporter construct in which the NF-κB sites in the E-selectin promotor were mutated (*21*). Thus, overexpression of TRAF2, but not of TRAF1 or TRAF3, is sufficient to initiate NF-κB activation in 293 cells. This observation is consistent with an activation mechanism in which TRAF2 clustering induced by its overexpression is similar to that induced by ligand-triggered receptor aggregation, thereby activating the NF-κB signaling pathway independently of TRAF2 association with TNF-R2.

TRAF2 and TRAF3 contain NH_2-terminal RING finger motifs that are not re-

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quired for interaction with TNF-R2 and CD40 (*7*, *9*–*11*). In particular, a truncated variant of TRAF2 which lacks the NH_2-terminal 86 amino acids that comprise the RING finger domain [TRAF2(87–501)] retains its ability to associate with the cytoplasmic domains of TNF-R2 and CD40, as well as with TRAF1 and wild-type TRAF2 (Table 1) (*7*). This otherwise functional TRAF2(87–501) protein was completely defective in the induction of NF-κB–dependent reporter gene activity in 293 cells (Fig. 2A). Thus, the RING finger domain of TRAF2 is required for NF-κB activation.

To confirm the results obtained in 293 cells, we performed similar transient transfection experiments in the murine cytotoxic T cell line, CT6. CT6 cells do not express detectable levels of TNF-R1 (*22*), and TNF-induced NF-κB activation in this cell line is mediated by TNF-R2 (*7*). As in 293 cells, expression of TRAF2 potently induced the E-selectin–luciferase reporter gene in CT6 cells, whereas TRAF1, TRAF3, and TRAF2(87–501) did not (Fig. 2B) (*23*). Furthermore, expression of mutant TRAF2(87–501), but not wild-type TRAF1, -2 or -3, suppressed the NF-κB–dependent reporter gene activity induced by TNF stimulation of CT6 cells (Fig. 2B). Thus, TRAF2(87–501) acts as a dominant-negative inhibitor of TNF-R2–mediated NF-κB activation in CT6 cells.

In contrast to CT6 cells, TNF-induced NF-κB activation in nontransfected 293 cells is mediated by TNF-R1 (above). Overexpression of mutant TRAF2(87–501) in 293 cells did not inhibit the TNF-induced E-selectin–luciferase reporter gene activity (Fig. 2A), which indicates that TNF-R1 signaling is not blocked by the dominant-negative TRAF2 mutation. The effect of TRAFs on TNF-R2 signaling in 293 cells was tested by transient coexpression of TNF-R2 with wild-type or mutant TRAFs. Overexpression of TNF-R2 alone was sufficient to initiate NF-κB–dependent reporter gene activity even without concomitant TNF stimulation (Fig. 3A) (*24*). This activity was dependent on the integrity of the cytoplasmic domain of TNF-R2 because overexpression of mutant TNF-R2 proteins defective in signal transduction (*7*) did not induce NF-κB activation (*7*, *21*). Coexpression of mutant TRAF2(87–501) completely abolished reporter gene activation induced by wild-type TNF-R2 (Fig. 3A). Thus, as in CT6 cells, TRAF2(87–501) exerts a dominant-negative effect on TNF-R2 signaling in 293 cells. However, NF-κB–dependent reporter gene activity in 293 cells that coexpressed TNF-R2 and TRAF2(87–501) could still be induced through the TNF-R1 pathway by the addition of TNF (Fig. 3A).

We next examined whether TRAF2 also mediates NF-κB activation by CD40, because it associates with both TNF-R2 and CD40 (above). As with TNF-R2, overexpression of CD40 in 293 cells was sufficient to induce NF-κB activation in the absence of ligand (Fig. 3B). In contrast, transient overexpression of a mutant CD40 protein defective in signal transduction (*13*) did not induce NF-κB activation (*21*). Coexpression of wild-type CD40 and mutant TRAF2(87–501) completely inhibited the CD40-induced reporter gene activity (Fig. 3B), which demonstrates that TRAF2(87–501) exerts a dominant-negative effect on CD40 signaling. These results indicate that TRAF2 is a common mediator of NF-κB activation for both TNF-R2 and CD40.

Whereas TRAF2 acted as a positive signal transducer, TRAF3 suppressed CD40-induced NF-κB–dependent reporter gene activity in 293 cells (Fig. 3B). TRAF3 also inhibited TNF-R2–induced NF-κB activation in 293 cells (Fig. 3A), although this effect was not observed in CT6 cells (Fig. 2B). Because no direct interaction of TRAF3 with TRAF1, TRAF2, or TNF-R2 was detected by two-hybrid analysis, the dominant-negative effect of TRAF3 in 293 cells might imply a weak interaction of TRAF3 with one of these components. Inhibition of TNF-R2–mediated NF-κB activation could then be explained by the high level of TRAF3 overexpression in 293 cells relative to CT6 cells. It is also possible that TRAF3 participates in signaling pathways distinct from the TRAF2-mediated NF-κB activation pathway. By analogy with TRAF2, the initiation of such a pathway may involve the RING finger domain of

Table 1. Interaction between TRAFs and members of the TNF receptor superfamily in the yeast two-hybrid assay. Yeast Y190 cells were cotransformed with expression vectors encoding the indicated Gal4 DNA-binding domain and Gal4 transcriptional activation domain fusion proteins (*14*). Each transformation mixture was plated on a synthetic dextrose plate lacking tryptophan and leucine. Filter assays for β-galactosidase activity were performed to detect interaction between fusion proteins. (+) Strong color development within 1 hour of the assay; (−) no development of color within 24 hours. Control transformations with empty Gal4 vectors were negative and are not listed.

DNA-binding domain hybrid	Activation domain hybrid			
	TRAF1	TRAF2	TRAF2(87–501)	TRAF3
TRAF1	+	+	+	−
TRAF2	+	+	+	−
TRAF3	−	−	−	+
TNF-R2	−	+	+	−
CD40	−	+	+	+
TNF-R1	−	−	−	−
Fas	−	−	−	−

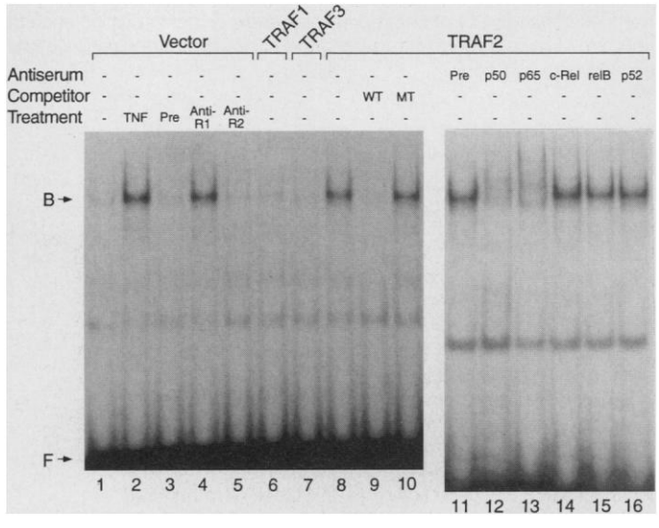

Fig. 1. Transcription factor NF-κB is activated by TRAF2 overexpression. 293 cells (2×10^6) were transfected with 7.5 μg of pRK control (lanes 1 to 5) or expression vectors for TRAF1 (lane 6), TRAF3 (lane 7), and TRAF2 (lanes 8 to 16) (*17*, *18*). Cells were treated with human TNF (100 ng/ml) (lane 2), preimmune serum (lane 3), antibodies to human TNF-R1 (anti–TNF-R1) (lane 4) or anti–human TNF-R2 (lane 5) at a dilution of 1:500 for 1 hour before harvest (*7*). Nuclear extracts were prepared 24 hours after transfection, and 6-μg aliquots were incubated with a radiolabeled double-stranded oligonucleotide containing two NF-κB binding sites (*26*). Individual reactions were supplemented with a 50-fold excess of unlabeled competitor oligonucleotide containing either wild-type (lane 9) or mutated (lane 10) NF-κB binding sites (*26*). Reaction mixtures were incubated with 1 μl of preimmune serum (lane 11), anti-p50 (lane 12), anti-p65 (lane 13), anti–c-Rel (lane 14), anti-relB (lane 15), or anti-p52 (lane 16) (*18*). F and B, free oligonucleotide probe or oligonucleotide probe in a complex with protein, respectively.

TRAF3. A truncated TRAF3 protein lacking the NH_2-terminal half of the molecule was observed to suppress CD40-mediated induction of CD23 (*10*). Although the effect of native TRAF3 was not examined in (*10*), our results could indicate that induction of CD23 expression by CD40 may be mediated by TRAF2 and inhibited by native TRAF3.

The COOH-terminal cytoplasmic domain of the Epstein-Barr virus–transforming protein, LMP1, has been reported to interact with TRAF1 and TRAF3 (*11*). The interaction between TRAF1 and LMP1 is indirect and is hypothesized to be mediated by an as yet unknown TRAF protein. Because TRAF2 mediates the interaction of TRAF1 with TNF-R2 (*7*) and TRAF2 also associates with CD40 (Table 1), we speculate that TRAF2 is likely to mediate the TRAF1:LMP1 interaction. LMP1 is a dominant oncogene that has multiple downstream effects on cell growth and gene expression, at least some of which require NF-κB activation (*25*). Our comparative analysis of all three known TRAFs suggests that TRAF2 initiates NF-κB activation as a common signal transducer for TNF-R2, CD40, and, by analogy, perhaps LMP1.

The recent identification of the TRAF protein family provided an important clue as to how members of the TNF receptor superfamily signal downstream responses. The findings outlined in the present study demonstrate that TRAF2 is a common mediator of TNF-R2 and CD40 signaling that is central to the activation of NF-κB. Conceptually, this signaling cascade is activated by TRAF2 aggregation, which can be triggered either by receptor clustering or TRAF2 overexpression. Recently, a protein, TRADD, was identified that is unrelated to the TRAF family and that mediates NF-κB activation by TNF-R1 (*8*). The identification of two signal transducers, TRAF2 and TRADD, provides molecular evidence for earlier indications that activation of NF-κB can be achieved through multiple, independent signaling pathways (*4*). The RING finger domain of TRAF2, which is required for NF-κB activation but not for other TRAF2 functions, represents a potential protein-protein interaction domain that may connect TRAF2 with subsequent steps in the signaling cascade.

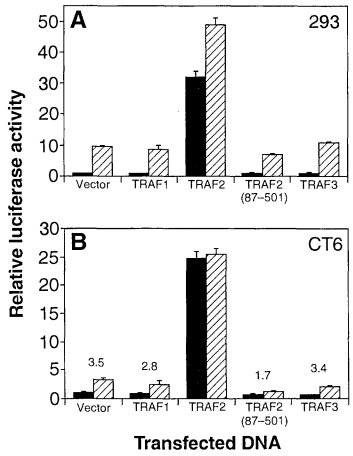

Fig. 2. Overexpression of TRAF2 induces NF-κB–dependent reporter gene activity. (**A**) Effect of TRAF overexpression on NF-κB–dependent reporter gene activity in 293 cells. Two hundred ninety-three cells were transiently cotransfected with an E-selectin–luciferase reporter gene plasmid (*19*) and TRAF expression vectors (1 μg) as indicated (*17, 18*). Cells were either untreated (closed bars) or stimulated for 8 hours with human TNF (100 ng/ml) (hatched bars) before harvest. Luciferase activities were determined and normalized on the basis of β-galactosidase expression (*18*). Values shown are averages (mean ± SD) of one representative experiment in which each transfection was done in triplicate. (**B**) Effect of TRAF expression on NF-κB–dependent reporter gene activity in CT6 cells. CT6 cells were transiently cotransfected with an E-selectin–luciferase reporter gene plasmid (*18*) and TRAF expression vectors (6 μg) as indicated (*23*). After 24 hours, cells were left untreated (closed bars) or stimulated for an additional 16 hours with murine TNF (100 ng/ml) (hatched bars) before harvest. Luciferase activities were determined and normalized on the basis of β-galactosidase expression (*18*). Values shown are averages (mean ± SD) of one representative experiment in which each transfection was performed in triplicate. Numbers represent the fold induction of reporter gene activity by TNF stimulation for the respective transfections.

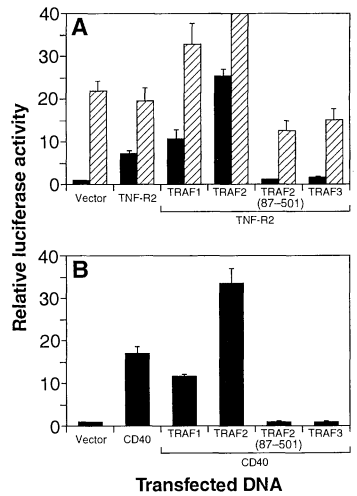

Fig. 3. TRAF2 mediates induction of NF-κB–dependent reporter gene activity by TNF-R2 and CD40. (**A**) Effect of coexpression of TNF-R2 and TRAFs on NF-κB–dependent reporter gene activity in 293 cells. Two hundred ninety-three cells were transiently cotransfected with an E-selectin–luciferase reporter gene plasmid (*19*) and expression vectors for TNF-R2 (1 μg) and TRAFs (1 μg) as indicated (*17, 18*). Cells were either untreated (closed bars) or stimulated for 8 hours with human TNF (100 ng/ml) (hatched bars) before harvest. Luciferase activities were determined and normalized on the basis of β-galactosidase expression (*18*). Values shown are averages (mean ± SD) of one representative experiment in which each transfection was performed in triplicate. The off-scale value is 92 ± 7. (**B**) Effect of coexpression of CD40 and TRAFs on NF-κB–dependent reporter gene activity in 293 cells. Two hundred ninety-three cells were transiently cotransfected with an E-selectin–luciferase reporter gene plasmid (*19*) and expression vectors for CD40 (1 μg) and TRAFs (1 μg) as indicated (*17, 18*). After 24 hours, cells were harvested and luciferase activities were determined and normalized (closed bars) (*18*). Values shown are averages (mean ± SD) of one representative experiment in which each transfection was done in triplicate.

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14. Expression vectors for TRAFs and the cytoplasmic domains of members of the TNF receptor superfamily in the yeast two-hybrid system have been reported previously (*7–9*). Two-hybrid interaction analysis between bait- and prey-encoded fusion proteins was done as described (*7, 9*).
15. TRAF2 had also been identified as a CD40-associated protein in the same yeast two-hybrid screen that had resulted in the identification of TRAF3 [H. M. Hu and V. M. Dixit, unpublished results].
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17. Expression vectors for TRAF1, TRAF2, and TRAF2(87–501) contained the respective coding regions (*7*) under the transcriptional control of the cytomegalovirus (CMV) immediate-early promotor-enhancer in pRK [T. J. Schall *et al.*, *Cell* **61**, 361 (1990)]. Expression of TRAF proteins in transiently transfected 293 cells (*18*) was confirmed by protein immunoblot analysis with polyclonal antibodies directed against TRAF1 and TRAF2, respectively (*21*). Expression vectors for TRAF3 and CD40 (*9*) and TNF-R2 [L. A. Tartaglia, M. Rothe, Y.-F. Hu, D. V. Goeddel, *Cell* **73**, 213 (1993)] have been described previously.
18. Transient transfection of 293 cells was done as described (*8*). After 24 hours, nuclear extracts were prepared, and NF-κB activation was analyzed by electrophoretic mobility–shift assay (*26*). The composition of the activated NF-κB complex was examined by supershift analysis with antibodies that recognize specific NF-κB subunits (Santa Cruz Biotechnology). For cotransfection experiments, 3×10^5 293 cells were transfected with 0.5 μg of E-selectin–luciferase reporter gene plasmid (*19*) and 1 μg of each expression construct (*17*). DNA concentrations were kept constant by supplementation with pRK. Cells were lysed 24 hours after transfection and reporter gene activity determined with the Luciferase Assay System (Promega). A pRSV–β-Gal vector (0.5 μg) was used for normalizing transfection efficiency.
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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).
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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].
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Inducible Gene Targeting in Mice

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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, *polβ*^{flox} (*2*), is inactivated upon excision of a *loxP*-flanked 1.5-kb segment containing the promoter region and first exon of the *polβ* 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 *polβ*^{flox} allele and the fragment derived from the deleted (*polβ*$^{\Delta}$) 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-

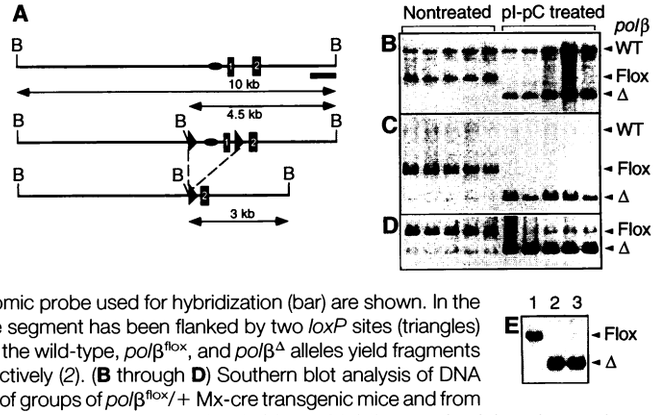

Fig. 1. Detection of Cre-mediated deletion. (**A**) Southern blot strategy to distinguish *polβ* wild-type (+) (upper line), *polβ*^{flox} (middle line), and *polβ*$^{\Delta}$ (lower line) alleles. Bam HI fragments (B) containing the promoter region (ellipses), the first two exons of the *polβ* gene (numbered rectangles), and an adjacent genomic probe used for hybridization (bar) are shown. In the *polβ*^{flox} allele a 1.5-kb gene segment has been flanked by two *loxP* sites (triangles) (*2*). Upon Bam HI digestion the wild-type, *polβ*^{flox}, and *polβ*$^{\Delta}$ 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 *polβ*^{flox}/+ Mx-cre transgenic mice and from liver (C) of *polβ*^{flox}/*polβ*^{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 *polβ* wild-type (WT), *polβ*^{flox} (Flox), and *polβ*$^{\Delta}$ (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.

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