An Intact Zinc Ring Finger Is Required for Tumor Necrosis Factor Receptor-associated Factor-mediated Nuclear Factor-κB Activation but Is Dispensable for c-Jun N-terminal Kinase Signaling*

(Received for publication, June 12, 1998, and in revised form, July 31, 1998)

Hajir Dadgostar§§ and Genhong Cheng¶¶

From the §Department of Microbiology and Molecular Genetics, Jonsson Comprehensive Cancer Center and the ¶¶Molecular Biology Institute, University of California, Los Angeles, California 90095

The diverse biological effects of the tumor necrosis factor (TNF) receptor superfamily are believed to be mediated in part through TNF receptor-associated factors (TRAFs), a family of cytoplasmic adaptor proteins which can activate intracellular signaling pathways, including the nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK) pathways. TRAFs 2, 5, and 6 strongly activate both pathways when overexpressed; however, TRAF 3 (a close homologue of TRAF 5) does not significantly activate either pathway. The current study addresses the structural basis for this difference by substituting corresponding domains of TRAF 5 into TRAF 3 and testing activation of both pathways. A small region of TRAF 5 (the first zinc finger and 10 residues of the second zinc finger) is sufficient to convert TRAF 3 into an activator of both pathways. Also, an intact zinc ring finger is required for NF-κB activation but not JNK activation. In agreement with this finding, TRAF 2A, a TRAF 2 splice variant with an altered ring finger, is a specific activator of JNK. These findings suggest that different domains of TRAFs may be involved in NF-κB and JNK signaling. Also, alternative splicing of TRAFs may represent a novel mechanism whereby TNF family receptors can mediate distinct downstream effects in different tissues.

The tumor necrosis factor receptor (TNFR) superfamily is a group of type I receptors expressed in a wide variety of cell types and is responsible for a vast range of biological responses, including leukocyte activation, proliferation and differentiation, local and systemic inflammation, metabolic regulation, bone development, neural growth, and programmed cell death (1, 2). They mediate various biological effects by activating a variety of intracellular signaling pathways such as the c-Jun N-terminal kinase (JNK) cascade, a mitogen-activated protein kinase cascade leading to the phosphorylation and activation of transcription factors such as c-Jun, Elk1, and ATF2, and the nuclear factor-κB (NF-κB) pathway, as well as pathways leading to cell death (3, 4).

* This work was supported by the UCLA Jonsson Comprehensive Cancer Center Development Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Recipient of UCLA Medical Science Training Program Training Grant GM 08042.

† To whom correspondence should be addressed. Tel.: 310-825-8896; Fax: 310-206-5503; E-mail: genhong@microbio.ucla.edu.

‡ The abbreviations used are: TNF, tumor necrosis factor receptor; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-κB; TRAF, TNF receptor-associated factor; PCR, polymerase chain reaction; HA, hemagglutinin; GST, glutathione S-transferase.

© 1998 by The American Society for Biochemistry and Molecular Biology, Inc.

During the past few years, a better understanding of TNFR signaling has developed with the discovery of a family of cytoplasmic adaptor molecules known as TNF receptor-associated factors (TRAFs) (5). To date, six members of this family have been described (5–13). TRAFs are grouped together on the basis of a conserved C-terminal protein-protein interaction domain known as the TRAF domain (5). Through this domain, TRAFs interact with the cytoplasmic tails of various TNFR family members (e.g. TNFR 2, CD40, CD30, CD27, LTβ-R) (5, 7, 12, 14–16). Different TRAF family members have different affinities for the various receptors; in addition, some TRAFs exhibit tissue-specific expression (e.g. TRAF 5 is expressed in the lung, thymus, and spleen; TRAF 4 is expressed in certain breast carcinomas) (10–12). Both of these factors may contribute to differences in signaling by distinct TNF receptor family members in different tissues.

All TRAFs exhibit some degree of structural homology, with the greatest degree of conservation being in the C-terminal TRAF domain. This domain is further subdivided into N- and C-terminal halves (the TRAF-N and TRAF-C domains, respectively) responsible for distinct protein-protein interactions (7). In addition, the N-terminal half of all TRAFs, except TRAF 1, consists of a predicted zinc-binding region containing a zinc ring finger, followed by five tandem zinc finger structures (7). Finally, TRAF 3 and TRAF 5 contain an isoform zipper domain between the last zinc finger and the TRAF-N domain (7, 11, 12).

Of the known TRAF family members, TRAFs 2, 5, and 6 have been shown to activate both the NF-κB and JNK pathways upon overexpression; however, neither pathway has been shown to be activated by overexpressed TRAF 1, 3, or 4 (17–18). Thus, it is thought that certain TRAFs may contribute to the activation of one or both of these pathways through certain receptor complexes, whereas other TRAFs may play other as yet undefined roles in signaling. The N-terminal putative zinc-binding domain has been shown to be required for activation of the NF-κB and JNK pathways (18); however, the nature of this domain’s contribution to signaling in either pathway remains a mystery.

The present study takes advantage of the high degree of sequence similarity between TRAF 5, which activates both NF-κB and JNK, and TRAF 3, which activates neither, to identify structural features which contribute to the activation of either signaling pathway. Through domain swapping within the N-terminal zinc-binding region, it is demonstrated that the structural basis for the differences in signaling by TRAF 3 and TRAF 5 can be overcome by replacing the first zinc finger and first 10 amino acids of the second zinc finger of TRAF 3 with those of TRAF 5. This region of TRAF 5 is sufficient to convert TRAF 3 into an activator of both pathways. In addition, it is shown that although the zinc ring finger is required for NF-κB
Signaling, the ring finger of TRAF 3 is able to substitute for that of TRAF 5 in this role. Finally, it is shown, through the generation of single amino acid substitutions within the ring finger, as well as through analysis of a TRAF 2 splice variant with an altered ring finger, that changes in the structure of the ring finger greatly reduce NF-κB but not JNK signaling, thus defining different structural requirements for activation of these two pathways.

**EXPERIMENTAL PROCEDURES**

**PCR and Plasmids**—All TRAF constructs were prepared by PCR between a forward primer containing a BamHI site and a reverse primer containing a NotI site. Domain swapping and point mutations were performed by a previously described PCR mutagenesis method (19). Constructs were digested with BamHI and NotI and cloned into pEBB, a high-expression vector utilizing an elongation factor 1 promoter carrying a 3′ HA tag. The luciferase reporter and β-galactosidase expression constructs have been described previously (20). For kinase assays, cells were transfected with an expression plasmid containing the p54B form of JNK with an HA tag (pMT-JNK) or a GST tag (pEBG-JNK).

**Luciferase Assay, in Vitro Kinase Assay, and Western Blotting Analysis**—Using a standard calcium phosphate method, approximately 2 × 10⁶ 293 cells/60-mm plate were transiently cotransfected with 2 μg of HA-tagged JNK expression vector, 15 ng of NF-κB luciferase reporter construct, 100 ng of β-galactosidase expression vector, and 4 μg of pEBB expression vector or various amounts of individual HA-tagged mutant TRAF constructs. Because transfecting the same amount of vector yielded different protein expression levels between TRAF 3 and TRAF 5, roughly equal protein expression levels were achieved, where possible, by transfecting different amounts of expression vector (for TRAF 3 and 5, as well as mutant constructs). Empty expression vector was added to make up any differences in the amount of DNA so that each plate was transfected with the same quantity of DNA. The NF-κB-dependent luciferase reporter assay, the in vitro GST-Jun phosphorylation assay, and Western blotting analysis were performed as described previously (20, 21). All results shown are representative experiments using extracts from the same population of cells for simultaneous determination of both NF-κB and JNK activities and for confirming comparable expression levels of tagged JNK as well as wild-type and mutant TRAFs; however, the activity of each mutant was confirmed in at least three independent experiments.

**RESULTS**

**Identification of a Minimum Sequence within the N-terminal Half of TRAF 5 That Is Sufficient for Strong Activation of Both NF-κB and JNK**—Recent work addressing the structural requirements for NF-κB activation by TRAF 2 suggests that the N-terminal putative zinc-binding region is necessary for this activity (22). This region consists of a predicted zinc ring finger, followed by five adjacent predicted zinc finger structures. It appears that if the ring finger and first two zinc fingers of TRAF 3, which ordinarily do not activate NF-κB, are replaced by the corresponding structures from TRAF 2, the resulting mutant is capable of activating NF-κB.

In Fig. 1, the sequences of TRAF 2, TRAF 3, and TRAF 5 within this region are aligned for comparison. Since TRAFs 3 and 5 share a high level of sequence homology (overall, as well as within this specific region), sequences within this region were exchanged between these two molecules to map the minimum sequence difference that distinguishes the two in terms of NF-κB and JNK activation. All constructs were marked with a C-terminal influenza HA tag and overexpressed from a high expression vector (pEBB) in the human embryonic kidney cell line, 293. For simultaneous analysis of NF-κB and JNK activation, 293 cells transfected with the various TRAF constructs were cotransfected with an NF-κB luciferase reporter construct as well as an HA-tagged JNK expression construct. Cytoplasmic extracts from these cells were analyzed for NF-κB and JNK activation by luciferase reporter and in vitro kinase assays, respectively.

As seen in Fig. 2, both the NF-κB and JNK pathways are activated by TRAF 5 but not TRAF 3. Using an N-terminal domain substitution, it was found that replacing the ring finger, first zinc finger, and first few residues of the second zinc finger of TRAF 3 with those of TRAF 5 (TF 53/148; Fig. 2, lane 3) was sufficient to enable the resulting mutant to activate both the NF-κB and JNK pathways to levels roughly comparable with those observed with wild-type TRAF 5.

Further mapping revealed that a 39-amino acid stretch comprising the first zinc finger and part of the second zinc finger of TRAF 5 (TF 353/110–148; Fig. 2, lane 5) was sufficient to strongly activate both pathways in the context of surrounding TRAF 3 sequences. Upon further dissection of this region, it was found that when the first zinc finger of TRAF 5 was substituted into TRAF 3 (TF 353/110–132), it was only capable
of relatively weak NF-κB activation and no JNK activation, whereas the adjacent partial second zinc finger sequence (TF 353/133–148) did not activate NF-κB and only weakly activated JNK (Fig. 2, lanes 6 and 7). Thus, it seems that amino acids 110–148 of TRAF 5 in the context of TRAF 3 are sufficient for strong activation of both pathways; however, further reduction of this region leads to significant loss of activity.

Since TF 353/110–148 contains no portion of the TRAF 5 ring finger sequence (Fig. 2) yet is capable of activating both NF-κB and JNK, it suggests that the full TRAF 3 ring finger is able to substitute for that of TRAF 5 in this respect. To confirm this, we designed a construct (TF 35/85) in which the only modification of TRAF 5 is the replacement of its ring finger with that of TRAF 3. As shown in Fig. 2C, this construct is also a strong activator of both pathways.

**An Intact Zinc Ring Finger Is Required for NF-κB but Not JNK Activation**—Early in the process of mapping the minimal TRAF 5 sequence required for activity, we generated a construct (TF 353/71–148) that activated JNK but not NF-κB. This result seemed surprising in light of the findings presented in Fig. 2; however, a distinguishing feature of this construct is that it contains a chimeric ring finger with TRAF 3 sequences on the N-terminal side fused to the TRAF 5 sequences at the C-terminal end. Another chimeric ring finger construct (TF 353/85–148) with a fusion point near the C-terminal end. Another chimeric ring finger construct (TF 353/85–148) with a fusion point near the end of the ring finger behaved similarly (Fig. 3, lanes 4 and 5). Based on these results, we reasoned that these hybrid ring fingers must somehow differ from the full-length TRAF 3 or TRAF 5 ring fingers and that this difference might contribute to the inability of constructs bearing such chimeric ring fingers to activate NF-κB.

Close examination of the sequences in this region revealed that TRAF 5 has an aspartic acid residue (Asp^84^) replacing one of the conserved cysteines of the TRAF 3 ring finger (Cys^90^). Although this sequence is most likely conducive to proper folding in the context of a TRAF 5 ring finger in wild-type TRAF 5, we reasoned that the TRAF 3 ring finger may require the cysteine at this position to chelate zinc and fold correctly. Since wild-type TRAF 3 does not activate NF-κB or JNK, we decided to test the effect of a point mutation at this position in the TRAF 3 ring finger in chimeric constructs that activated both pathways, with the full ring finger of TRAF 3 replacing that of TRAF 5 (see Fig. 2, lanes 4 and 5). As shown in Fig. 3, lanes 6–9, replacement of this cysteine of TRAF 3 (Cys^90^) with the corresponding aspartic acid from the TRAF 5 ring finger (Asp^84^) resulted in a selective reduction in NF-κB signaling. To test the requirement for an intact ring finger in the context of full-length wild-type TRAF 5, we next generated two constructs, each bearing a cysteine to alanine mutation at one of the conserved zinc-chelating positions of the ring finger (TF 5/C45A and TF 5/C81A). In agreement with our findings above, both of these mutants retained JNK signaling, whereas NF-κB activation was lost (Fig. 4).

**TRAF 2A Is a Naturally Occurring Pathway-specific TRAF**—Identification of an alternatively spliced form of TRAF 2 has been reported recently (23). This molecule, designated TRAF 2A, differs structurally from TRAF 2 in that it bears a seven-amino acid insertion in its ring finger domain, and functionally in its inability to activate NF-κB.

Based on the above findings in TRAF 5, it seemed possible that alterations in the ring finger of TRAF 2 might similarly lead to a selective effect on NF-κB activity. To test this possibility, a construct encoding TRAF 2A was generated by PCR, based on the published sequence, and this molecule was compared with TRAF 2 in its ability to activate NF-κB and JNK. It has been observed previously in COS-7 cells that, despite transfection of identical quantities of expression vector, TRAF 2A protein is expressed at lower levels than TRAF 2 protein (23). We observed the same phenomenon in 293 cells and compensated by titrating the levels of TRAF 2A transfected. When equal levels of the two proteins were expressed (Fig. 5) it was found that TRAF 2A does not activate NF-κB to any significant extent, yet it remains a potent activator of JNK kinase activity.

**DISCUSSION**

Using the strategy of domain swapping between homologous regions of the closely related family members, TRAF 3 and TRAF 5, we have identified a minimum sequence within the putative zinc-binding region of TRAF 5 (amino acids 110–148), which when used to replace the corresponding region of TRAF...
is capable of converting the latter into a potent activator of both NF-κB and JNK. This region comprises the entire first zinc finger and the first 10 residues of the second zinc finger of TRAF 5.

Since this construct (TF 353/110–148) contains the full ring finger of TRAF 3, and since previous work as well as the results of the present study suggest that the ring finger is required for activation of NF-κB, it appears that the TRAF 3 ring finger is functional and able to substitute for that of TRAF 5 in NF-κB signaling. This finding contrasts with those of a previous study in which NF-κB was not activated by a construct in which the TRAF 2 ring finger was replaced by that of TRAF 3 (22). We find that an analogous construct consisting of full-length TRAF 5, with the ring finger replaced by that of TRAF 3, is a potent activator of NF-κB. One possible explanation for this discrepancy is the fact that TRAF 3 shares greater sequence homology with TRAF 5 than it does with TRAF 2. Since the folding of one domain may affect the overall folding of the chimeric protein, it is possible that certain TRAF 3/TRAF 2 hybrids may fold abnormally and become nonfunctional, whereas the corresponding domains in analogous TRAF 3/TRAF 5 hybrids may match each other sufficiently to produce correctly folded domains.

More importantly, the present study demonstrates that different structural features of TRAF molecules are required for TRAF-mediated NF-κB and JNK activation and that these two signal transduction pathways can be dissected at the level of the TRAFs. We showed that single amino acid substitutions targeting cysteine residues in the ring finger of TRAF 3 (in chimeric constructs) or TRAF 5 (in wild-type TRAF 5) result in mutants capable of activating JNK but not NF-κB. This suggests that correct folding of the ring finger (whether it is that of TRAF 3 or TRAF 5) may be essential for NF-κB activation but dispensable for JNK activation. This finding may appear to conflict with previous work demonstrating that TRAF 2 lacking a ring finger not only fails to activate JNK but also acts as a dominant negative inhibitor of TNF-mediated JNK activation (24); however, that study used a truncation that may have affected the folding of adjacent domains to a greater extent than the single amino acid substitutions used in the present study.

Since both the NF-κB and JNK pathways signal through cascades of serine/threonine phosphorylation but TRAFs have no known enzymatic activity, it is generally thought that TRAFs function as adaptor molecules facilitating the recruitment of other proteins into an active signaling complex. In addition to the various receptor cytoplasmic tails, TRAFs have been shown to interact with over 10 distinct intracellular proteins, including TRADD, TRIP, RIP, TANK/I-TRAF, NIK, GCK, GCKR, A20, c-IAP1, c-IAP2, and Peg3/Pw1 (14, 15, 20, 25–30). It is not clear whether all of these TRAF-interacting proteins are present in a single complex involved in both NF-κB and JNK signaling, distinct complexes mediate these two signals, or one complex sends both signals, whereas a subset of this complex can activate a single pathway (e.g. JNK alone). The results of the present study tend to support one of the latter models; however, resolution of this issue awaits further experimentation.

Interestingly, all of the known TRAF-interacting proteins...
FIG. 5. TRAF 2A is an activator of JNK but not NF-κB. A. diagrammatic representation of full-length TRAF 2 and TRAF 2A, a splice variant of TRAF 2 with a 7-amino acid insertion in the ring finger. B. transfection conditions were similar to those described in the experimental procedures. Top panel, luciferase assays; middle panel, JNK kinase assays; bottom panel, Western blot of cytoplasmic extracts probed with anti-TRAF 2 (N19) Ab indicating expression levels of TRAFs 2 and 2A. Luciferase activities represent the results of three separate experiments.

associate with TRAFs through the C-terminal TRAF domain; however, we have demonstrated that the N-terminal domains of TRAFs play essential roles in the activation and branching of the NF-κB and JNK pathways. One possible explanation for this is that the N-terminal portion of TRAFs may determine the overall conformation of the entire protein and thus dictate the composition of the signaling complex that is formed. Alternatively, the N-terminal ring finger and zinc finger domains of TRAFs may participate in as yet uncharacterized protein-protein interactions important for the activation of downstream pathways, and differential interactions with these domains may determine specificity in TRAF-mediated NF-κB or JNK activation.

Finally, the present study demonstrates that TRAF 2A is the first naturally occurring pathway-restricted TRAF. This recently identified TRAF 2 splice variant is the first alternatively spliced TRAF family member to be described (23). TRAF 2A is unable to activate NF-κB when compared with TRAF 2; however, as demonstrated above, it is a strong activator of JNK. Interestingly, recent studies indicate that cells from TRAF 2-deficient mice (which should also be deficient in TRAF 2A) are unable to activate JNK in response to TNF, but NF-κB activation is only delayed (31).

TNF is a pleiotropic cytokine with widely varying effects in various tissues. Since TNFR 1 is expressed in nearly all tissues and leads to the activation of multiple signal transduction pathways, the existence of alternatively spliced, pathway-specific TRAFs with different levels of expression in different tissues suggests a novel level at which TNF signaling can be regulated in a tissue-specific manner. Both TRAFs 2 and 2A are expressed in a wide variety of tissues; however, the ratio of TRAF 2A mRNA to that of TRAF 2 varies between tissues with very low levels in the brain, lung, and heart and relatively higher levels in spleen (23). Regulation of signaling at this level may apply to all members of the TNFR superfamily that signal through TRAFs.

It is important to explore factors regulating the splicing of TRAF 2 and thus the ratio of TRAF 2A to TRAF 2 in a given tissue at a given developmental stage. In addition, it will be important to identify any other TRAF splice variants which mediate pathway-specific signals, perhaps including some with NF-κB-specific activity.

Acknowledgments—We thank Dr. Jun-ichiro Inoue for providing mouse TRAF5 cDNA and Drs. Stephen Smale and Daniel Kaufman for their helpful suggestions regarding this manuscript.

REFERENCES
1. Smith, C. A., Farrah, T., and Goodwin, R. G. (1994) Cell 76, 959–962
2. Simonet, W., Lacey, D., Dunstan, C., Kelley, M., Chang, M., Luthy, R., Nguyen, H., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colombi, A., Tan, H., Traill, G., Sullivan, J., Davy, E., Bucay, N., Renshaw-Gegg, L., Hughes, T., Hill, D., Pattison, W., Campbell, P., and Boyle, W. (1997) Cell 89, 309–319
3. Karin, M., Liu, Z., and Zandi, E. (1997) Curr. Opin. Cell Biol. 9, 240–246
4. Bauereuer, P., and Baltimore, D. (1996) Cell 87, 13–20
5. Rothe, M., Wong, S. C., Henzel, W. J., and Goeddel, D. V. (1994) Cell 78, 681–692
6. Hu, H. M., O’fourke, K., Boguski, M. S., and Dixit, V. M. (1994) J. Biol. Chem. 269, 30069–30072
7. Cheng, G., Cleary, A. M., Ye, Z., Hong, D. I., Lederman, S., and Baltimore, D. (1995) Science 267, 1484–1488
8. Mosialos, G., Birkenbach, M., Yalamanchili, R., VanArsdale, T., Ware, C., and Kieff, E. (1995) Cell 80, 389–399
9. Sato, T., Irie, S., and Reed, J. C. (1995) FEMS Lett. 358, 113–118
10. Regnier, C., Temaseetto, C., Mogo-Lutz, C., Chenard, M., Wendling, C., Basset, P., and Rio, M. (1995) J. Biol. Chem. 270, 25715–25721
11. Ishida, T., Tojo, A., Ashikaga, S., Ohara, T., Watanabe, T., Yamamoto, T., and Inoue, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9437–9442
12. Nakano, H., Oshima, H., Chung, W., Williams-Abbott, L., Ware, C., Yagita, H., and Okumura, K. (1996) J. Biol. Chem. 271, 14661–14664
13. Cao, Z., Xiong, J., Takeuchi, M., Kurama, T., and DV, G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4947–4952
14. Hsu, H., Shu, H., Pan, M., and Goeddel, D. (1996) Cell 84, 299–308
15. Hsu, H., Huang, J., Shu, H., Baichwal, V., and Goeddel, D. (1996) Immunity 4, 387–396
16. Akiba, H., Nakano, H., Nishinaoka, S., Shindo, M., Kobata, T., Atsuta, M., Morimoto, C., Ware, C., Matlin, N., Wallach, D., Yagita, H., and Okumura, K. (1998) J. Biol. Chem. 273, 13353–13358
17. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995) Science 269, 1424–1427
Distinct TRAF Domains for NF-κB Versus JNK Activation

18. Song, H., Regnier, C., Kirschning, C., Goeddel, D., and Rothe, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9792–9796
19. Higuchi, R., Krummel, B., and Saiki, R. (1988) Nucleic Acids Res. 16, 7351–7367
20. Cheng, G., and Baltimore, D. (1996) Genes Dev. 10, 963–973
21. Hibi, M., Lin, A., Smeal, T., Minden, A., and Karin, M. (1993) Genes Dev. 7, 2135–2148
22. Takeuchi, M., Rothe, M., and Goeddel, D. (1996) J. Biol. Chem. 271, 19935–19942
23. Brink, R., and Lodish, H. (1998) J. Biol. Chem. 273, 4129–4134
24. Liu, Z., Hsu, H., Goeddel, D., and Karin, M. (1997) Cell 87, 565–576
25. Lee, S., Lee, S., and Choi, Y. (1997) J. Exp. Med. 185, 1275–1285

26. Rothe, M., Xiong, J., Shu, H., Williamson, K., Goddard, A., and Goeddel, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8241–8246
27. Rothe, M., Pan, M., Henzel, W., Ayres, T., and Goeddel, D. (1995) Cell 7, 1243–1252
28. Relaix, F., Wei, X., Wu, X., and Sassoon, D. (1998) Nat. Genet. 18, 287–291
29. Song, H., Rothe, M., and Goeddel, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6721–6725
30. Shi, C., and Kehrl, J. (1997) J. Biol. Chem. 272, 32102–32107
31. Yeh, W., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J., Ferrick, D., Hum, B., Iscove, N., Ohashi, P., Rothe, M., Goeddel, D., and Mak, T. (1997) Immunity 7, 715–725