Tumor necrosis factor (TNF) receptor-associated factors (TRAFs) are critical signaling adaptors downstream of many receptors in the TNF receptor and interleukin-1 receptor/Toll-like receptor superfamilies. Whereas TRAF2, 5, and 6 are activators of the canonical NF-κB signaling pathway, TRAF3 is an inhibitor of the noncanonical NF-κB pathway. The contribution of the different domains in TRAFs to their respective functions remains unclear. To elucidate the structural and functional specificities of TRAF3, we reconstituted TRAF3-deficient cells with a series of TRAF3 mutants and assessed their abilities to restore TRAF3-mediated inhibition of the noncanonical NF-κB pathway as measured by NF-κB-inducing kinase (NIK) protein levels and processing of p100 to p52. We found that a structurally intact RING finger domain of TRAF3 is required for inhibition of the noncanonical NF-κB pathway. In addition, the three N-terminal domains, but not the C-terminal TRAF domain, of the highly homologous TRAF5 can functionally replace the corresponding domains of TRAF3 in suppression of the noncanonical NF-κB pathway. This functional specificity correlates with the specific binding of TRAF3, but not TRAF5, to the previously reported TRAF3 binding motif in NIK. Our studies suggest that both the RING finger domain activity and the specific binding of the TRAF domain to NIK are two critical components of TRAF3 suppression of NIK protein levels and the processing of p100 to p52.
Activation of the noncanonical NF-κB pathway requires NF-κB-inducing kinase (NIK) to induce the processing of p100 (or NF-κB2) to p52 (28). Then p52-associated NF-κB complexes, predominantly p52-ReIB heterodimers, are translocated into the nucleus and activate transcription of genes with preference functions in lymphoid organogenesis (14, 28). The mechanism by which TRAF3 negatively regulates the noncanonical NF-κB pathway is unclear. Several studies have suggested that TRAF3 suppresses p100 processing by inducing NIK degradation. TNF receptor activation of the noncanonical NF-κB pathway may involve inhibition of this TRAF3 function, allowing for an increase in NIK protein levels and resulting in processing of p100 to p52 via the proteasome (22, 24).

In this study, we explored the structural and functional specificities between TRAF3 and its close homologue TRAF5 in suppressing NIK protein levels and p100 processing to p52. We reconstituted TRAF3−/− mouse embryonic fibroblasts (MEFs) with a series of TRAF3 mutants including TRAF3/5 chimeric molecules and then examined TRAF3-mediated suppression of p100 processing. We report that both the RING finger and TRAF domains of TRAF3 are essential for its negative regulation of NIK protein levels and p100 processing. In addition, the TRAF domain of TRAF3 confers its specificity in suppression of the noncanonical NF-κB pathway by its association with the TRAF3 binding motif (T3BM) within NIK.

EXPERIMENTAL PROCEDURES

Reagents—Anti-p100/p52 and anti-NIK antibodies were purchased from Cell Signaling Technology (Beverly, MA); anti-FLAG (M2) and anti-GST (B-14) antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). Anti-FLAG (M2) and anti-β actin antibodies were obtained from Sigma. The anti-hemagglutinin (16B12) antibody was purchased from Covance (Berkeley, CA).

Constructs—GST-NIK(T3BM) was constructed as previously described (29). Briefly, synthetic DNA oligos corresponding to amino acid residues 73–94 of human NIK (GenBankTM accession number Y10256) containing the TRAF3 binding motif were annealed and ligated into the BamH1 and EcoRI sites of pGEX-2T. Murine TRAF3 (GenBankTM accession number Y10256), TRAF5 (GenBankTM accession number D78141), and mutant constructs were generated as described previously (8). Briefly, TRAF3 deletion mutants were cloned into pBABE-puro (pBABEpuro) or pBABE-puro-TAP (Tandem Affinity Purification tag) retroviral vector with an N-terminal FLAG tag. TRAF3/TRAF5 chimeras were constructed using a QuikChange kit (Stratagene) and the numbering below each domain indicates the predicted amino acid residues for the RING finger, zinc fingers, isoleucine zipper, and TRAF domains. B. TRAF3−/− MEFs were reconstituted with the pBABEpuro-TAP-FLAG or pBABEpuro-TAP-FLAG-TRAF3 constructs described in panel A. Basal p100 processing to p52 and expression of each TRAF3 construct in whole cell extracts were detected by immunoblotting. * indicates expression of each FLAG-tagged TRAF3 construct. Total β actin is shown as a loading control.

FIGURE 1. The N terminus of TRAF3 is required for negative regulation of p100 processing. A. diagram of full-length TRAF3 and N terminus truncation mutant constructs. The numbering below each domain indicates the predicted amino acid residues for the RING finger, zinc fingers, isoleucine zipper, and TRAF domains. B. TRAF3−/− MEFs were reconstituted with the pBABEpuro-TAP-FLAG or pBABEpuro-TAP-FLAG-TRAF3 constructs described in panel A. Basal p100 processing to p52 and expression of each TRAF3 construct in whole cell extracts were detected by immunoblotting. * indicates expression of each FLAG-tagged TRAF3 construct. Total β actin is shown as a loading control.
GST-NIK(T3BM) for 2 h. Glutathione beads were then washed and incubated for 1.5 h with lysates of 293T cells expressing the indicated TRAF3 or TRAF5 constructs. After washing, proteins were eluted from the beads and separated by 10% SDS-PAGE.

RESULTS

Intact RING Finger and TRAF Domains of TRAF3 Are Required for Suppression of p100 Processing—Recently, TRAF3 has been shown to be an essential negative regulator of p100 processing to p52 (22). To map the domain(s) in TRAF3 that are required for this function, we generated TRAF3 mutants with successive truncations at the N terminus (Fig. 1A). These TRAF3 mutant constructs were cloned into the low expression pBABE retroviral vector and were transduced into TRAF3/H11002/H11002 MEFs. We then assessed the ability of TRAF3 mutants to suppress noncanonical NF-κB activity by immunoblotting for p100 and p52 protein in the reconstituted TRAF3/H11002/H11002 MEFs. As seen in Fig. 1B, whereas wild-type MEFs showed low basal levels of p52, TRAF3−/− MEFs showed constitutive, high levels of p100 processing to p52. Reconstitution of TRAF3−/− MEFs with the full-length TRAF3 restored basal p100 processing levels. However, reconstitution with the N-terminal deletion mutants of TRAF3 failed to reduce the high basal p52 levels in TRAF3−/− MEFs to wild-type levels. All these truncation mutants lack the N-terminal sequence (amino acid residues 1–107) of TRAF3, indicating that this region is required for the negative regulation of p100 processing.

Amino acid residues 1–107 of TRAF3 contain a RING finger domain that is thought to form a conformation necessary for zinc chelation. To determine whether an intact RING finger structure is required for TRAF3 to suppress p100 processing, we mutated the highly conserved cysteines and a histidine to alanines (C52A/C55A and C67A/H69A) to generate two point mutations in TRAF3 that disrupt the zinc-chelating positions of the RING finger (Fig. 2A). As shown in Fig. 2B, TRAF3−/− MEFs reconstituted with either TRAF3 (C52A/C55A) or TRAF3 (C67A/H69A) showed high basal p52 levels, like the TRAF3−/− MEFs reconstituted with the vector control. Thus, these two RING finger mutants could not restore the TRAF3-mediated suppression of basal p100 processing to p52 to wild-type levels. Collectively, these data suggest that proper folding of the RING finger domain is critical for TRAF3-mediated suppression of the noncanonical NF-κB pathway.
Previously, TRAF3 association with NIK via the TRAF domain was shown to be critical for suppression of p100 processing in a 293T cell line under conditions of overexpression (24). To further determine the role of the TRAF domain of TRAF3 in suppression of p100 processing, we reconstituted TRAF3^{-/-} MEFs with a TRAF3 construct lacking the TRAF domain (Fig. 3A, T3(1–300)). As seen in Fig. 3B, TRAF3^{-/-} MEFs reconstituted with the T3(1–300) mutant are unable to reduce the high basal p52 levels to the wild-type levels, indicating that this region containing the NIK binding site is indeed required for TRAF3-mediated suppression of p52 activity.

**Specificity of TRAF3 in Negative Regulation of p100 Processing**

Among all the TRAF family members, TRAF5 shares the same domain organization as TRAF3 (1). However, TRAF5 cannot functionally replace TRAF3 in suppressing p100 processing using our reconstitution assay (Fig. 4A), implying that different functions exist for the domains in these two TRAF members. To determine which domains of TRAF3 and TRAF5 are functionally interchangeable, we reconstituted TRAF3^{-/-} MEFs with a series of TRAF3/5 chimeric molecules where individual domains of TRAF3 are substituted with the corresponding domain of TRAF5 (Fig. 4B). As shown in Fig. 4C, reconstitution of TRAF3^{-/-} MEFs with the TRAF3 chimeras containing the RING finger, zinc fingers, or isoleucine zipper of TRAF5 could reduce the high basal p100 processing to p52. Interestingly, the TRAF3/5TD chimera containing the TRAF domain of TRAF5 could not suppress the high basal p52 levels in the TRAF3^{-/-} MEFs, indicating that the TRAF domain of TRAF3 is functionally distinct from that of TRAF5 for its ability to negatively regulate p100 processing. To confirm this observation, we replaced the TRAF domain of TRAF5 with the corresponding region of TRAF3 and found that this mutant TRAF5/3TD could now suppress constitutive p100 processing in TRAF3^{-/-} MEFs (Fig. 4C). Thus, our studies have demonstrated that the difference in the functional specificity between TRAF3 and TRAF5 in negatively regulating the noncanonical NF-κB pathway is conferred by the C-terminal TRAF domain of TRAF3.

**Conclusion**

The study demonstrates that the TRAF domain of TRAF3 plays a crucial role in the negative regulation of p100 processing, specifically through its interaction with NIK. The differential functional specificity between TRAF3 and TRAF5 in the NF-κB pathway is attributed to differences in their TRAF domains. These findings have implications for understanding the regulatory mechanisms of the NF-κB pathway and could guide future therapeutic strategies.
the functional specificity between the TRAF domain of TRAF3 and TRAF5 is that TRAF5 cannot associate with NIK at the T3BM. To test this hypothesis, we constructed GST fusion proteins with a 22-amino acid peptide encompassing the T3BM sequence (Fig. 5). We then performed a GST pulldown assay on TRAF3 and TRAF5. As shown in Fig. 5, unlike TRAF3, TRAF5 cannot associate with NIK(T3BM).

The RING Finger and TRAF Domains of TRAF3 Are Required for Suppression of NIK Protein Levels—We have previously demonstrated that TRAF3-/- cells have an accumulation of NIK protein and that NIK is responsible for the constitutive p100 processing in TRAF3-/- MEFs (22). In the current study, we have concluded that both the RING and TRAF domains of TRAF3 are required for suppression of p100 processing to p52. To test whether these domains are also important for suppression of NIK protein levels, we compared NIK expression in TRAF3-/- MEFs reconstituted with the TRAF3 RING finger mutants or the TRAF3/5 chimeras. As shown in Fig. 6A, reconstitution with full-length TRAF3, but not the vector control, suppressed the high NIK levels in TRAF3-/- MEFs. However, the TRAF3 RING finger mutants (C52A/C55A and C67A/H69A) failed to suppress NIK levels to the same extent as the full-length TRAF3, suggesting that an intact RING finger domain is important for inducing NIK degradation. Similarly, reconstitution of the TRAF3-/- MEFs with the TRAF3/5TD mutant or TRAF5 showed high NIK protein levels comparable with those of the vector control. However, TRAF3-/- MEFs reconstituted with TRAF3/5 chimeras T3/5RD, ZF, IZ, and T5/3TD showed greatly reduced NIK protein levels comparable with the cells reconstituted with the full-length TRAF3 (Fig. 6B). Moreover, NIK expression levels in these reconstituted cells correlated well with the amount of p100 processing to p52 (Fig. 6, A and B, top two panels). Therefore, the RING finger and TRAF domains of TRAF3, which are critical for inhibiting p100 processing, are also important for suppressing NIK protein levels.

DISCUSSION

The structural similarities between TRAF proteins prompt the interesting question of the specificity of each TRAF protein in regulation of downstream signal transduction pathways. A specific example is that TRAF5 activates the canonical NF-kB pathway, whereas its closest structural homologue, TRAF3, functions as a negative regulator of the nonca-

FIGURE 5. TRAF3, but not TRAF5, interacts with the T3BM of NIK. Whole cell extract containing hemagglutinin-(HA)-TRAF3 or HA-TRAF5 was subjected to GST pulldown assay using GST alone or GST fused to the indicated 22-amino acid peptide that contains the T3BM sequence (bold letters) of NIK as a bait. Amounts of GST, GST-T3BM, HA-TRAF3, and HA-TRAF5 used in the assay were monitored by immunoblotting.

FIGURE 6. Both the TRAF and RING finger domains are required for TRAF3 to negatively regulate NIK expression. TRAF3-/- MEFs were reconstituted with pBABEpuro-TAP-FLAG, pBABEpuro-TAP-FLAG-TRAF3 or TRAF3 RING domain mutant constructs (A) and pBABEpuro-TAP-FLAG -TRAF5 or TRAF3/5 chimeric molecules (B). Basal NIK and p100/p52 protein levels and expression of each reconstituted TRAF molecule in whole cell extracts were detected by immunoblotting. Whole cell extracts from NIK-/- and NIK-/- MEFs incubated with 25 μM MG132 for 2 h were served as a control for NIK expression. Total β actin is shown as a loading control. Blots of p100 and p52 were quantitated, and the band intensities were normalized to background. The resulting relative p100 and p52 protein levels were calculated as p52/p100 ratios and presented in the bar graph.
nonical NF-κB pathway. In this study, we determined that the N-terminal domains of TRAF5, but not the C-terminal TRAF domain, are functionally interchangeable with those of TRAF3 in suppression of NIK protein levels and p100 processing. Furthermore, we clearly showed that an intact RING finger domain of TRAF3 is required for the suppression of the noncanonical NF-κB pathway. Therefore, the TRAF and RING finger domains are two important determinants for the role and the specificity of TRAF3 in its negative regulation of the noncanonical NF-κB pathway.

By reconstituting TRAF3<sup>−/−</sup> MEFs with two TRAF3 mutants (C52A/C55A and C67A/H69A), we observed that an intact RING finger domain is required for TRAF3 to suppress p100 processing to p52. The RING finger domain is a common motif found in many E3 ubiquitin ligases, including TRAF2 and TRAF6 (18, 31). Thus, it is tempting to speculate that TRAF3 is also an E3 ubiquitin ligase, particularly considering that TRAF3 has been hypothesized to trigger NIK ubiquitination and degradation (24). However, thus far, we and other groups have been unable to demonstrate by in vitro assays that TRAF3 acts as an E3 ubiquitin ligase for NIK (24). Based on the previous work in isolating the TRAF6 ubiquitin ligase complex, there may be additional components that are required for NIK ubiquitination (18, 32). In support of this, Grech et al. (33) have reported that TRAF2<sup>−/−</sup> B cells exhibit constitutive p100 processing to p52, suggesting that TRAF2 is also a negative regulator of the noncanonical NF-κB pathway. We have examined whether TRAF2 can functionally replace TRAF3 in the suppression of p100 processing by retrovirally transducing TRAF2 into TRAF3<sup>−/−</sup> MEFs. As shown in supplemental Fig S1, TRAF3<sup>−/−</sup> MEFs with overexpression of TRAF2 still display high NIK protein levels and constitutive processing of p100 to p52. Hence, TRAF2 and TRAF3 perform distinct roles in suppressing the noncanonical NF-κB pathway. Furthermore, Act1 and TNAP, two other intracellular proteins known to associate with TRAF2 and TRAF3, have been shown to suppress CD40- and NIK-mediated p100 processing (34, 35). How TRAF2, Act1, or TNAP negatively regulate the noncanonical NF-κB pathway and whether any of these molecules participate in TRAF3-mediated suppression of p100 processing has yet to be elucidated. Moreover, the mechanism by which TNF receptors such as CD40, BAFFR, and LTβR lead to activation of NIK remains controversial. Some reports suggest that CD40 and BAFF stimulation of B cell lines induces TRAF3 degradation, thereby allowing NIK accumulation and activation of p100 processing (24). However, this observation is inconsistent in the case of LTβR stimulation of MEFs or CD40 and BAFF stimulation of primary B cells where TRAF3 levels are not significantly reduced upon receptor ligation and induction of p100 processing to p52 (33, 36). These discrepancies point to the need for further experimentation to decipher the mechanism by which TRAF3 negatively regulates the noncanonical NF-κB pathway during homeostasis as well as during cell activation by TNF receptors.

By reconstituting TRAF3<sup>−/−</sup> MEFs with TRAF3/5 chimeras, we have determined that the TRAF domain is the structural feature that differentiates the functionality between TRAF3 and TRAF5. In support of the significance and specificity of the TRAF domain of TRAF3, we observed that TRAF3, but not TRAF5, associates with the TRAF3 binding motif in NIK. These data correspond to the study by Liao et al. (24) that showed that the binding of NIK and TRAF3 at the TRAF domain is required for TRAF3 to induce NIK degradation. Together, this evidence suggests that a specific TRAF3 interaction with NIK is critical for regulating basal protein levels of NIK. Moreover, recent studies indicate that TRAF3 is also a positive regulator of the interferon pathway (6–8). Thus, TRAF3 functions not only as an essential regulator of basal noncanonical NF-κB activity but also as a critical mediator of the cellular response to viral infections. Interestingly, similar structural requirements and specificity were observed for TRAF3 in both of these functions (8). Thus, it is unclear what mechanism directs TRAF3 to act as an activator of the type 1 interferon pathway or an inhibitor of the noncanonical NF-κB pathway. Future characterization of endogenous TRAF3 complexes involved in these two distinct pathways will provide us with a better understanding of the role of TRAF3 in the antiviral response and could possibly identify novel targets to treat immune disorders and cancers caused by constitutive activation of the noncanonical NF-κB pathway (28, 37, 38).

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REFERENCES

1. Grech, A., Quinn, R., Srinivasan, D., Badoux, X., and Brink, R. (2000) Mol. Immunol. 37, 721–734
2. Wajant, H., Muhlenbeck, F., and Scheurich, P. (1998) J. Mol. Evol. 47, 656–662
3. Dempsey, P. W., Doyle, S. E., He, J. Q., and Cheng, G. (2003) Cytokine Growth Factor Rev. 14, 193–209
4. Chung, J. Y., Park, Y. C., Ye, H., and Wu, H. (2002) J. Cell Sci. 115, Pt. 4, 679–688
5. Inoue, J., Ishida, T., Tsukamoto, N., Kobayashi, N., Naito, A., Azuma, S., and Yamamoto, T. (2000) Exp. Cell Res. 254, 14–24
6. Hacker, H., Redecke, V., Blagoev, B., Kratchmarova, I., Hsu, L.-C., Wang, G. G., Kamps, M. P., Raz, E., Wagner, H., Hacker, G., Mann, M., and Karin, M. (2006) Nature 439, 204–207
7. Oganesyan, G., Saha, S. K., Guo, B., He, J. Q., Shahangan, A., Zarnegar, B., Perry, A., and Cheng, G. (2006) Nature 439, 208–211
8. Saha, S. K., Pietras, E. M., He, J. Q., Kang, J. R., Liu, S. Y., Oganesyan, G., Shahangan, A., Zarnegar, B., Shiba, T. L., Wang, Y., and Cheng, G. (2006) EMBO J. 25, 3257–3263
9. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995) Science 269, 1424–1427
10. Ishida, T. K., Tojo, T., Aoki, T., Kobayashi, N., Ohishi, T., Watanabe, T., Yamamoto, T., and Inoue, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9437–9442
11. Nakano, H., Oshima, H., Chung, W., Williams-Abbott, L., Ware, C. F., Yagita, H., and Okumura, K. (1996) J. Biol. Chem. 271, 14661–14664
12. Cao, Z., Xiong, J., Takeuchi, M., Kurama, T., and Goeddel, D. V. (1996) Nature 383, 443–446
13. Hayden, M. S., and Ghosh, S. (2004) Genes Dev. 18, 2195–2224
14. Bonizzi, G., and Karin, M. (2004) Trends Immunol. 25, 280–288
15. Arch, R. H., Gedrich, R. W., and Thompson, C. B. (1998) Genes Dev. 12, 2821–2830
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16. Takeuchi, M., Rothe, M., and Goeddel, D. (1996) *J. Biol. Chem.* **271**, 19935–19942
17. Brink, R., and Lodish, H. (1998) *J. Biol. Chem.* **273**, 4129–4134
18. Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C., and Chen, Z. J. (2000) *Cell* **103**, 351–361
19. Tsitsikov, E. N., Laouini, D., Dunn, I. F., Sannikova, T. Y., Davidson, L., Alt, F. W., and Geha, R. S. (2001) *Immunity* **15**, 647–657
20. Dadgostar, H., and Cheng, G. (1998) *J. Biol. Chem.* **273**, 24775–24780
21. Song, H. Y., Regnier, C. H., Kirschning, C. J., Goeddel, D. V., and Rothe, M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 9792–9796
22. He, J. Q., Zarnegar, B., Oganesyan, G., Saha, S. K., Yamazaki, S., Doyle, S. E., Dempsey, P. W., and Cheng, G. (2006) *J. Exp. Med.* **203**, 2413–2418
23. Hauer, J., Puschner, S., Ramakrishnan, P., Simon, U., Bongers, M., Federle, C., and Engelmann, H. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 2874–2879
24. Liao, G., Zhang, M., Harhaj, E. W., and Sun, S. C. (2004) *J. Biol. Chem.* **279**, 26243–26250
25. Claudio, E., Brown, K., Park, S., Wang, H., and Siebenlist, U. (2002) *Nat. Immunol.* **3**, 958–965
26. Coope, H. J., Atkinson, P. G., Huhe, B., Belich, M., Janzen, J., Holman, M. J., Klaus, G. G., Johnston, L. H., and Ley, S. C. (2002) *EMBO J.* **21**, 5375–5385
27. Dejardin, E., Droin, N. M., Delhase, M., Haas, E., Cao, Y., Makris, C., Li, Z. W., Karin, M., Ware, C. F., and Green, D. R. (2002) *Immunity* **17**, 525–535
28. Dejardin, E. (2006) *Biochem. Pharmacol.* **72**, 1161–1179
29. Li, C., Ni, C. Z., Havert, M. L., Cabezas, E., He, J., Kaiser, D., Reed, J. C., Satterthwait, A. C., Cheng, G., and Ely, K. R. (2002) *Structure (Camb.)* **10**, 403–411
30. Cheng, G., and Baltimore, D. (1996) *Genes Dev.* **10**, 963–973
31. Pickart, C. M. (2001) *Annu. Rev. Biochem.* **70**, 503–533
32. Sun, L., Deng, L., Ea. C. K., Xia, Z. P., and Chen, Z. J. (2004) *Mol. Cell* **14**, 289–301
33. Grech, A. P., Amesbury, M., Chan, T., Gardam, S., Basten, A., and Brink, R. (2004) *Immunity* **21**, 629–642
34. Qian, Y., Qin, J., Cui, G., Naramura, M., Snow, E. C., Ware, C. F., Fairchild, R. L., Omori, S. A., Rickert, R. C., Scott, M., Kotzin, B. L., and Li, X. (2004) *Immunity* **21**, 575–587
35. Hu, W.-H., Mo, X.-M., Walters, W. M., Brambilla, R., and Bethea, J. R. (2004) *J. Biol. Chem.* **279**, 35975–35983
36. Kim, Y. S., Nedospasov, S. A., and Liu, Z. G. (2005) *Mol. Cell. Biol.* **25**, 2130–2137
37. Mackay, F., Woodcock, S. A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., Tschopp, J., and Browning, J. L. (1999) *J. Exp. Med.* **190**, 1697–1710
38. Ishikawa, H., Carrasco, D., Claudio, E., Ryseck, R. P., and Bravo, R. (1997) *J. Exp. Med.* **186**, 999–1014