one of the family of Gadd45 proteins, which are implicated in growth arrest, DNA-damage repair and programmed cell death[8,9]. De Smaele *et al.* showed that ectopic overexpression of Gadd45β in mouse-embryo fibroblasts (MEFs) and in NF-κB-deficient cell lines antagonizes TNF-α-induced cell death and increases cell survival. In addition, ectopic expression of antisense *gadd45β* messenger RNA, which presumably blocks Gadd45β expression, was found to decrease cell survival and prolong JNK activity; this is similar to the response in cells lacking NF-κB. The authors conclude[6] that TNF-α-mediated activation of NF-κB induces Gadd45β, which inhibits TNF-α-mediated cell death and JNK signalling and promotes cell survival; however, this conclusion contradicts an earlier study[10] that implicates Gadd45β as an activator of JNK.

We used *gadd45β*-null mice, in which the *gadd45β* gene is ablated (D.L. and A.F., unpublished results), to assess further the effect of *gadd45β* deficiency on TNF-α-mediated cellular responses, including cell survival and JNK signalling. We found that TNF-α induced *gadd45β* expression in wild-type but not in *gadd45β*-deficient MEFs (Fig. 1a). Like wild-type MEFs, *gadd45β*-deficient (*gadd45β*$^{-/-}$) MEFs were not susceptible to TNF-α-mediated cell death (Fig. 1b). However, in the presence of the protein-synthesis inhibitor cycloheximide, which prevents the expression of the pro-survival genes induced by NF-κB, both *gadd45β*$^{-/-}$ and wild-type MEFs were equally susceptible to TNF-α-mediated cell death.

Our findings indicate that *gadd45β* expression is not essential for the NF-κB pro-survival function. Furthermore, the kinetics of downregulation of JNK activity were similarly rapid in *gadd45β*$^{-/-}$ MEFs and in wild-type cells (Fig.1c), as well as in another cell type, splenic lymphocytes (data not shown).

Our results indicate that other NF-κB target genes[5,7] are more likely than *gadd45β* to be primary mediators of the survival function of NF-κB. The discrepancy between our observations and those of De Smaele *et al.*[6] might reflect limitations in their experimental approach — for example, ectopic overexpression of *gadd45β* or of its antisense RNA in cells stimulated with TNF-α might have affected cell survival and JNK activity in some indirect or nonspecific way. Further work is needed to assess what role, if any, Gadd45β has in the cell's response to TNF-α.

**Arshad Amanullah*†, Naiyer Azam*, Arthur Balliet*, Christine Hollander‡, Barbara Hoffman*, Albert Fornace Jr‡, Dan Liebermann***

**Fels Institute for Cancer Research and Molecular Biology and Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, USA*
*e-mail: lieberma@unix.temple.edu*
*‡Gene Response Section, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892-4255, USA*
*†Present address: HTB/Integrative Biology Initiative, GlaxoSmithKline, King of Prussia, Pennsylvania 19406, USA*

1. Baud, V. & Karin, M. *Trends Cell Biol.* **11,** 372–377 (2001).
2. Locksley, R. M., Killeen, N. & Lenardo, M. J. *Cell* **104,** 487–501 (2001).
3. Chen, G. & Goeddel, D.V. *Science* **296,** 1634–1635 (2002).
4. Kyriakis, J. M. *Nature* **414,** 265–266 (2001).
5. Karin, M. & Lin, A. *Nature Immunol.* **3,** 221–227 ( 2002).
6. De Smaele, E. *et al. Nature* **414,** 308–313 (2001).
7. Tang, G. *et al. Nature* **414,** 313–317 (2001).
8. Liebermann, D. A. & Hoffman, B. *Leukemia* **16,** 527–541 (2002).
9. Fornace, A. J. Jr, Jackman, J., Hollander, M. C., Hoffman-Liebermann, B. & Liebermann, D. A. *Ann. NY Acad. Sci.* **663,** 139–153 (1992).
10. Takekawa, M. & Saito, H. *Cell* **95,** 521–530 (1998).

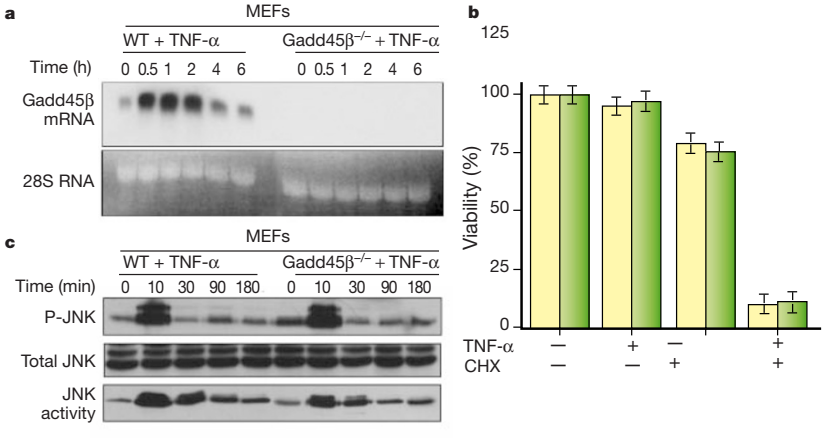


**Figure 1** Gadd45β deficiency does not alter TNF-α-mediated cell survival or JNK signalling. **a,** TNF-α induces expression of *gadd45β* in wild-type (WT) but not in *gadd45β*$^{-/-}$ mouse-embryo fibroblasts (MEFs). RNA from cells collected at different times after treatment with TNF-α (1,000 U ml$^{-1}$) was resolved on a 2% agarose–formaldehyde gel and analysed by northern blotting using a $^{32}$P-labelled *gadd45β* probe. 28S RNA, loading control. **b,** *gadd45β*$^{-/-}$ cells are not susceptible to TNF-α-mediated cell death in the absence of cycloheximide (CHX). Viability of WT (left bars) and *gadd45β*$^{-/-}$ MEFs (right bars) was determined by trypan-blue staining after 14-h treatment with TNF-α (1,000 U ml$^{-1}$), CHX (1 mg ml$^{-1}$) or both. **c,** Gadd45β is not required for rapid downregulation of JNK after TNF-α treatment. MEFs from WT and *gadd45β*$^{-/-}$ mice were treated with TNF-α (1,000 U ml$^{-1}$) and, at the indicated times, cell lysates were resolved by SDS–PAGE and western blotted (antibody probes: antiphospho-JNK, anti-JNK; Cell Signaling, Inc). A fusion protein of glutathione-*S*-transferase and c-Jun was used as substrate in the JNK assay (bottom).

*De Smaele* et al. *reply* — We and others have shown that the control of TNF-α-induced apoptosis by NF-κB/Rel transcription factors involves suppression of the JNK enzyme cascade[1–3], and we have proposed that this suppression is mediated in part by Gadd45β/Myd118 (refs 1,4). Amanullah *et al.* suggest that the ablation of *gadd45β* has no effect on JNK activation and apoptosis by TNF-α and argue that the protective effects of NF-κB are mediated by factors other than Gadd45β.

However, caution is needed in drawing inferences from these provocative findings about the role of Gadd45β in the cell. Under the conditions used by Amanullah *et al.*, knockout mutation of any of the NF-κB targets identified so far[5,6] — including those of the putative JNK inhibitor XIAP (ref. 7) and of NF-κB/RelA itself[8] (our unpublished observations) — would not have affected TNF-α-induced killing. This is because cytokine treatment of fibroblasts was far too short and was performed in the absence of low doses of cycloheximide (about 0.1 μg ml$^{-1}$; ref. 1) , which is needed to downregulate functionally redundant factors.

Our antisense experiments[1] indicate that in certain cells, such as lymphoid cell lines, downregulation of *gadd45β* leads to exaggerated JNK signalling and apoptosis in response to TNF-α. It is likely that the pro-survival programme that is activated by NF-κB depends on tissue-specific elements[5,6], so the relevance of Gadd45β to this protective activity of NF-κB might be more marked in certain cell types. As the analysis of Amanullah *et al.* is limited to the fibroblastoid lineage, it might not be appropriate to generalize conclusions about the effects of Gadd45β on the JNK pathway and apoptosis to other cell types. We agree with Amanullah *et al.* that further investigation is needed to define the precise contribution of this factor and of other targets to the anti-apoptotic function of NF-κB.

**Francesca Zazzeroni, Salvatore Papa, Enrico De Smaele*, Guido Franzoso**

*Ben May Institute for Cancer Research, and Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, Illinois 60637, USA*
*e-mail: gfranzos@midway.uchicago.edu*
**Present address: Department of Experimental Medicine and Pathology, University of Rome 'La Sapienza', 00161 Rome, Italy*

1. De Smaele, E. *et al. Nature* **414,** 308–313 (2001).
2. Tang, G. *et al. Nature* **414,** 313–317 (2001).
3. Javelaud, D. & Besancon, F. *Oncogene* **20,** 4365–4372 (2001).
4. Franzoso, G., Zazzeroni, F. & Papa, S. *Cell Death Diff.* **10,** 13–15 (2003).
5. Baldwin, A. S. *J. Clin. Invest.* **107,** 241–246 (2001).
6. Karin, M. & Lin, A. *Nature Immunol.* **3,** 221–227 (2002).
7. Harlin, H., Reffey, S. B., Duckett, C. S., Lindsten, T. & Thompson, C. B. *Mol. Cell. Biol.* **21,** 3604–3608 (2001).
8. Beg, A. A. & Baltimore, D. *Science* **274,** 782–784 (1996).