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and some of them can be entirely bypassed when there are high concentrations of Cu salts in the medium. Thus, yeast strains missing the gene for Cox17 cannot respire in normal growth media because CCO is Cu deficient, but are rescued when the medium is made 0.4% $CuSO_4$ (*2*). Likewise, increasing the Cu concentration in the medium allows Cu to be delivered to Fet3 in yeast strains missing the gene for Atx1 (*1*). These results indicate that neither Cox17 nor Atx1 is required for proper Cu trafficking when Cu levels are high and that their presence is not absolutely required to detoxify Cu. The observation that high levels of Cu in the growth medium can under some circumstances be beneficial to yeast is counterintuitive but reminiscent of the ability of similarly high levels of Cu to enable strains lacking CuZnSOD to grow well in air, as long as metallothionein is present (*11*). Metallothionein is a key player in Cu detoxification for yeast, probably acting as a Cu buffer to keep intracellular "free" Cu concentrations low; its synthesis is induced by Cu.

Multiple Cu-binding equilibria must be present in the cell, since Cu-binding proteins such as metallothionein, Atx1, Cox17, and Lys7 are all cytosolic and must presumably compete for available Cu. It will be interesting to learn to what extent this competition regulates cellular Cu distribution, and whether competitive success is determined by kinetic or thermodynamic factors. Although many questions remain, the convergence of chemistry, biochemistry, and genetics on this problem has set the stage for a complete understanding of cellular copper and iron metabolism, from the molecular details of the intermolecular transfer reactions to the genetic control of the relevant proteins in both yeast and humans.

References

1. S. J. Lin, R. A. Pufahl, A. Dancis, T. V. O'Halloran, V. C. Culotta, *J. Biol. Chem.* **272**, 9215 (1997).
2. D. M. Glerum, A. Shtanko, A. Tzagoloff, *ibid.* **271**, 14504 (1996).
3. R. A. Pufahl *et al.*, *Science* **278**, 853 (1997).
4. V. C. Culotta *et al.*, *J. Biol. Chem.* **272**, 23469 (1997).
5. A. Dancis, D. Haile, D. S. Yuan, R. D. Klausner, *ibid.* **269**, 25660 (1994).
6. D. S. Yuan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2632 (1995).
7. L. W. J. Klomp *et al.*, *J. Biol. Chem.* **272**, 9221 (1997).
8. R. Amaravadi, D. M. Glerum, A. Tzagoloff, *Hum. Genet.* **99**, 329 (1997).
9. I. H. Hung *et al.*, *J. Biol. Chem.* **272**, 21461 (1997).
10. B. Zhou, and J. Gitschier, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7481 (1997).
11. K. T. Tamai, E. B. Gralla, L. M. Ellerby, J. S. Valentine, D. J. Thiele, *ibid.* **90**, 8013 (1993).
12. D. M. Glerum, A. Shtanko, A. Tzagoloff, *J. Biol. Chem.* **271**, 20531 (1996).

SIGNAL TRANSDUCTION

Catalysis by a Multiprotein IκB Kinase Complex

Tom Maniatis

The transcriptional activator protein NF-κB mediates key immune and inflammatory responses (*1*). NF-κB, present in the cytoplasm of most cell types, is normally bound to a member of a family of inhibitor proteins known as IκB (Inhibitor κB). The best-characterized member of this family, IκB-α, binds the p50/p65 heterodimer of NF-κB in the cytoplasm. When cells are exposed to inducers of NF-κB, such as the cytokines tumor necrosis factor–α (TNF-α) or interleukin-1 (IL-1), two serine residues of IκB-α (Ser^{32} and Ser^{36}) are specifically phosphorylated. This phosphorylation is a signal for ubiquitination and degradation of IκB-α by the 26S proteasome (*2*, *3*). NF-κB is thus released to translocate to the nucleus and activate transcription of target genes.

Because NF-κB can be activated by an extraordinarily large number of different signals, ranging from ultraviolet irradiation to T cell activation (*1*), the mechanism by which these signals converge on IκB-α is of wide interest. During the past 2 years, many components from certain signaling pathways that lead to activation of NF-κB (*3*, *4*) have been described. The recent identification of a high molecular mass IκB kinase complex (*5*) and the identification of two unusual IκB kinases, reported in this issue on pages 860 and 866 (*6*, *7*), and elsewhere (*8*, *9*) now provide a framework for resolving the problem of integrating multiple NF-κB signaling pathways.

A number of cell-surface proteins interact specifically with the intracellular domains of the TNF-α and IL-1 receptors and are intermediates in the activation of NF-κB (*3*, *4*). One of these, TRAF2, is recruited to the TNF-α receptor and another, TRAF6, is recruited to the IL-1 receptor (*10*); both TRAFs interact with a MAP kinase kinase kinase (MAPKKK) known as NIK (*11*, *12*). Cell transfection experiments with wild-type and dominant-negative mutants of NIK suggest that this kinase is required for the TNF-α– and IL-1–dependent activation of NF-κB (*11*, *12*). Another MAPKKK, MEKK1, has been implicated in the TNF-α pathway, but its role is more controversial and its mechanism of activation not well understood (*12–15*).

Many kinases have been suggested to phosphorylate IκB-α, but until recently, none was shown to phosphorylate the critical serine residues. Last year, a kinase activity capable of specifically phosphorylating Ser^{32} and Ser^{36} of IκB-α was identified in cytoplasmic extracts from uninduced HeLa cells (*5*). Remarkably, this kinase fractionated as high molecular mass complex (~700 kD). Although it is inactive alone, this complex can be activated in vitro by treatment with purified recombinant MEKK1, directly implicating a MAPKKK in its activation (*5*, *14*). Quite unexpectedly, this complex could also be activated independently by ubiquitination (*5*, *14*). Finally, an already active IκB kinase complex of approximately the same size could be detected in extracts from TNF-α–induced HeLa cells (*14*). These studies, however, did not identify a specific protein kinase that directly interacts with and phosphorylates IκB-α.

A major breakthrough in the search for IκB kinases is now provided by three different groups, which report the identification and cloning of two closely related protein kinases that appear to directly phosphorylate IκB-α (*6–9*). Both Karin's group at University of California in San Diego and Mercurio, Rao, and their co-workers at Signal Pharmaceuticals and the Harvard Medical School approached the problem by purifying the high molecular mass IκB kinase complex from TNF-α–induced cells (size estimates from the different labs range from 500 to 900 kD) (*6–9*). Independently, Rothe, Goeddel, and their co-workers at Tularik identified one of the kinases by using the NIK kinase as bait in a yeast two-hybrid screen (*8*), and the other by virtue of

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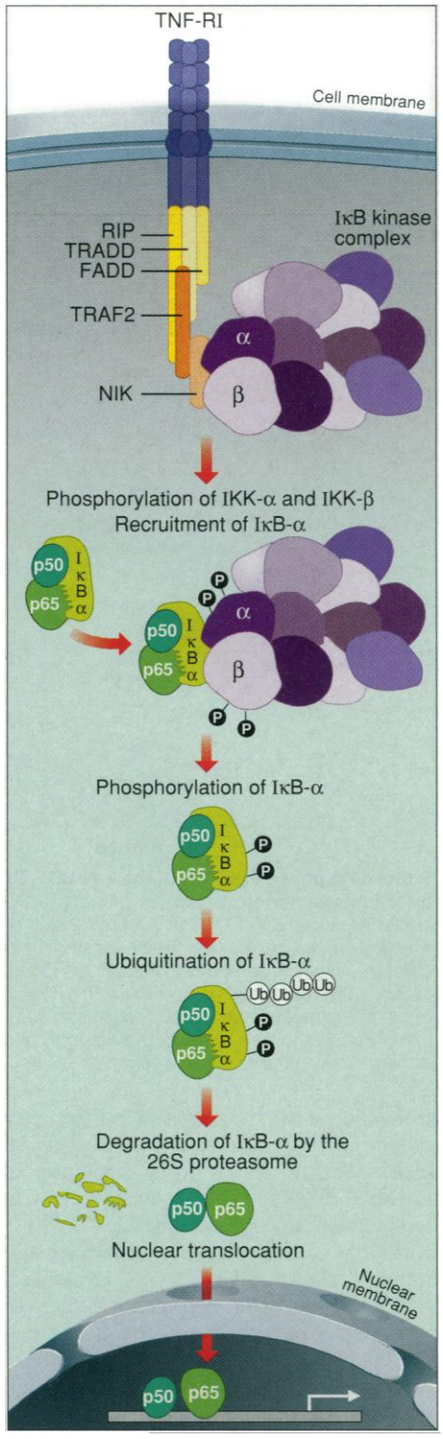
its similarity to the first in a DNA sequence database search (*7*).

One of the kinases is identical to a previously cloned serine-threonine kinase of unknown function (*16*). This kinase, known as CHUK, differs from other serine-threonine kinases in that it contains helix-loop-helix (HLH) and leucine zipper sequences (*16*), structural motifs originally shown to function as protein-protein interaction domains in transcriptional activator proteins. The second kinase in the complex is distinct, but closely related to CHUK. The CHUK has been renamed IKK-α or IKK-1 (IκB kinase α or 1), while the related kinase is designated IKK-β or IKK-2. For convenience, I refer to these kinases as IKK-α and IKK-β.

Both kinases contain a canonical MAPKK activation loop motif (SxxxS), suggesting that they are direct targets of MAPKKKs such as NIK or MEKK1 (*6*). IKK-α and IKK-β form homo- and heterodimers with each other (*6*, *7*, *9*), but the active form of the protein in vivo may be the heterodimer (*9*). The formation of heterodimers requires the leucine zipper motif, while the helix-loop-helix motif may mediate interactions between the IKKs and other proteins in the IκB kinase complex (*9*).

The conclusion that IKK-α and IKK-β are critical components in the NF-κB activation pathway is based on the results of mammalian transfection experiments. First, both kinases activate NF-κB when overexpressed, and dominant negative mutations in the kinase domain of either protein can suppress TNF-α or IL-1 induction of NF-κB (albeit with different efficiencies) (*6*–*9*). Mutations in the MAP kinase activation loop of IKK-β, but not IKK-α, suppress NF-κB activation in the same assay (*6*). Finally, expression of antisense to IKK-α RNA suppresses the TNF-α– or IL-1–mediated activation of NF-κB (*9*). Although the exact role of each kinase in the activation of NF-κB may be different, these observations implicate both proteins in the site-specific phosphorylation of IκB-α.

The conclusion that both kinases directly phosphorylate IκB-α is based on experiments in which immunoprecipitates of the kinases were mixed with purified IκB-α protein. Both kinases were epitope-tagged and expressed by in vitro translation in reticulocyte extracts or by transfection in mammalian cells. When the proteins were immunoprecipitated, the proteins that were recovered could phosphorylate Ser^{32} and Ser^{36} of IκB-α (*6*–*9*). In addition, the immunoprecipitates contained IκB-α, indicating that the kinases interact directly or indirectly with IκB-α. Of course, these observations do not rule out the formal possibility that IKK-α and IKK-β activate the "real" IκB kinase, which coimmunoprecipitates with the IKKs as a part of the multiprotein IκB kinase complex. In vitro studies with purified recombinant IKK-α and IKK-β proteins will be required to resolve this question.



NF-κB activation. Two newly identified kinases, IκB kinases α and β, add to the pathway.

The model in the figure is consistent with the data obtained so far. The TNF-dependent trimerization of the TNF receptor leads to the recruitment of TRADD, RIP, TRAF2, and NIK to the cell membrane. This association results in the activation of NIK, which in turn activates the IκB kinase complex through phosphorylation of IKK-α and IKK-β at the MAPKK activation loop. This activation could occur at the membrane or after an activated NIK was released from the receptor complex. IκB-α is then recruited to the activated IκB kinase complex where it is phosphorylated by the IKK-α/IKK-β heterodimer. The phosphorylation of IκB-α leads to its ubiquitination and degradation by the proteasome. In the case of the IL-1 receptor, an interaction between TRAF6 and NIK would lead to the activation of the kinase complex. An alternative signaling pathway that involves MEKK1 or other MAPKKK is also possible, because MEKK1 is recruited to the TNF-α–activated IκB complex (*6*), and recombinant MEKK1 can activate the complex isolated from uninduced cells (*14*).

Intriguing questions about the composition, regulation, and function of the IκB kinase complex remain. One puzzle is the role of ubiquitination in the activation of the IκB complex isolated from uninduced cells (*5*). Two groups report that they were unable to observe ubiquitin-dependent activation of the IκB kinase complex (*6*, *9*), but in those studies, the IκB kinase was already active, because it was isolated from TNF-α–induced cells. The possibility that the IκB kinase complex can be activated by NIK, MEKK1, or ubiquitination suggests that the complex processes signals from the large number of inducers that activate NF-κB. If so, do signals from different pathways converge on the same or different targets within the same complex, or are there distinct complexes that respond to different signals? Answers to these questions will require the identification and characterization of other components of the IκB kinase complex.

References

1. P. A. Baeuerle and D. Baltimore, *Cell* **87**, 13 (1996); A. S. Baldwin, *Annu. Rev. Immunol.* **14**, 649 (1996).
2. D. Thanos and T. Maniatis, *Cell* **80**, 529 (1995).
3. I. M. Verma *et al.*, *Genes Dev.* **9**, 2723 (1995).
4. C. A. Smith *et al.*, *Cell* **76**, 959 (1994); C. A. Dinarello, *Blood* **87**, 2095 (1996).
5. Z. J. Chen *et al.*, *Cell* **84**, 853 (1996).
6. F. Mercurio *et al.*, *Science* **278**, 860 (1997).
7. J. D. Woronicz *et al.*, *ibid.*, p. 866.
8. C. H. Regnier *et al.*, *Cell* **90**, 373 (1997).
9. J. A. DiDonato *et al.*, *Nature* **388**, 548 (1997); E. Zandi *et al.*, *Cell* **91**, 243 (1997).
10. H. Hsu *et al.*, *Cell* **84**, 299 (1996); Z. Cao *et al.*, *Nature* **383**, 443 (1996).
11. N. L. Malinin *et al.*, *Nature* **385**, 540 (1997).
12. H. Y. Song *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9792 (1997).
13. M. Hirano *et al.*, *J. Biol. Chem.* **271**, 13234 (1996).
14. F. S. Lee *et al.*, *Cell* **88**, 213 (1997).
15. Z.-g. Liu *et al.*, *Cell* **87**, 565 (1996).
16. M. A. Connelly and K. B. Marcu, *Cell. Mol. Biol. Res.* **41**, 537 (1995).