Nod2, a Nod1/Apaf-1 Family Member That Is Restricted to Monocytes and Activates NF-κB*

Yasunori Ogura**, Naohiro Inohara‡, Adalberto Benito‡, Felicia F. Chen‡, Shoji Yamaoka‡, and Gabriel Núñez**

From the Department of Pathology and Comprehensive Cancer Center, the University of Michigan Medical School, Ann Arbor, Michigan 48109 and Department of Microbiology, Tokyo Medical and Dental University, School of Medicine, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8519, Japan

Received for publication, September 4, 2000, and in revised form, November 2, 2000
Published, JBC Papers in Press, November 21, 2000, DOI 10.1074/jbc.M008072200

Apaf-1 and Nod1 are members of a protein family, each of which contains a caspase recruitment domain (CARD) linked to a nucleotide-binding domain, which regulate apoptosis and/or NF-κB activation. Nod2, a third member of the family, was identified. Nod2 is composed of two N-terminal CARDs, a nucleotide-binding domain, and multiple C-terminal leucine-rich repeats. Although Nod1 and Apaf-1 were broadly expressed in tissues, the expression of Nod2 was highly restricted to monocytes. Nod2 induced nuclear factor κB (NF-κB) activation, which required Iκκγ and was inhibited by dominant negative mutants of Iκκα, Iκκα, Iκκβ, and Iκκγ. Nod2 interacted with the serine-threonine kinase RICK via a homophilic CARD-CARD interaction. Furthermore, NF-κB activity induced by Nod2 correlated with its ability to interact with RICK and was specifically inhibited by a truncated mutant form of RICK containing its CARD. The identification of Nod2 defines a subfamily of Apaf-1-like proteins that function through RICK to activate a NF-κB signaling pathway.

Apaf-1 and Nod1 (also called CARD4) are members of a family of intracellular proteins that are composed of an N-terminal caspase recruitment domain (CARD),1 a centrally located nucleotide-binding domain (NBD), and a C-terminal regulatory domain (1, 2). Although Apaf-1 possesses WD40 repeats, Nod1 contains leucine-rich repeats (LRRs) in its C terminus (1, 2). The structural and functional similarities between Apaf-1 and Nod1 suggest that these proteins share a common molecular mechanism for activation and effector function. In the case of Apaf-1, the WD-40 repeats act as a recognition domain for mitochondrial damage through binding to cyclochrome c, allowing Apaf-1 to oligomerize and interact with procaspase-9 through a CARD-CARD homophilic interaction (3, 4). Apaf-1 oligomerization is mediated by the NBD and is thought to induce the proximity and proteolytic activation of procaspase-9 molecules in the apoptosome complex (5, 6).

Previous studies showed that Nod1 promotes apoptosis when overexpressed in cells, but unlike Apaf-1, it induces NF-κB activation (1, 2). NF-κB activation induced by Nod1 is mediated by the association of the CARD of Nod1 with the corresponding CARD of CARD (also called RIP2 and CARDI4), a protein kinase that activates NF-κB (1, 2, 7–9). Analyses with wild type (wt) and mutant forms of both Nod1 and RICK have suggested that Nod1 and RICK act in the same pathway of NF-κB activation, where RICK functions as a downstream mediator of Nod1 signaling (1, 2, 10). Nod1 self-associates through its NBD and Nod1 oligomerization promotes proximity of RICK molecules and NF-κB activation (10). Nod1 also displays striking similarity to a class of disease resistance (R) proteins found in plants (11, 12). Like Nod1, these intracellular R proteins contain N-terminal effector domains linked to a NBD and share with Nod1 the presence of multiple LRRs located C-terminally of the NBD (1, 12). After specific recognition of pathogen products, these R proteins mediate a defense response associated with metabolic alterations and localized cell death at the site of pathogen invasion (12). The LRRs of R proteins are highly diverse and appear to be involved in the recognition of a wide array of pathogen components (11, 12). The binding partner of the LRRs of Nod1 remains unknown. The structural homology of Nod1 with plant R proteins suggest that other LRR-containing Nod-1-like molecules may exist in the human genome to allow activation of these molecules by different sets of intracellular stimuli. We report here the identification and characterization of Nod2, another LRR-containing protein with structural and functional similarity to Nod1. These studies indicate that Nod2 activates NF-κB, but unlike Nod1, this new homologue is primarily expressed in monocytes.

MATERIALS AND METHODS

Isolation of the Nod2 cDNA—Nucleotide sequences encoding peptides with homology to Nod1 (GenBank™ accession numbers AC007728 and AQP546856) were found in the public genomic data base using the TBLASTN program. The coding region of human nod2 was obtained by RT-PCR amplification and 5’ RACE using Nod2-specific oligonucleotide primers cDNA fragments and mRNA from primary mammary tissue as a template. 5’ RACE was performed using a commercial kit (Roche Molecular Biologicals). For PCR, three sets of primers were used: 5’-ATGTTGCTCCAAAGGACGGCTTCTCAGGCA-3’ and 5’-CGCCTTACACCACCACCAACGTG-3’; 5’-CATGGTCTGGACCCCGACAGGACCCCA-3’ and 5’-CA-TGCCCGGGTGTTCTACTCTGGCTACTCCGG-3’; and 5’-GCCATGCGGCGTTCTACGTGGCCATC-3’ and 5’-TGAACGTCGACATGGGGAAGCTCGTCTTC-3’. For 5’ RACE, the initial primer 5’-AGACGCTCCAGGCCGCTGCTGGTGC-3’.

* This work was supported in part by Grants CA-64556 from the National Institutes of Health. 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.

** To whom correspondence should be addressed. Tel.: 734-764-8509; Fax: 734-647-9654; E-mail: bclx@umich.edu.

† Supported by funds from Tokushima University.

‡ Supported by a fellowship from the Spanish Ministry of Education and Science.

§ Supported by a fellowship from the Spanish Ministry of Education and Science.

1 The abbreviations used are: CARD, caspase recruitment domain; HA, hemagglutinin; Iκκ, inhibitor of NF-κB; Iκκα, Iκκα, Iκκβ, and Iκκγ, NF-κB nuclear factor κB; TNFRtumor necrosis factor α; wt, wild type; RT, reverse transcriptase; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends.
was fractionated into lymphocytes and monocytes by adherence to plastic incubation with hypotonic lysis buffer. The mononuclear cell population by Ficoll-Paque (Amersham Pharmacia Biotech) density gradient centrifugation were obtained from heparinized venous blood from healthy volunteers according to the manufacturer’s instructions. Peripheral blood leukocytes from various tissues (CLONTECH Laboratories, Palo Alto, CA) accord-

**FLAG-IKK**

**fig. 1. Deduced amino acid sequence and domain structure of human Nod2.** A, amino acid sequence of Nod2. CARD1 and CARD2, NBD, and LRRs are indicated by reverse highlighting, underlining, and arrows, respectively. The consensus sequence of the P-loop (Walker A box) and the Mg$^{2+}$ binding site (Walker B box) are indicated by boxes. B, domain structure of Nod2. Numbers corresponding to amino acid residues are shown in A. The region homologous to the CARDs, NBD, and LRRs are indicated by black, shaded, and hatched boxes, respectively.

Northern Blot and RT-PCR Analysis of nod2 Expression—A 3.7-kilobase fragment containing the entire nod2 coding region was radio-labeled by random priming using a commercial kit (Roche Molecular Biochemicals) and applied for analysis of human poly(A)$^+$ RNA blots from various tissues (CLONTECH Laboratories, Palo Alto, CA) according to the manufacturer’s instructions. Peripheral blood leukocytes were obtained from heparinized venous blood from healthy volunteers by Ficoll-Paque (Amersham Pharmacia Biotech) density gradient centrifugation. Granulocytes were separated from red blood cells by brief incubation with hypotonic lysis buffer. The mononuclear cell population was fractionated into lymphocytes and monocytes by adherence to plastic dishes. For RT-PCR analysis, 2 μg of total RNA from each tissue preparation were used to generate first strand cDNA using a commercially available kit (Life Technologies, Inc.). Nod2 cDNA fragments corresponding to the Nod2 coding region were amplified by PCR using two sets of specific primers: P1, 5′-AGTGGTGCACGAGGCAGTTTCAGGCA-3′; P2, 5′-CGCTTACACCCACCA-CGACACAGTGT-3′; P3, 5′-ATGTCGCTCGAGGACGACACACT-3′; and P4, 5′-CGCCACACACACACGTG-3′. As a control, a DNA fragment of the human glyceraldehyde-3-phosphate dehydrogenase was amplified using the primers 5′-GAGTCAACGGATTTGGTCGTAT-3′ with significant homology to Nod1. To determine the ends of the coding region, we performed 5′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene. To confirm the partial sequences we performed 5′ and 3′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene. 3′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene. 3′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene.

**RESULTS AND DISCUSSION**

Identification of nod2—To identify novel nod1/Apaf-1-like molecules, we searched public genomic data bases for genes encoding proteins with homology to Nod1 (2). We found a genomic sequence in human chromosome 16 (GenBank™ accession number AC007728) that encodes a peptide with significant homology to the NBD of Nod1. Analysis with GeneFinder of the genomic region predicted a gene encoding a novel protein with significant homology to Nod1. To determine the ends of the coding region, we performed 5′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene. 3′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene. 3′ RACE using an oligonucleotide complementary to sequences encoding the N terminus of the predicted protein and sequenced several expressed sequence tag cDNAs which contain partial sequences of the gene.

To test the interaction between wt Nod2 and RICK mutants, HEK293T cells were cotransfected with pcDNA3-HA-Nod2 and pcDNA3-FLAG-RICK, pcDNA3-FLAG-RICK (1–374), or pcDNA3-FLAG-RICK (374–540) (2). Proteins communoprecipitated with anti-HA antibody were detected with anti-FLAG antibody. Proteins in total lysate were detected by anti-FLAG antibody. Proteins in total lysate were detected by anti-FLAG antibody.
the Apaf-1/Nod1 superfamily (Fig. 1). Analysis of the nucleotide sequence revealed two potential in-frame translation initiation sites separated by 81 nucleotides. Further analysis revealed that both translation initiation sites can be utilized in cells, although the longer open reading frame is preferentially used (see below). For simplicity, we refer here to the longer open reading frame as Nod2 and the product encoded by the shorter open reading frame as Nod2b. A BLAST search and domain analyses revealed that Nod2 is composed of two N-terminal CARDs (residues 28–220) fused to a centrally located NBD domain (residues 273–577) containing consensus nucleotide-binding motifs followed by 10 tandem LRRs (residues 744–1020) (Figs. 1 and 2). Each of the 10 LRRs of Nod2 contained predicted α helix and β sheet sequences that are consistent with the prototypical horseshoe-shaped structure of LRRs (Ref. 14 and Fig. 2 C). To our knowledge, Nod2 is the first protein to contain two CARDs.

Chromosomal Localization and Genomic Organization of the Human Nod2 Gene—We identified two human BAC clones, RP11-327F22 and RP11-401P9, containing the genomic sequence of human Nod2 (GenBank™ accession numbers AC007728 and AC007608, respectively). These BAC clones mapped to chromosome 16 at q12.

The Expression of Nod2 Is Most Abundant in Monocytes—Northern blot analysis showed Nod2 to be expressed as two ~7.0- and ~5.5-kilobase transcripts in peripheral blood leukocytes with little or no detectable expression in various human tissues (Fig. 3A). This highly restricted pattern of expression is in contrast to that of Nod1 and Apaf-1 that are expressed in virtually all adult tissues although at different levels (1, 2, 3).

To determine the cells that express Nod2, we fractionated peripheral blood leukocytes into granulocyte, lymphocyte, and monocyte populations and performed RT-PCR analysis with two different sets of oligonucleotide primers complementary to Nod2 coding sequences. The analysis showed that Nod2 was

2 Y. Ogura, N. Inohara, and G. Núñez, unpublished results.
expressed primarily in monocytes (Fig. 3B). Because the Nod2 sequence contained two potential in-frame translation initiation sites separated by 81 nucleotides (Fig. 3C), we determined their usage by transfection of a Nod2 construct containing both translation initiation sites into HEK293T cells. Two potential in-frame translation initiation sites separated by 81 nucleotides are indicated by arrows. D, immunoblotting of nod2 gene products expressed in HEK293T cells. Cells were transfected with control plasmid (lane 1), constructs containing both potential translation initiation sites of Nod2 (lane 2), as a control the second translation initiation site corresponding to that of Nod2b (lane 3), or the most N-terminal translation initiation site (lane 4) in the context of a canonical translation initiation motif. In all cases, a Nod2 protein lacking residues 302–1040 and HA-tagged at its C terminus was expressed to facilitate detection of nod2 gene products. Nod2 proteins were detected by immunoblotting with anti-HA antibody and indicated by a and b.

Nod2 Activates NF-κB—Because Nod2 shows the highest homology to Nod1 and the latter protein activates NF-κB, we...
first tested whether expression of Nod2 activates NF-κB by transfection of Nod2 plasmids into HEK293T cells. Transfection of the wt Nod2 cDNA induced potent activation of NF-κB, as measured with a reporter luciferase construct (see below). In addition, we tested the Nod2b cDNA and obtained similar results to those observed with Nod2. We generated a panel of Nod2 mutants to determine the regions of Nod2 that are required for NF-κB activation (Fig. 4A). Immunoblotting analysis revealed that these mutant constructs were expressed when transiently transfected into HEK293T cells (Fig. 4B). Expression of as little as 3 ng of wt Nod2 induced 18-fold activation of NF-κB (Fig. 4C). Expression of a Nod2 mutant form lacking the LRRs resulted in enhanced NF-κB activation, whereas mutants expressing the LRRs or the NBD alone were inactive (Fig. 4C). The enhanced activity of the Nod2 mutant lacking the LRRs could not be explained by increased expression of the mutant (Fig. 4A). Consistent with these results, it was shown previously that deletion of the LRRs of Nod1 and WD-40 repeats of Apaf-1 results in enhanced NF-κB activation and increased ability to activate procaspase-9, respectively (2, 5, 6). Deletion of the CARDs of Nod2, either singly or in combination, resulted in total loss of NF-κB activity (Fig. 4C). However, expression of both CARDs alone, but not each CARD separately, was sufficient for NF-κB activation (Fig. 4C). Thus, both CARDs of Nod2 are necessary and sufficient for NF-κB activation, suggesting that the CARDs acts as an effector domain in Nod2 signaling. The conserved lysine residue in the P-loop of Nod1 and Apaf-1 is important for the activities of these proteins (2, 10, 15). Similarly, replacement of the corresponding lysine for arginine in Nod2 resulted in diminished NF-κB activity that was rescued at least in part by deletion of the LRRs (Fig. 4C).

We also investigated the ability of Nod2 to induce apoptosis. We found that overexpression of Nod2 did not induce apoptosis by itself but enhanced apoptosis induced by caspase-9 expression. These results are similar to those reported for Nod1 and Apaf-1 (1, 2).

NF-κB Activation Induced by Nod2 Requires IKKγ and Is Inhibited by Dominant Negative Forms of IKKs and RICK—A main pathway of NF-κB activation is mediated by IkB kinases (IKKs) resulting in IkB phosphorylation and release of cytoplasmic NF-κB (16). To determine whether Nod2 activates an IKK-dependent pathway, Nod2 was coexpressed with mutant

![Image](https://example.com/image.png)
forms of IKKα, IKKβ, and IκB that have been shown to act as dominant inhibitors of their corresponding endogenous counterparts and/or the IKK complex (16). In addition, we used a truncated mutant of IKKγ/Nemo (residues 134–419) that is defective in IKKα and IKKβ binding and acts as an inhibitor of NF-κB activation induced by RIP and RICK (10). The NF-κB activity induced by Nod2 as well as that induced by TNFα stimulation were greatly inhibited by mutant IKKα, IKKβ, IKKγ, and IκBa (Fig. 5A). Because RICK has been shown to serve as a downstream target of Nod1 (1, 2, 10), we used a truncated form of RICK containing its CARD (residues 406–540) that acts as a dominant inhibitor of Nod1 activity (1) to test whether NF-κB activation induced by Nod2 is similarly inhibited by this RICK mutant. We found that NF-κB activation induced by Nod2 was inhibited by mutant RICK but not by a mutant form of RIP that expresses its death effector domain (Fig. 5A). The inhibition by the CARD of RICK was specific in that it did not interfere with ability of TNFα to induce NF-κB, an activity that was inhibited by the RIP mutant (Fig. 5A). To verify that Nod2 acts upstream of the IKK complex to activate NF-κB, we tested the ability of Nod2 to activate NF-κB in parental Rat1 fibroblasts and 5R cells, a Rat1-derived cell line that is defective in IKKγ, an essential subunit of the IKKs (13). We found that Nod2, as well as TNFα, induced NF-κB activity in parental Rat1 cells but not in IKKγ-deficient 5R cells (Fig. 5B). As a control, expression of IKKβ, which functions downstream of IKKγ, induced NF-κB activation in both Rat1 and 5R cell lines (Fig. 5B). These results indicate that Nod2 acts through IKKγ/IKKα/IKKβ to activate NF-κB.

Nod2 Associates with RICK via a Homophilic CARD-CARD Interaction—The CARD motifs function as an effector domain that mediates specific homophilic interaction with downstream CARD-containing molecules (17). Because NF-κB activation induced by Nod2 was inhibited by a RICK truncated mutant, we tested whether RICK could act as a direct downstream mediator of Nod2 signaling. To test a physical association between Nod2 and RICK, HEK293T cells were cotransfected with plasmids expressing HA-tagged wt or mutant forms of Nod2 and FLAG-tagged RICK, and cellular extracts were immunoprecipitated with anti-HA antibody. Immunoblotting with anti-FLAG antibody revealed that RICK associated with Nod2 (Fig. 6A). The association was mediated by both CARDs of Nod2, because only Nod2 proteins containing both CARDs were capable of interacting with RICK (Fig. 6A, A and B). The association of Nod2 with RICK was specific in that Nod2 did not associate with several CARD-containing proteins including Apaf-1, caspase-1, caspase-4, c-IAP-1, c-IAP2, procaspase-9, Bcl-10, RAIDD, and Ced-4 nor with several molecules that activate NF-κB including TRAF-1, TRAF-2, TRAF-5, TRAF-6, RIP, NIK, TRADD, IKKα, IKKβ, or IκBα (Fig. 6B). To determine the region of RICK that associates with Nod2, mutant forms of RICK expressing the CARD (residues 374–540) or lacking the CARD (residues 1–374) were coexpressed with Nod2, and the cell extracts were immunoprecipitated with anti-FRAP antibody. The analysis showed that only the CARD of RICK coimmunoprecipitated with Nod2 (Fig. 6C). Thus, Nod2 and RICK associate via a homophilic CARD-CARD interaction.

Enforced Oligomerization of Nod2 Induces NF-κB Activation—Previous studies showed that the NBD of Nod1 and Apaf-1 mediates oligomerization of these molecules, an activity that is critical for NF-κB and caspase-9 activation, respectively (5, 6, 10). In the case of Nod1, its oligomerization appears to promote proximity of RICK and NF-κB activation (10). To test a similar role for Nod2, we constructed plasmids to express chimeric proteins in which wt or Nod2 mutants were fused to three tandem repeated dimerization domains of Fpk (Fpk3), which can be oligomerized by the cell-permeable ligand AP1510 (18). Immunoblotting analysis showed that the chimeric Fpk3-Nod2 constructs were expressed when transfected in HEK293T cells (Fig. 7A). Because wt Nod2 alone induces NF-κB activation, we expressed suboptimal amounts of the chimeric Fpk3-Nod2 constructs into HEK293T cells. Under these experimental conditions, expression of Nod2-Fpk3 induced NF-κB activation in a ligand-dependent manner (Fig. 7B). Consistent with the results shown in Fig. 4C, enforced oligomerization of both CARDs but not each CARD singly induced NF-κB activation. Similarly, NF-κB activation induced by a Nod2 P-loop mutant lacking the LRRs (K305ΔLRR), which have reduced ability to induce NF-κB activation, was enhanced by enforced oligomerization (Fig. 7B). A Nod2-Fpk3 construct lacking the
LRRs induced NF-κB activation in the absence and presence of AP1510 (Fig. 7B). The latter result might be explained by our observations that Nod2 lacking the LRRs has enhanced activity to self-associate and induce NF-κB (Ref. 10 and Fig. 4C). To demonstrate that Nod2 can function in monocytic cells, we transfected Nod2 constructs into HL60 cells stimulated with phorbol 12-myristate 13-acetate. Similarly to the case of HEK293T cells, transient expression of Nod2 induced NF-κB activation in monocytic HL60 cells (Fig. 7C, left panel). Furthermore, enforced oligomerization of wild type Nod2-Fpk3 or CARDs-Fpk3 was sufficient for NF-κB activation in monocytic HL60 cells (Fig. 7C, right panel).

We have shown that Nod2 is a member of the Nod1/Apaf-1 family that activates NF-κB through interactions with its N-terminal CARDs, as these domains were necessary and sufficient for NF-κB activation. Nod2 associated with RICK via a homophilic CARD-CARD interaction. The NF-κB-inducing activity of Nod2 correlated with its ability to associate with RICK and was inhibited by a RICK mutant, suggesting that RICK is a direct downstream target of Nod2. Thus, the signaling pathways of both Nod1 and Nod2 appear to utilize RICK as a downstream mediator of NF-κB activation. To our knowledge, Nod2 is the first molecule to contain two CARDs. The molecular basis underlying the requirement of both CARDs of Nod2 for RICK binding remains unclear. The presence of both CARDs may enhance the affinity for the CARD of RICK. Another possibility is that upon an initial interaction involving a CARD of Nod2 and the CARD of RICK, Nod2 may undergo a conformational change that allows the second CARD to associate with high affinity to RICK. The intermediate region of RICK associates with IKKγ (10), providing a direct link between Nod1/Nod2 and the IKK complex. Consistent with this model, we have shown that NF-κB activation induced by Nod2 as well as that induced by Nod1 required IKKγ and was inhibited by dominant negative forms of IKKγ, IKKα, and IKKβ. The functional role for the LRRs of Nod1 and Nod2 remains unclear. The LRR is a repeated protein-protein interaction module that is presumably involved in the activation of Nod1 and Nod2 by upstream signals. In the case of plant NBD/LRR-containing R proteins, their LRRs appear to be important for the recognition of pathogen components, and their N-terminal domains appear to mediate a signaling cascade that regulates gene expression (11, 12). Recent results indicate that Nod1 and Nod2 can confer responsiveness to bacterial components, suggesting that these proteins are functional counterparts of plant R proteins (20). Because both Nod1 and Nod2 activate NF-κB, their LRRs may act to recognize a different set of intracellular stimuli that mediate Nod1 and Nod2 oligomerization and association with RICK. Consistent with this model, Nod1 and Nod2 conferred a different pattern of response to bacterial products including lipopolysaccharides (20), suggesting that they are activated by different microbial stimuli. Because Nod2 is expressed primarily in monocytes, Nod2 might serve as an intracellular receptor for bacterial lipopolysaccharides and/or other bacterial products transducing signals in the monocyte/macrophage that lead to activation of NF-κB and transcription of regulatory genes.

Acknowledgments—We thank Peter Lucas for critical review of the manuscript. We thank Steve Ethier for mammary cells, Victor Rivera (Ariad Pharmaceuticals) for Fpk plasmids and dimerization agent AP1510, and David Goeddel and Marshall Horwitz for plasmids.

REFERENCES
1. Bertin, J., Nir, W. J., Fischer, C. M., Tayber, O. V., Errada, P. R., Grant, J. R., Keilty, J. J., Gesselein M. L., Robinson, K. E., Weng, G. H., Glucksmann, M. A., and DiStefano, P. S. (1999) J. Biol. Chem. 274, 12955–12958
2. Inohara, N., Koseki, T., del Peso, L., Hu, Y., Yee, C., Chen, S., Carrio, R., Merino, Liu, D., Ni, J., and Núñez, G. (1999) J. Biol. Chem. 12470–12456
3. Li, P., Nijhawan, D., Budiardjo, I., Srinivasula, S., Ahmad, M., Alnemri, E. S., and Wang, X. (1997) Cell 91, 479–489
4. Zeu, H., Li, Y., Liu, X., and Wang, X. (1999) J. Biol. Chem. 274, 11549–11556
5. Srinivasula, S. M., Ahmad, M., Fernandes-Alnemri, T., and Alnemri, E. S. (1998) Mol. Cell 1, 949–957
6. Hu, Y., Ding, L., Spencer, D. M., and Núñez, G. (1998) J. Biol. Chem. 273, 33489–34494
7. Inohara, N., del Peso, L., Koseki, T., Chen, S., and Núñez, G. (1998) J. Biol. Chem. 273, 12,296–12,300
8. McCarthy, J. Y., Ni, J., and Dixit, V. M. (1998) J. Biol. Chem. 273, 16968–16975
9. Thome, M., Hofmann, K., Burns, K., Martinson, F., Bodmer, J. L., Mattmann, C., and Tschopp, J. (1998) Trends Biochem. Sci. 22, 155–156
10. MacCorkle, R. A., Freeman, K. W., and Spencer, D. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3655–3660
11. Vlahos, S., Mateau, H., Duan, H., and Wagner, G. (1998) Cell 94, 171–180
12. Inohara, N., Oyura, Y., Chen, F. M., Futo, A., and Núñez, G. (2000) J. Biol. Chem. 275, in press