Receptor activator of NF-κB recruits multiple TRAF family adaptors and activates c-Jun N-terminal kinase

Hong-Hee Kim[a], Da Eun Lee[a], Jin Na Shin[a], Yong Soo Lee[a], Yoo Mi Jeon[a], Chae-Heon Chung[a], Jian Ni[b], Byoung Se Kwon[c], Zang Hee Lee[a,*]

[a] *Department of Microbiology and Immunology, Chosun University Dental School, 375 Seosuk-Dong, Dong-Ku, Kwangju 501-759, South Korea*
[b] *Human Genome Sciences, Inc., Rockville, MD 20850, USA*
[c] *Department of Life Sciences, Ulsan University, Ulsan, South Korea*

Received 7 December 1998

Abstract Receptor activator of NF-κB (RANK) is a recently cloned member of the tumor necrosis factor receptor (TNFR) superfamily, and its function has been implicated in osteoclast differentiation and dendritic cell survival. Many of the TNFR family receptors recruit various members of the TNF receptor-associated factor (TRAF) family for transduction of their signals to NF-κB and c-Jun N-terminal kinase. In this study, the involvement of TRAF family members and the activation of the JNK pathway in signal transduction by RANK were investigated. TRAF1, 2, 3, 5, and 6 were found to bind RANK in vitro. Association of RANK with each of these TRAF proteins was also detected in vivo. Expression of RANK in cultured cells also induced the activation of JNK, which was blocked by a dominant-negative form of JNK. Furthermore, by employing various C-terminal deletion mutants of RANK, the regions responsible for TRAF interaction and JNK activation were identified. TRAF5 was determined to bind to the C-terminal 11 amino acids and the other TRAF members to a region N-terminal to the TRAF5 binding site. The domain responsible for JNK activation was localized to the same region where TRAF1, 2, 3, and 6 bound, which suggests that these TRAF molecules might mediate the RANK-induced JNK activation.

Key words: Receptor activator of neclear factor κB; Tumor necrosis factor receptor-associated factor; c-Jun N-terminal kinase; Signal transduction

1. Introduction

Receptor activator of NF-κB (RANK) is a recently cloned member of the tumor necrosis factor receptor (TNFR) family [1]. The RANK protein consists of 616 amino acids containing the cysteine residues conserved in the extracellular domain of the TNFR family proteins. Its relatively long cytoplasmic domain (383 amino acids) has little homology with other TNFR family members. The ligand for RANK, RANKL [1], was found to be identical to TRANCE (TNF-related activation-induced cytokine), ODF (osteoclast differentiation factor), and OPGL (osteoprotegerin ligand) [2–4]. The expression level of RANK mRNA is high in thymus, lung, and trabecular bone and lower in spleen and bone marrow [3], which suggests physiological functions of RANK in the regulation of bone and immune systems. Consistent with this suggestion, treatment with RANKL/TRANCE was shown to enhance the function and survival of T cells and dendritic cells [1,5]. In addition, RANKL/ODF has been demonstrated to cause the differentiation of hematopoietic precursor cells into osteoclasts and to stimulate the bone resorbing activity of mature osteoclasts [4].

The TNF receptor-associated factor (TRAF) family molecules are cytoplasmic adaptor proteins known to mediate signaling events specifically for members of the TNFR family, which includes TNFR1, TNFR2, CD30, CD40, CD27, Fas, 4-1BB, Ox40, TR2, and LMP-1 [6,7]. To date six members of the TRAF family have been identified. The TRAF2 protein is recruited to TNFR1 through a death domain-containing molecule TRADD, whereas all TRAF proteins except TRAF4, which is localized to the nucleus, have been shown to directly bind to several TNFR family receptors [8–17]. TRAF family proteins have a similar structural array consisting of an N-terminal zinc RING finger, multiple zinc fingers, and C-terminal TRAF-N and TRAF-C domains. The TRAF1 molecule is unique in that it lacks the RING finger domain [9]. The N-terminal zinc binding regions of TRAFs serve as effector domains, whereas the TRAF domain appears to be involved in binding to the cytoplasmic tail of TNFR proteins [12,18,19]. Characterization of binding sites for TRAFs in TNFR family proteins has led to the identification of two types of sequence motif: PXQXT/S [20,21] and EED/EEE [15,20,22]. The PXQXT/S motif seems to be capable of binding to TRAFs 1, 2, 3, and 5 and the EED/EEE motif binds TRAFs 1, 2, and 3. Characteristic cellular responses to the stimulation of TNFR family receptors include the activation of the NF-κB transcription factor and c-Jun N-terminal kinase (JNK) of the mitogen-activated protein kinase family. The N-terminal zinc binding motifs of TRAF2, TRAF5, and TRAF6 have been shown to be responsible for the NF-κB activation induced by TNFR family proteins [13–15,18,23–25]. Overexpression of TRAF2, 5, or 6 was reported to activate JNK [26], and TRAF2 has been implicated in the TNFα-induced activation of JNK [27]. The role of TRAF2 in the TNFR-induced JNK activation was further supported by a study with transgenic mice expressing a dominant-negative TRAF2 [28].

The activation of NF-κB appears to be involved in RANK signaling as other TNFR family proteins. Ectopic overexpression of RANK in a cultured cell line and ligation of endogenous RANK in T cells pretreated with phytohemagglutinin and interleukin (IL)-4 resulted in NF-κB activation [1]. Involvement of JNK in RANK signaling pathways might also

*Corresponding author. Fax: (82) (62) 227-6589.
E-mail: jhblee@mail.chosun.ac.kr

Abbreviations: RANK, receptor activator of NF-κB; TNFR, tumor necrosis factor receptor; TRAF, tumor necrosis factor receptor-associated factor; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor κB; GST, glutathione *S*-transferase; ICD, intracellular domain; SEK, SAPK (JNK)/ERK kinase

PII: S0014-5793(98)01731-1

be suggested as RANKL/TRANCE stimulation was shown to cause a modest increase in the JNK activity in T cells [2]. As TRAF2, 5, and 6 were implicated in the activation of NF-κB and JNK for some TNFR family members, it may be anticipated that some TRAF molecules mediate the RANK-induced responses. In support of this, the amino acid sequence analysis of the cytoplasmic domain of human RANK reveals several potential TRAF binding sites: four PXQXT/S-like motifs (^{302}PDQGG, ^{353}PSQPT, ^{569}PVQEE, and ^{607}PVQEQ) and three acidic residue triplets (^{336}EED, ^{346}EDE, and ^{481}EEE). To gain insight into the mechanism by which RANK transmits its signal to the nucleus, the interaction between RANK and TRAFs and the effect of RANK signaling on JNK activation were characterized in this study.

2. Materials and methods

2.1. Cell culture and transfection

HeLa cells were cultured in DMEM containing 10% FCS and 293-EBNA cells (Invitrogen) were maintained in DMEM containing 10% FCS and 250 μg/ml G418. For transient transfection, 2×10^5 cells per well were plated onto 6-well plates. The next day, transfection was carried out with a mixture of 1–2 μg DNA and 10 μl SuperFect reagent (Qiagen) following the manufacturer's instructions. 40–48 h after transfection, cells were harvested. For co-immunoprecipitation experiments, 1.5×10^6 cells plated in T-25 flasks were transfected with 4–7 μg DNA and 20 μl SuperFect.

2.2. Plasmid constructions

The cDNA encoding the full-length human RANK was obtained from Human Genome Sciences (Rockville, MD). The T7-tagged mammalian expression vector of hRANK, pSRa-RANK-T7, was constructed by PCR. The sequence of PCR product was verified by automated DNA sequencing (Perkin Elmer, ABI 310). RANK C-terminal deletion mutants, pSRa-RANK-605 and -432, were generated using *Xho*I (amino acid 605 position) and *Msc*I (amino acid 432 and 513 positions) sites, respectively. A third deletion mutant, pSRa-RANK-352, was constructed by PCR.

The GST fusion construct of the entire cytoplasmic domain (amino acids 235–616) of RANK was produced by subcloning PCR amplified DNA into pGEX4T-1 (Pharmacia). The cytoplasmic domain deletion mutants of GST fusion plasmids, pGEX-RANK-ICD-605 and pGEX-RANK-ICD-432, were constructed by similar approaches as for pSRa-RANK mutants. GST fusion proteins were purified as described previously [29].

Flag-tagged human TRAF1, 2, 3, and 6 and murine TRAF5 mammalian expression vectors, mammalian GST expression vectors (pEBG) of JNK1 and SEK-K/R, and GST-c-Jun-77 construct were generously provided by Drs. H.Y. Song (Lilly Co. Center, Indianapolis, IN, USA), J.S. Gutkind (NIH, Bethesda, MD, USA) and R.K. Park (Wonkwang University, Iksan, South Korea), respectively.

2.3. Immunoprecipitation, Western blotting, and in vitro binding assays

Cell lysates were prepared using a lysis buffer containing 1% Triton X-100 and immunoprecipitated with anti-Flag mAb M2 (Eastman Kodak), immunoprecipitates were resolved by SDS-PAGE, and co-precipitated proteins were blotted with either anti-T7-HRP or anti-T7 (Novagen) and anti-mouse Ig-HRP (Amersham) as previously described [30]. Rabbit anti-GST and anti-RANK sera were produced using as immunogen GST and GST-RANK cytoplasmic domain proteins, respectively. A small fraction of total cell lysate (1/50–1/100) was subjected to Western blotting with anti-Flag M2, anti-T7, anti-RANK or anti-GST.

For in vitro binding experiments, 2–5 μg of purified GST-RANK proteins were incubated with cell lysates from the Flag-TRAF-transfected HeLa or 293-EBNA cells and glutathione beads. Extensively washed precipitates were separated by SDS-PAGE and subjected to Western blotting with anti-Flag M2.

2.4. JNK activity assay

For JNK activity assays, 250 μg of lysates were incubated with glutathione beads for 2 h at 4°C, precipitates washed twice in the lysis buffer and twice in the kinase reaction buffer, and kinase reaction performed as previously described [31] with the omission of [γ-^{32}P]ATP. Samples were resolved by SDS-PAGE and Western blotted with an antibody specific for phosphorylated c-Jun (New England Biolabs, Cat. #9161S). Alternatively, the activity of endogenous JNK was measured by the solid-phase kinase assay [32] using 3 μg of GST-c-Jun-77.

3. Results and discussion

3.1. Interaction of RANK with TRAF family proteins in vitro

Some TNFR family receptors recruit TRAF2, 5, and 6 to activate NF-κB [23–25], while this activation appears to be antagonized by TRAF3 binding [17,23,25]. To determine whether RANK can also utilize TRAF family proteins in signal transduction, the potential interaction between RANK and TRAFs was examined by in vitro binding experiments, employing the GST fusion protein of the cytoplasmic domain of human RANK. When the GST-RANK protein was incubated with lysates from HeLa or 293-EBNA cells expressing Flag-tagged TRAF molecules (Fig. 1A) and the precipitates were subjected to Western blotting with anti-Flag, TRAF1, 2, 3, 5, and 6 were found to bind RANK (Fig. 1B). The binding of TRAF to GST-RANK was specific as the GST control and the GST fusion of protein X, which does not possess any homology to RANK, showed little binding, even though comparable amounts of the GST proteins were used (data not shown). Although the possibility of the involvement of a third cellular protein mediating the interactions between RANK and TRAFs cannot be excluded, given

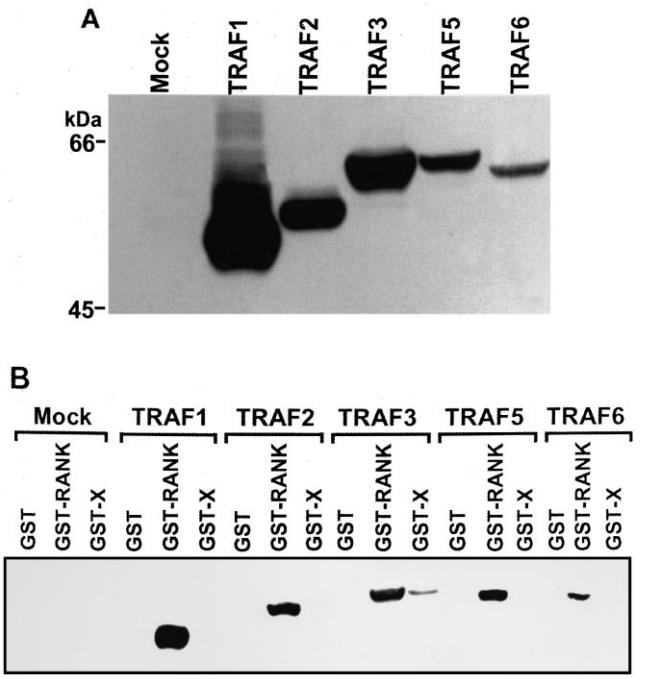

Fig. 1. In vitro binding of TRAF family proteins to RANK. Flag-tagged human TRAF1, 2, 3, or 6, mouse TRAF5, or control vector cDNA was transfected into HeLa cells, and a fraction of each cell lysate was subjected to Western blotting with anti-Flag (A). The cell lysates were incubated with 2 μg of either GST, GST fusion protein of the cytoplasmic domain of RANK (GST-RANK), or that of an irrelevant protein X (GST-X) and then precipitated using glutathione beads. The precipitates were extensively washed, resolved by SDS-PAGE, and blotted with anti-Flag (B).

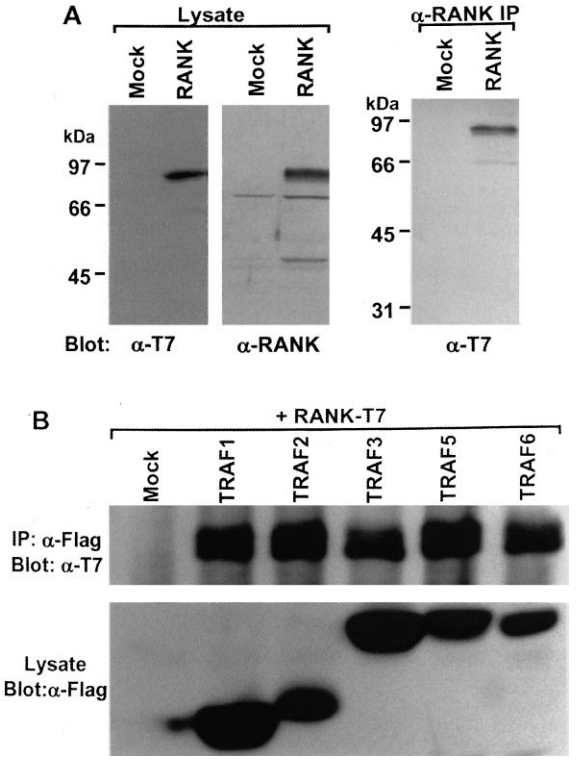

Fig. 2. Association of the RANK protein with TRAF family members in cultured cells. A: T7-tagged RANK cDNA or control vector plasmid was transfected into 293-EBNA cells and a fraction of cell lysate was analysed for RANK expression by Western blotting with either monoclonal anti-T7 or polyclonal anti-RANK (left panel). The lysates were also immunoprecipitated with anti-RANK and the precipitates were blotted with anti-T7 (right panel). B: 293-EBNA cells were transfected with RANK-T7 and each of the TRAF plasmids, cell lysates were immunoprecipitated with anti-Flag, and the precipitates were subjected to Western blotting with anti-T7 (top panel). A fraction of each cell lysate was analysed by blotting with anti-Flag to verify the expression of all TRAF proteins (bottom panel).

the high levels of TRAFs expressed in this system, the TRAF binding to RANK is likely to be direct. Consistent with this, TRADD, known to mediate TRAF2 association with TNFR1 [8], did not show any binding to GST-RANK (data not shown). These results indicate that RANK has the potential to bind multiple TRAF family members and utilize various TRAF molecules for its signal transduction.

3.2. Interaction of TRAFs and RANK in mammalian cells

To investigate the signal transduction pathways used by RANK, we expressed the full-length RANK protein as a T7-tagged form in 293-EBNA cells. When the lysate was Western blotted with anti-T7 or anti-RANK, the RANK protein appeared to have the relative molecular mass of about 90 kDa (Fig. 2A, left panel), which was larger than the calculated size and suggested that RANK may be subject to glycosylation or other posttranslational modifications. Immunoprecipitation with anti-RANK or anti-T7 also yielded a 90 kDa immunoreactive protein (Fig. 2A, right panel and data not shown). Analyses of the expressed RANK protein by non-denaturing electrophoresis showed a band of approximately 270 kDa (data not shown). Trimerization of TNFR upon TNF stimulation has been thought to be the mechanism by which the signal of ligand engagement is transmitted to the receptor [33,34], and the RANK proteins apparently also showed the potential to trimerize. To determine whether the intracellular events in the RANK signal transduction include the recruitment of TRAF molecule(s), co-immunoprecipitation analyses were performed with cells transfected with the RANK-T7 and each of the Flag-tagged TRAF molecule cDNAs. Blotting of the anti-Flag precipitates with anti-T7 showed that TRAF1, 2, 3, 5, and 6 proteins associated with RANK (Fig. 2B). Results of the reverse analysis, immunoprecipitation with anti-T7 and Western blotting with anti-Flag, were in agreement (data not shown). In some cases, the expressed RANK protein migrated as a doublet in SDS-PAGE, and TRAF3 preferentially bound the lower band (Fig. 2B and data not shown). It is possible that some RANK proteins existed as a phosphorylated form with retarded mobility under certain conditions, and TRAF3 binds more efficiently to the non-phosphorylated form. These co-immunoprecipitation results indicated that RANK can associate with TRAF1, 2, 3, 5, and 6 in vivo, which suggested the involvement of the multiple TRAF family proteins in the signal transduction by RANK.

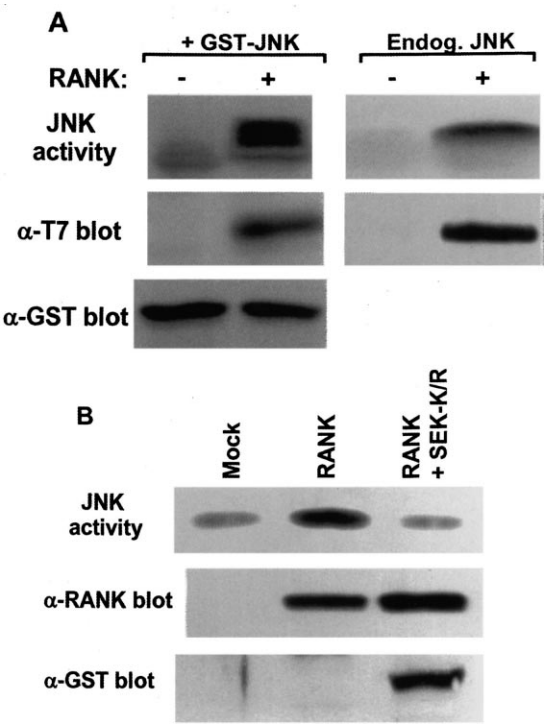

Fig. 3. Activation of JNK induced by RANK expression. A: 293-EBNA cells were transfected with RANK-T7 and a mammalian GST expression construct of JNK1, and the JNK activity was measured using equal amounts of cell lysates as described in Section 2 (left top panel). Expression of RANK and JNK was confirmed by Western blotting with anti-T7 and anti-GST, respectively, of aliquots of cell lysates (left middle and bottom panels). Alternatively, cells were transfected with RANK-T7 and the activity of endogenous JNK was measured by solid-phase kinase assays as described in Section 2 (right top panel). Expression of RANK was analyzed by anti-T7 blotting of aliquots of these lysates (right bottom panel). B: Cells were transfected with indicated plasmids. JNK activity was determined by solid-phase kinase assays (top panel), and the expression of RANK and SEK-K/R was verified by Western blotting of the lysates with anti-RANK (middle panel) and anti-GST (bottom panel), respectively.

3.3. RANK signaling to JNK

TRAF2 and TRAF6 have been shown to mediate the activation of JNK by TNFR1 and IL-1R, respectively [27,35]. Transient overexpression of TRAF2, 5, and 6 has also been found to induce the activation of this kinase [26]. To determine whether JNK activation is involved in RANK signal transduction pathways, RANK and GST-JNK1 were transfected into 293-EBNA cells and the activity of JNK was measured after precipitation with glutathione beads. The transfection of RANK led to the elevated activity of the ectopically expressed kinase compared to the vector transfected control (Fig. 3A, left panel). This increase in JNK activity was not due to differential levels of expression of the kinase as proven by an anti-GST blot (Fig. 3A, left bottom panel). The RANK-induced JNK activation was also observed with the kinase endogenously present when the cells were transfected with RANK cDNA alone and the solid-phase kinase assay was performed (Fig. 3A, right panel). Furthermore, this activation was blocked by the coexpression of SEK-K/R, a kinase-inactive form of SEK/JNKK which is an upstream activating kinase of JNK (Fig. 3B). These results imply that the signaling pathways utilized by RANK include one leading to JNK activation, and that the conventional JNK activation cascade involving SEK may lie in the pathway transducing RANK signals to the nucleus.

3.4. Effects of C-terminal deletions on TRAF binding to and JNK activation by RANK

To locate the regions in the cytoplasmic tail of RANK responsible for TRAF binding and JNK activation, the C-terminal deletion mutants RANK-605, RANK-432, and RANK-352 were generated as mammalian expression plasmids and GST fusion proteins of the intracellular domain segments of the RANK variants (GST-RANK-ICD-605 and GST-RANK-ICD-432) were produced (Fig. 4A). When the various GST-RANK fusion proteins were incubated with lysates from 293-EBNA cells expressing Flag-tagged TRAF molecules, GST-RANK-ICD-605 showed binding to TRAF1, 2, 3 and 6 but not to TRAF5, whereas binding of all tested TRAFs was observed with the wild type RANK fusion protein (Fig. 4B). The GST-RANK-ICD-432 failed to bind to any of the TRAF molecules (Fig. 4B). The lack of TRAF binding to GST-RANK-ICD-432 was not due to an insufficient amount of this fusion protein as comparable amounts of the wild type and mutant RANK fusion proteins were used (data not shown). These results indicate that the binding site for TRAF5 is located in the C-terminal 11 amino acids of RANK, and that for TRAF1, 2, 3, and 6 it lies in amino acids 433–605. Next, using the wild type and mutant RANK constructs encoding the extracellular and transmembrane domains as well as the cytoplasmic domain, identification of the region responsible for the activation of JNK was pursued. The deletion mutant RANK-605 could induce JNK activation to an extent comparable to that caused by the wild type RANK protein (Fig. 4C). In contrast, the deletion mutants RANK-432 and -352 could not elevate the JNK activity above the basal level (Fig. 4C). These results map the JNK activation domain to amino acids 433–605 in the cytoplasmic tail of RANK which was also required for the binding of TRAF1, 2, 3, and 6.

In this study, we investigated the signal transduction pathways of RANK. The RANK protein was found to bind

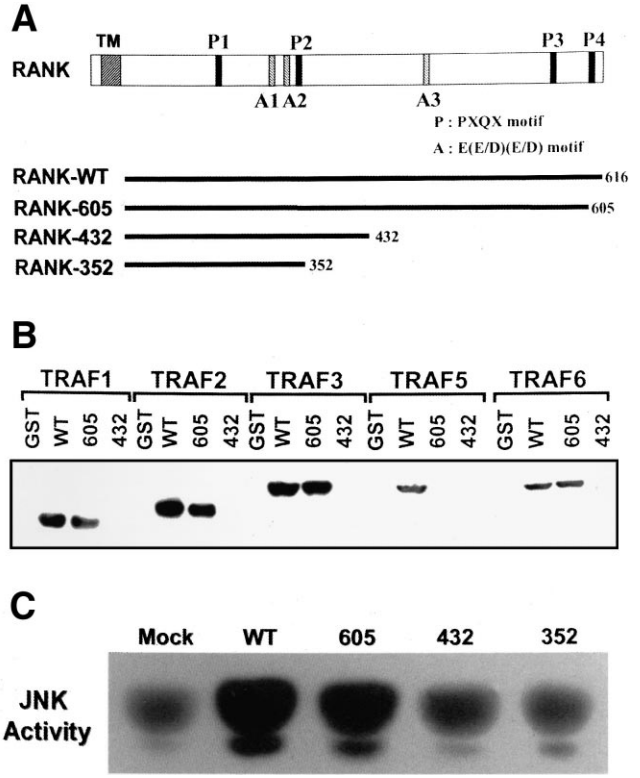

Fig. 4. Identification of the regions in the RANK cytoplasmic domain responsible for binding of TRAFs and JNK activation. A: The schematic structure of the cytoplasmic domain of hRANK is shown. P1, P2, P3, and P4 denote the PXQX motifs, and A1, A2, and A3 indicate the acidic triplet motifs present in RANK. The intracellular domains of the wild type and C-terminal deletion mutants (RANK-ICD-WT, -605, and -432) were produced as GST fusion proteins. In addition, mammalian expression plasmids (pSRa) containing the extracellular and transmembrane domains as well as the cytoplasmic tail were generated for the wild type RANK and RANK-605, -432, and -352 deletion mutants. B: The GST fusion proteins of the wild type and deletion mutants of RANK intracellular domain were incubated with lysates from 293-EBNA cells expressing Flag-tagged TRAF proteins. The complexes were precipitated with glutathione beads and subjected to Western blotting with anti-Flag. C: 293-EBNA cells were transfected with the indicated pSRa-RANK construct or the control vector. The JNK activity was measured with equal amounts of cell lysates by the solid-phase kinase assay as described in Section 2.

TRAF1, 2, 3, 5, and 6 in vitro (Fig. 1B) and to be associated with these TRAF molecules in vivo (Fig. 2B). In experiments with C-terminal deletion mutants of RANK, the binding site for TRAF5 was determined to be the C-terminal end (amino acids 606–616), and that for the other TRAFs amino acids 433–605 (Fig. 4B). The TRAF5 binding region of RANK contains the sequence ^{607}PVQEQ similar to the PXQXT motif shown to be important for the interaction of CD40, CD30, or LMP-1 with TRAF1, 2, 3, and 5 [15,17,20,21]. Similar sequences, PIQEE and PIQED, have been implicated in the binding of TRAF1, 2, and 3 to CD27 and Ox40, respectively [36,37], which indicates the minimal sequence requirement for TRAF binding might be proline and glutamine with an intervening amino acid. Another PXQX motif (^{569}PVQEE) is present in the C-terminal portion of RANK where TRAF1, 2, 3, and 6 were found to bind. In addition, there is an acidic triplet

sequence ^{481}EEE which was shown to mediate the binding of TRAF1, 2, and 3 to CD30 and 4-1BB [20,22]. Whether the ^{569}PVQEE and/or ^{481}EEE sequences are critical for the interaction between RANK and TRAF1, 2, 3, or 6 is under investigation by introduction of point mutations in those sequences.

The JNK activation cascade was found to be involved in RANK signal transduction (Fig. 3). The region responsible for the stimulation of JNK activity was mapped to amino acids 433–605 of RANK (Fig. 4C), where also the association of TRAFs 1, 2, 3, and 6, but not that of TRAF5, apparently occurred (Fig. 4B), excluding the role of TRAF5 in this aspect of RANK signaling. Considering that TRAF2, 5, and 6 have been reported to be involved in the JNK activation induced by some TNFR superfamily receptors [9,13,23,25,27,28,38], the RANK-dependent JNK activation might be expected to be mediated by TRAF2 and/or TRAF6. Recently, TRAF3 was reported to participate in the CD40-induced JNK activation [39], implying that the mediation of JNK activation by TRAF3 is possible in RANK signal transduction. In addition, the possibility that other TRAF molecules yet to be identified bind to the same region of RANK and link RANK to the JNK cascade or that the RANK-induced JNK activation is TRAF-independent cannot be ruled out.

During the preparation of this article, studies on TRAF binding to human and mouse RANK were reported [40,41]. In those studies, TRAF2, 5, and 6 were found to bind hRANK and additional TRAF1 and 3 to bind mRANK in vitro. The in vivo association was demonstrated only with TRAF2 and hRANK. Here, we showed the in vivo interaction of hRANK with TRAF1, 3, 5, and 6, in addition to TRAF2. The binding sites for TRAFs and the role of TRAF binding in the signal transduction appeared different for human and mouse RANK proteins. The binding site for TRAF6 in hRANK was mapped to the C-terminal 85 amino acids [40], whereas that for mRANK seems to be N-terminal to that region [41]. In our study, TRAF5 was found to bind the very C-terminal 11 amino acids that contain the PXQX motif (PVQEQ at 607–611 of hRANK, corresponding to amino acids 604–608 of mRANK). However, in mRANK, TRAF5 binding appeared to occur in a more upstream portion [41]. In this regard, it is intriguing to note that mRANK contains an additional PXQX motif (^{595}PRQKD) in the TRAF5 binding region. Additionally, the domain responsible for NF-κB activation signaling was identified as the C-terminal 85 amino acids in hRANK [40], which was least effective among multiple regions that induced NF-κB activation for mRANK [41]. Further investigations on RANK signal transduction and comparative studies on physiological functions of the RANK protein in mice and humans will be required to resolve these discrepancies and to understand the structure-function relationships of this new TNFR family receptor.

Acknowledgements: The authors thank Drs. Ho Y. Song (Lilly Co. Center, Indianapolis, IN, USA), J. Silvio Gutkind (NIH, Bethesda, MD, USA), and Rae K. Park (Wonkwang University, Iksan, South Korea) for generously providing constructs. We also thank Drs. Kyung K. Kim (Chonnam University, Kwangju, South Korea) and Ho Y. Song for helpful suggestions. This research was supported by the academic research fund of the Ministry of Education, Republic of Korea BM-97-250 and in part by research funds from Chosun University, 1996.

References

[1] Anderson, D.M., Maraskovsky, E., Billingsley, W.L., Dougall, W.C., Tomesko, M.E., Roux, E.R., Teepe, M.C., DuBose, R.F., Cosman, D. and Galibert, L. (1997) Nature 390, 175–179.
[2] Wong, B.R., Rho, J., Arron, J., Robinson, E., Orlinick, J., Chao, M., Kalachikov, S., Cayani, E., Bartlett III, F.S., Frankel, W.N., Lee, S.Y. and Choi, Y. (1997) J. Biol. Chem. 272, 25190–25194.
[3] Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S.-I., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N. and Suda, T. (1998) Proc. Natl. Acad. Sci. USA 95, 3597–3602.
[4] Lacey, D.L., Timms, E., Tan, H.-L., Kelley, M., Dunstan, C.R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y.-X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J. and Boyle, W.J. (1998) Cell 93, 165–176.
[5] Wong, B.R., Josien, R., Lee, S.Y., Sauter, B., Li, H.-L., Steinman, R.M. and Choi, Y. (1997) J. Exp. Med. 186, 2075–2080.
[6] Baker, S.J. and Reddy, E.P. (1996) Oncogene 12, 1–9.
[7] Kwon, B.S., Tan, K.B., Ni, J., Oh, K.-O., Lee, Z.H., Kim, K.K., Kim, Y.-J., Wang, S., Gentz, R., Yu, G.-L., Harrop, J., Lyn, S.D., Silverman, C., Porter, T.G., Truneh, A. and Young, P.R. (1997) J. Biol. Chem. 272, 14272–14276.
[8] Hsu, H., Shu, H.-B., Pan, M.-G. and Goeddel, D.V. (1996) Cell 84, 299–308.
[9] Rothe, M., Wong, S.C., Henzel, W.J. and Goeddel, D.V. (1994) Cell 78, 681–692.
[10] Sato, T., Irie, S. and Reed, J.C. (1995) FEBS Lett. 358, 113–118.
[11] Ishida, T.K., Tojo, T., Aoki, T., Kobayashi, N., Ohishi, T., Watanabe, T., Yamamoto, T. and Inoue, J. (1996) Proc. Natl. Acad. Sci. USA 93, 9437–9442.
[12] Cheng, G., Cleary, A.M., Ye, Z.S., Hong, D.I., Lederman, S. and Baltimore, D. (1995) Science 267, 1494–1498.
[13] Ishida, T., Mizushima, S., Azuma, S., Kobayashi, N., Tojo, T., Suzuki, K., Aizawa, S., Watanabe, T., Mosialos, G., Kieff, E., Yamamoto, T. and Inoue, J. (1996) J. Biol. Chem. 271, 28745–28748.
[14] Nakano, H., Oshima, H., Chung, W., Williams-Abbott, L., Ware, C.F., Yagita, H. and Okumura, K. (1996) J. Biol. Chem. 271, 14661–14664.
[15] Gedrich, R.W., Gilfillan, M.C., Duckett, C.S., van Dongen, J.L. and Thompson, C.B. (1996) J. Biol. Chem. 271, 12852–12858.
[16] Regnier, C.H., Tomasetto, C., Moog-Lutz, C., Chenard, M.P., Wendling, C., Basset, P. and Rio, M.C. (1995) J. Biol. Chem. 270, 25715–25721.
[17] Devergne, O., Hatzivassiliou, E., Izumi, K.M., Kaye, K.M., Kleijnen, M.F., Kieff, E. and Mosialos, G. (1996) Mol. Cell. Biol. 16, 7098–7108.
[18] Mosialos, G., Birkenbach, M., Yalamanchili, R., van Arsdale, T., Ware, C. and Kieff, E. (1995) Cell 80, 389–399.
[19] Takeuchi, M., Rothe, M. and Goeddel, D.V. (1996) J. Biol. Chem. 271, 19935–19942.
[20] Boucher, L.-M., Marengere, L.E.M., Lu, Y., Thukral, S. and Mak, T.W. (1997) Biochem. Biophys. Res. Commun. 233, 592–600.
[21] Devergne, O., McFarland, E.C., Mosialos, G., Izumi, K.M., Ware, C.F. and Kieff, E. (1998) J. Virol. 72, 7900–7908.
[22] Jang, I.K., Lee, Z.H., Kim, Y.J., Kim, S.H. and Kwon, B.S. (1998) Biochem. Biophys. Res. Commun. 242, 613–620.
[23] Rothe, M., Sarma, V., Dixit, V.M. and Goeddel, D.V. (1995) Science 269, 1424–1427.
[24] Aizawa, S., Nakano, H., Ishida, T., Horie, R., Nagai, M., Ito, K., Yagita, H., Okmura, K., Inoue, J. and Watanabe, T. (1997) J. Biol. Chem. 272, 2042–2045.
[25] Cao, Z., Xiong, J., Takeuchi, M., Kurama, T. and Goeddel, D.V. (1996) Nature 383, 443–446.
[26] Song, H.Y., Regnier, C.H., Kirschning, C.J., Goeddel, D.V. and Rothe, M. (1997) Proc. Natl. Acad. Sci. USA 94, 9792–9796.
[27] Natoli, G., Antonio, C., Ianni, A., Templeton, D.J., Woodgett, J.R., Balsano, C. and Levrero, M. (1997) Science 275, 200–203.

[28] Lee, S.Y., Reichlin, A., Santana, A., Sokol, K.A., Nussenzweig, M.C. and Choi, Y. (1997) Immunity 7, 703–713.
[29] Kwon, B.S., Wang, S., Udagawa, N., Haridas, V., Lee, Z.H., Kim, K.K., Oh, K.-O., Greene, J., Li, Y., Su, J., Gentz, R., Aggarwal, B.B. and Ni, J. (1998) FASEB J. 12, 845–854.
[30] Kim, H.-H., Sierke, S.L. and Koland, J.G. (1994) J. Biol. Chem. 269, 24747–24755.
[31] Kim, H.-H., Tharayil, M. and Rudd, C.E. (1998) J. Biol. Chem. 273, 296–301.
[32] Hibi, M., Lin, A., Smeal, T., Minden, A. and Karin, M. (1993) Genes Dev. 7, 2135–2148.
[33] Tartaglia, L.A. and Goeddel, D.V. (1992) Immunol. Today 13, 151–153.
[34] Smith, C.A., Farrah, T. and Goodwin, R.G. (1994) Cell 76, 959–962.
[35] Regnier, C.H., Song, H.Y., Gao, X., Goeddel, D.V., Cao, Z. and Rothe, M. (1997) Cell 90, 373–383.
[36] Yamamoto, H., Kishimoto, T. and Minamoto, S. (1998) J. Immunol. 161, 4753–4759.
[37] Arch, R.H. and Thompson, C.B. (1998) Mol. Cell. Biol. 18, 558–565.
[38] Liu, Z.G., Hsu, H., Goeddel, D.V. and Karin, M. (1996) Cell 87, 565–576.
[39] Grammer, A.C., Swantek, J.L., McFarland, R.D., Miura, Y., Geppert, T. and Lipsky, P.E. (1998) J. Immunol. 161, 1183–1193.
[40] Darnay, B.G., Haridas, V., Ni, J., Moore, P.A. and Aggarwal, B.B. (1998) J. Biol. Chem. 273, 20551–20555.
[41] Wong, B.R., Josien, R., Lee, S.Y., Vologodskaia, M., Steinman, R.M. and Choi, Y. (1998) J. Biol. Chem. 273, 28355–28359.