Evidence for a Role of a Tumor Necrosis Factor-α (TNF-α)-converting Enzyme-like Protease in Shedding of TRANCE, a TNF Family Member Involved in Osteoclastogenesis and Dendritic Cell Survival*

Lawrence Lum,a,b,c,d Brian R. Wong, a,d,e,f Régis Josien,g J. David Becherer,h Hediye Erdjument-Bromage,i Johannes Schlöndorf, a,b,i Paul Tempst, i,k Yongwon Choi,i,l,m and Carl P. Blobelb,j,h,m

From the “Tri-Institutional (Cornell / Rockefeller University / Memorial Sloan-Kettering Cancer Center) M.D. / Ph.D. Training Program, New York, New York 10021, the "Cellular Biochemistry and Biophysics Program and Molecular Biology Program, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, the "Department of Molecular Biochemistry, Glaxo Wellcome Research and Development Inc., Research Triangle Park, North Carolina 27709, and the "Laboratory of Immunology, Laboratory of Cellular Physiology and Immunology, and 1Howard Hughes Medical Institute, Rockefeller University, New York, New York 10021

Tumor necrosis factor (TNF)-related activation-induced cytokine (TRANCE), a member of the TNF family, is a dendritic cell survival factor and is essential for osteoclastogenesis and osteoclast activation. In this report we demonstrate (i) that TRANCE, like TNF-α, is made as a membrane-anchored precursor, which is released from the plasma membrane by a metalloprotease; (ii) that soluble TRANCE has potent dendritic cell survival and osteoclastogenic activity; (iii) that the metalloprotease-disintegrin TNF-α convertase (TACE) can cleave immunoprecipitated TRANCE in vitro in a fashion that mimics the cleavage observed in tissue culture cells; and (iv) that in vitro cleavage of a TRANCE ectodomain/CD8 fusion protein and of a peptide corresponding to the TRANCE cleavage site by TACE occurs at the same site that is used when TRANCE is shed from cells into the supernatant. We propose that the TRANCE ectodomain is released from cells by TACE or a related metalloprotease-disintegrin, and that this release is an important component of the function of TRANCE in bone and immune homeostasis.

Received for publication, February 2, 1999

1 The abbreviations used are: TNF, tumor necrosis factor; TRANCE, TNF-related activation-induced cytokine; TRANCE-R, TRANCE receptor; TACE, TNF-α convertase; OPG/OCIF, osteoprotegerin/osteoclast inhibitory factor; βAPP, β-amyloid precursor protein; PBS, phosphate-buffered saline; GST, glutathione S-transferase; mAb, monoclonal antibody; DC, dendritic cell; PAGE, polyacrylamide gel electrophoresis; STI, soybean trypsin inhibitor; TPCN, N-tosyl-l-phenylalanine chloromethyl ketone; TPA, 12-O-tetradecanoylphorbol-13-acetate.

* This work was supported in part by a grant from Glaxo-Wellcome (to C. P. B.), by National Institutes of Health NIAID Grant AI44264 (to Y. C.), and by National Science Foundation Grant DBI-942013 (to P. T.). 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.

** Supported in part by National Institutes of Health MSTP Training Grant 5T32GM07739-17 and the Robert Wood Johnson Jr. Charitable Trust Endowment Fund.

*** The first and second authors contributed equally to this report.

† Supported by National Institutes of Health Medical Scientist Training Program Training Grant 5T32GM07739-17.

‡ Supported by National Institutes of Health Medical Scientist Training Program Training Grant 5ST2GM07739-17 and the Louis and Rachel Rudin Family Foundation.

§ Supported by Memorial Sloan-Kettering Cancer Center Support Grant NCI-P30-CA-08748.

‖ An investigator of the Howard Hughes Medical Institute.

* To whom correspondence should be addressed: Cellular Biochemistry and Biophysics Program, Sloan-Kettering Inst., Memorial Sloan-Kettering Cancer Center, Box 368, 1275 York Ave., New York, NY 10021. Tel.: 212-639-2915; Fax: 212-717-3047; E-mail: c-blobel@ski.mskcc.org.
TRANCE Ectodomain Shedding

To generate full-length TRANCE, COS-7 terminal Sequence Analysis—eluted with sample loading buffer and boiling at 95 °C for 5 min prior to drying and exposure to autoradiography film (Kodak XAR). M2 anti-FLAG mAb was purchased from Sigma. A cDNA fragment encoding for the human TACE cytotail (corresponding to amino acids 695–824) was cloned in frame to the coding region of GST in the pGEX-4T-1 vector (Amersham Pharmacia Biotech). The GST-TACE cytotail fusion protein was expressed and purified from BL21 bacteria and used as an immunogen to raise rabbit polyclonal antisera as described previously (15).

**MATERIALS AND METHODS**

**cDNA Constructs and Reagents**—A FLAG-tagged full-length murine TRANCE expression vector (pFLAG-TRANCE) has been described (5). hCD8-TRANCE was expressed in baculovirus and purified on an α-hCD8-Sepharose column as described (1). Mouse TRANCE-R fused to human IgG1 (TR-Fc) was cloned into the vector PVLI1392 and expressed in baculovirus. Purification was performed by binding to a protein A-Sepharose column, eluting with glycine (0.2 M, pH 2.9), and dialyzing against PBS. M2 anti-FLAG mAb was purchased from Sigma. A cDNA fragment encoding for the human TACE cytotail (corresponding to amino acids 695–824) was cloned in frame to the coding region of GST in the pGEX-4T-1 vector (Amersham Pharmacia Biotech). The GST-TACE cytotail fusion protein was expressed and purified from BL21 bacteria and used as an immunogen to raise rabbit polyclonal antisera as described previously (15).

**Cell Cultures**—The T cell hybridoma KMLS-8.3.5.1 (5) was grown in S-minimal essential medium supplemented with 10% fetal bovine serum. COS-7 and 293T cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. 

**Transient Transfections**—COS-7 cells were transiently transfected with pcDNA3 vector or pFLAG-TRANCE vector with LipofectAMINE (Life Technologies, Inc.) following the manufacturer’s suggestions. Human 293T cells were transiently transfected with similar constructs using a standard calcium phosphate method.

**Pulse-Chase Analysis**—COS-7 cells transiently transfected with either pcDNA3 vector or pFLAG-TRANCE were subjected to pulse-chase with 200 μCi of [35S]-labeled methionine and cysteine (EXPRESS, NEN Life Sciences) as described previously (16). After chasing in Opti-MEM (Life Technologies, Inc.) for different amounts of time, supernatant and lysates were separated on a 10% SDS-PAGE, and visualized with band rainbow. The bands were cut out and analyzed by automated N-terminal amino acid residues were analyzed by automated

**RESULTS AND DISCUSSION**

To determine if TRANCE, like TNF-α, is shed from the plasma membrane by a protease, pulse-chase analysis was performed in COS-7 cells transiently expressing full-length TRANCE with a cytoplasmic FLAG epitope tag (FLAG-TRANCE). Immediately after the pulse labeling, two closely co-migrating bands of 46 and 48 kDa could be immunoprecipitated from the extracts with an anti-FLAG mAb (Fig. 1, lane 2). After deglycosylation of an identical sample with peptide:N-glycosidase F, only a 35-kDa band was detected, corresponding to the predicted molecular weight of membrane-anchored TRANCE (data not shown). As the duration of the chase increased from 3 to 12 h (Fig. 1A, lanes 3–5), the relative amount of 46- and 48-kDa proteins immunoprecipitated with anti-FLAG mAb decreased. Simultaneously, a 26-kDa band, which could be precipitated from the culture supernatant using a soluble TRANCE-receptor Fc fusion protein (TR-Fc), increased in intensity (Fig. 1A, lanes 7–9). N-terminal sequence analysis (see below) confirmed that the 26-kDa protein is a
soluble form of TRANCE (referred to as ecto-TRANCE hereafter). After a 12-h chase period, an additional band of 24 kDa, which most likely represents a minor shed TRANCE product, could also be seen in the supernatant (Fig. 1A, lane 9). These observations indicate that TRANCE, like TNF-α, can be released from the cell surface.

To confirm that TRANCE shedding also occurs in non-transfected cells, a similar experiment was performed using the T cell hybridoma KMLs-8.3.5.1 (5) from which TRANCE was originally cloned. Hybridoma cells were labeled overnight with [35S]methionine/cysteine and then stimulated for 3 h with 500 ng/ml ionomycin and 50 ng/ml phorbol 12-myristate 13-acetate following labeling overnight with [35S]methionine/cysteine. Supernatant (lanes 3 and 4) and lysate samples (lanes 1 and 2) were collected and incubated with TR-Fc followed by protein A (lanes 2 and 4), or protein A beads alone (lanes 1 and 3). All samples were reduced prior to electrophoresis.

Fig. 1. Release of a soluble TRANCE ectodomain from transfected COS-7 cells and from a T cell hybridoma. A, pulse-chase analysis of COS-7 cells transiently transfected with pcDNA3 (lanes 1 and 6) or FLAG-TRANCE (lanes 2–5 and 7–9). At the indicated time points, full-length TRANCE was immunoprecipitated from cell lysates with the anti-FLAG mAb (lanes 1–5), and soluble TRANCE was precipitated from supernatants with TR-Fc (lanes 6–9). B, T cell hybridoma cells (KMLs-8.3.5.1) were stimulated for 3 h with 500 ng/ml ionomycin and 50 ng/ml phorbol 12-myristate 13-acetate following labeling overnight with [35S]methionine/cysteine. Supernatant (lanes 3 and 4) and lysate samples (lanes 1 and 2) were collected and incubated with TR-Fc followed by protein A (lanes 2 and 4), or protein A beads alone (lanes 1 and 3). All samples were reduced prior to electrophoresis.

To address whether ecto-TRANCE is indeed functional, its activity was tested in osteoclast differentiation and in DC survival assays. Bone marrow precursors cultured in macrophage colony-stimulating factor (30 ng/ml) were incubated in medium with hCD8-TRANCE (1 μg/ml), 293T/Vec (1:50 dilution), or 293T/TRANCE (1:50 dilution) with or without purified TR-Fc (10 μg/ml) or hIgG1 (10 μg/ml). Six days later, osteoclast differentiation was assessed in bone marrow cultures by measuring tartrate-resistant acid phosphatase activity in the cell lysates as described under “Materials and Methods.” A representative result of the absorbance values (OD405) of three independent experiments is shown. Error bars denote the standard deviations of conditions performed in triplicate. B, mature bone marrow-derived dendritic cells incubated with medium with hCD8-TRANCE (1 μg/ml), 293T/Vec (1:50), or 293T/TRANCE (1:50) with or without purified TR-Fc (10 μg/ml) or hIgG1 (10 μg/ml). 48 h after treatment, the cell viability was assessed by trypan blue exclusion. A representative result of three independent experiments is shown. Error bars denote the standard deviations of conditions performed in triplicate.

TRANCE ectodomain shedding is a potent osteoclastogenic and dendritic cell survival factor. Supernatants from TRANCE-transfected (293T/TRANCE) or vector-transfected (293T/Vec) 293T cells were filtered and centrifuged (100,000 x g) to remove cells and cellular membranes. A, bone marrow cells cultured in macrophage colony-stimulating factor (30 ng/ml) were incubated in medium with hCD8-TRANCE (1 μg/ml), 293T/Vec (1:50 dilution), or 293T/TRANCE (1:50 dilution) with or without purified TR-Fc (10 μg/ml) or hIgG1 (10 μg/ml). Six days later, osteoclast differentiation was assessed in bone marrow cultures by measuring tartrate-resistant acid phosphatase activity in the cell lysates as described under “Materials and Methods.” A representative result of the absorbance values (OD405) of three independent experiments is shown. Error bars denote the standard deviations of conditions performed in triplicate. B, mature bone marrow-derived dendritic cells incubated with medium with hCD8-TRANCE (1 μg/ml), 293T/Vec (1:50), or 293T/TRANCE (1:50) with or without purified TR-Fc (10 μg/ml) or hIgG1 (10 μg/ml). 48 h after treatment, the cell viability was assessed by trypan blue exclusion. A representative result of three independent experiments is shown. Error bars denote the standard deviations of conditions performed in triplicate.

Since shedding of many cell surface proteins can be stimulated with saturating doses of a soluble receptor, TR-Fc. Fig. 2B shows that ecto-TRANCE-containing supernatants enhanced dendritic cell survival and this effect could also be inhibited by the addition of TR-Fc, indicating that the shed form of TRANCE is functionally active as a survival factor for mature DCs.

Fig. 2. Shed TRANCE is a potent osteoclastogenic and dendritic cell survival factor. Supernatants from TRANCE-transfected (293T/TRANCE) or vector-transfected (293T/Vec) 293T cells were filtered and centrifuged (100,000 x g) to remove cells and cellular membranes. A, bone marrow cells cultured in macrophage colony-stimulating factor (30 ng/ml) were incubated in medium with hCD8-TRANCE (1 μg/ml), 293T/Vec (1:50 dilution), or 293T/TRANCE (1:50 dilution) with or without purified TR-Fc (10 μg/ml) or hIgG1 (10 μg/ml). Six days later, osteoclast differentiation was assessed in bone marrow cultures by measuring tartrate-resistant acid phosphatase activity in the cell lysates as described under “Materials and Methods.” A representative result of the absorbance values (OD405) of three independent experiments is shown. Error bars denote the standard deviations of conditions performed in triplicate. B, mature bone marrow-derived dendritic cells incubated with medium with hCD8-TRANCE (1 μg/ml), 293T/Vec (1:50), or 293T/TRANCE (1:50) with or without purified TR-Fc (10 μg/ml) or hIgG1 (10 μg/ml). 48 h after treatment, the cell viability was assessed by trypan blue exclusion. A representative result of three independent experiments is shown. Error bars denote the standard deviations of conditions performed in triplicate.

Since shedding of many cell surface proteins can be stimulated with saturating doses of a soluble receptor, TR-Fc. Fig. 2B shows that ecto-TRANCE-containing supernatants enhanced dendritic cell survival and this effect could also be inhibited by the addition of TR-Fc, indicating that the shed form of TRANCE is functionally active as a survival factor for mature DCs.

Since shedding of many cell surface proteins can be stimulated with saturating doses of a soluble receptor, TR-Fc. Fig. 2B shows that ecto-TRANCE-containing supernatants enhanced dendritic cell survival and this effect could also be inhibited by the addition of TR-Fc, indicating that the shed form of TRANCE is functionally active as a survival factor for mature DCs.
residual component TRANCE shedding is not inhibited even by high doses of BB-94 in the TPA-treated sample. Since BB-94 also does not inhibit TRANCE shedding in unstimulated cells, other proteases besides metalloproteases may play a role in the constitutive release of TRANCE into the supernatant. A similar observation has been reported for the β-amylloid precursor protein (βAPP) (23). In fibroblasts lacking TACE, the phorbol 12-myristate 13-acetate-dependent shedding of βAPP is abolished, while a low level of constitutive shedding of βAPP, which is not inhibited by the hydroxamate-based metalloprotease inhibitor TAPI-2, is still present. Only a small percentage of total TRANCE was released, as levels of TRANCE in the cell lysate did not decrease with TPA stimulation compared with untreated cells (Fig. 3a, lane 1) or increase in BB-94-treated cells. Taken together, these results provide the first evidence that TACE, like TNF-α, βAPP, and other shed proteins can be released in response to phorbol esters (12, 24, 25), and that this release can be inhibited by a hydroxamate-based metalloprotease inhibitor.

To test for a potential role of TACE in the shedding of TRANCE, metabolically labeled full-length TRANCE was immunoprecipitated and incubated in vitro with recombinant TACE. This treatment yielded polypeptides of 23 and 26 kDa (Fig. 3c, lane 3) that were not visible in the untreated sample (Fig. 3c, lane 2). A likely explanation for the relatively inefficient processing of immunoprecipitated TRANCE in Nonidet P-40 by soluble TACE is that TACE and its substrate may both need to be membrane-anchored for optimal cleavage to occur. The 26-kDa band generated in vitro by TACE co-migrated with the ecto-TRANCE isolated from the supernatant of transfected COS-7 cells (Fig. 3c, lane 1), whereas the 23-kDa product did not. As a control for specificity, immunoprecipitated full-length TRANCE was also incubated with MMP-1, a member of the matrix metalloprotease family (26) and media with TR-Fc (lane 1). Immunoprecipitated full-length TRANCE on protein A beads was washed into PBS and incubated alone (lanes 2 and 4), with 2.5 μg/ml TACE (lanes 3 and 5) or 0.1 μg/ml MMP-1 (lane 6) at 37°C for 5 h. Solid arrow, products of in vitro cleavage with TACE; open arrow, products of in vitro cleavage with MMP-1. D, Western blot analysis of cell lysates of untransfected COS-7 cells (lanes 1 and 2), of monocytic THP-1 cells as a positive control (lanes 3 and 4), and of 293T cells (lanes 5 and 6) probed with an antibody raised against a GST fusion protein with the cytoplasmic domain of TACE (lanes 2, 3, and 6) or a preimmune antiserum (lanes 1, 4, and 5). Samples in A–C, but not D, were reduced prior to electrophoresis.
below and Ref. 3), whereas the other corresponded to the N terminus of human CD8 after removal of its signal sequence. N-terminal sequence analysis of the 24-kDa band (Fig. 4A, lane 2) revealed only the hCD8 N terminus (data not shown). The higher molecular weight bands seen in Fig. 4A (lane 2) presumably represent intermediate in vitro cleavage products. N-terminal sequencing of ecto-TRANCE released into the supernatant of transfected 293T cells (Fig. 4A, lane 5) confirmed the previously reported cleavage site of TRANCE, and was identical to the cleavage site generated by TACE (Fig. 4B and Ref. 3). These results demonstrate that a major in vitro cleavage site for TACE in the ectodomain of TRANCE is identical to the cleavage site of the protease that releases ecto-TRANCE from 293T cells (see diagram in Fig. 4C).

To further evaluate the cleavage of TRANCE, we determined the cleavage site and kinetics for processing of TRANCE and TNF-α peptides by TACE. The TRANCE peptide was cleaved in the correct position by TACE, but the specificity constant was 1000-fold lower than for the TNF-α peptide (Table I). MMP-1 did not cleave the TRANCE peptide (data not shown). This is consistent with the observation that TACE cleavage of immunoprecipitated pro-TRANCE produced a protein that co-migrated with TRANCE shed from cells, whereas cleavage with MMP-1 did not (see Fig. 2C). With respect to the peptide cleavage specificity of metalloprotease-disintegrins, we note that both ADAM10 (KUZ/MADM) and TACE cleave a TNF-α peptide at the physiological position (9, 10, 27), while MDC9 has a clearly distinct specificity compared with TACE (21). The correct cleavage of the TRANCE peptide by TACE therefore suggests that TACE, or a metalloprotease with a similar substrate specificity, cleaves TRANCE in cells. The difference in specificity constants of TACE for the TRANCE and TNF-α peptides (1000-fold) is similar to the reported difference in peptide cleavage efficiency of TACE for TNF-α and the putative TACE substrate L-selectin (2250-fold) (11). A general question raised by these observations is whether TACE substrate recognition in cells involves additional targeting events between the protease and the substrate (11, 21, 28, 29), or alternatively if TRANCE is actually cleaved by another related metalloprotease with a higher specificity constant for the TRANCE cleavage site.

The results presented here demonstrate that a phorbol ester-inducible metalloprotease can release ecto-TRANCE from the plasma membrane. The finding that incubation of the hCD8-TRANCE ectodomain or of a TRANCE peptide with recombinant TACE generates a fragment with the identical N terminus as ecto-TRANCE suggests that TACE, or a related metalloprotease, mediates TRANCE shedding. Soluble TRANCE has potent functional activity in promoting dendritic cell survival and osteoclast differentiation. In analogy to the regulation of TNF-α function by shedding from the plasma membrane (9, 10), our results suggest that TRANCE shedding may be an important aspect of the functional regulation of this protein. We propose that released TRANCE may mediate signaling as a soluble cytokine, and that the release of TRANCE may be an important factor in the TRANCE/TRANCE-R/osteonectin signaling axis.

Acknowledgments—We thank Masha Vologodskaya and Angela Santana for excellent technical assistance.

REFERENCES
1. 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
2. Anderson, D. M., Maraskovsky, E., Billingsley, W. L., Dougall, W. C., Tomatsco, M. E., Roux, E. R., Tepee, M. C., Dufrose, R. F., Cosman, D., and Galibert, L. (1997) Nature 390, 175–179
3. Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colomberto, 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., Gao, J., Delaney, J., and Boyle, W. J. (1990) Cell 63, 165–176
4. Yasuda, H., Shima, N., Nakagawa, N., Yamagaki, K., Kinosita, M., Mochizuki, S., Tomoyasu, A., Yanaka, K., Go, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N., and Suda, T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3597–3602
5. Wong, B. R., Rho, J., Arron, J., Robinson, E., Orlando, J., Choi, M., Kalashnikov, S., Cayani, E., Bartletti, P. S., iii, Frankel, W. N., Lee, S. Y., and Choi, H. (1997) J. Biol. Chem. 272, 25190–25194
6. Yasuda, H., Shima, N., Nakagawa, N., Mochizuki, S., I. Yano, K., Fujise, N., Sato, Y., Go, M., Yamagaki, K., Kuriyama, M., Kanno, T., Murakami, A., Tsuda, E., Morinaga, T., and Higashio, K. (1998) Endocrinology 139, 1329–1337
7. Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Luthy, R., Nguyen, H. Q., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colomberto, A., Tan, H. L., Trail, G., Sullivan, J.,

### Table I

| Substrate | Sequence | N-terminal product | $k_{cat}/K_m$ |
|-----------|----------|--------------------|--------------|
| TRANCE    | IVGQPR+FGSAPPA | IVGQPR             | 2 x $10^4$   |
| TNF-α     | SPLAQΑ+VRSSSR | SPLAQα             | 2 x $10^4$   |
Davy, E., Bucay, N., Renshaw-Gegg, L., Hughes, T. M., Hill, D., Pattison, W., Campbell, P., Boyle, W. J., et al. (1997) Cell 89, 309–319
8. Bucay, N., Sarosi, I., Dunstan, C. R., Morony, S., Tarpley, J., Capparelli, C., Scully, S., Tan, H. L., Xu, W., Lacey, D. L., Boyle, W. J., and Simonet, W. S. (1998) Genes Dev. 12, 1260–1268
9. Black, R., Rauch, C. T., Kozlosky, C. J., Peschon, J. J., Slack, J. L., Wolfson, M. F., Castner, B. J., Stocking, K. L., Reddy, P., Srinivasan, S., Nelson, N., Boiani, N., Schooley, K. A., Gerhart, M., Davis, R., Fitzner, J. N., Johnson, R. S., Paxton, R. J., March, C. J., and Cerretti, D. P. (1997) Nature 385, 729–733
10. Moss, M. L., Jin, S.-L. C., Milla, M. E., Burkhart, W., Cartner, H. L., Chen, W.-J., Clay, W. C., Didibus, J. R., Hassler, D., Hoffman, C. R., Kost, T. A., Lambert, M. H., Lessnitzer, M. A., McCauley, P., McGeehan, G., Mitchell, J., Moyer, M., Pahel, G., Roque, W., Overton, L. K., Schoenen, F., Seaton, T., Su, J.-L., Warner, J., Willard, D., and Becherer, J. D. (1997) Nature 385, 733–736
11. Peschon, J. J., Slack, J. L., Reddy, P., Stocking, K. L., Sunnarborg, S. W., Lee, D. C., Russel, W. E., Castner, B. J., Johnson, R. S., Fitzner, J. N., Boyce, R. W., Nelson, N., Kozlosky, C. J., Wolfson, M. F., Rauch, T. C., Cerretti, D. P., R. J. P., March, C. J., and Black, R. A. (1998) Science 282, 1281–1284
12. Hooper, N. M., Karran, E. H., and Turner, A. J. (1997) Biochem. J. 321, 265–279
13. Tanaka, M., Itai, T., Adachi, M., and Nagata, S. (1999) Nat. Med. 4, 31–36
14. Tracey, K. J., and Cerami, A. (1994) Annu. Rev. Med. 45, 491–503
15. Kratzschmar, J., Lum, L., and Blobel, C. P. (1996) J. Biol. Chem. 271, 4593–4596
16. Lum, L., Reid, M. S., and Blobel, C. P. (1998) J. Biol. Chem. 273, 26236–26247
17. Botos, I., Scapozza, L., Zhang, D., Liotta, L. A., and Meyer, E. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2749–2754
18. Westkap, G., Kratzschmar, J., Reid, M., and Blobel, C. P. (1996) J. Cell Biol. 132, 717–726
19. Becherer, J. D., Howe, A., Patel, I., Wisely, B., LeVine, H., and McGeehan, G. M. (1997) J. Biol. Chem. Suppl. 15G, 139
20. Tempest, P., Geronmanos, S., Elcone, C., and Erdjument-Bromage, H. (1994) Methods Comp. Methods Enzymol. 6, 248–261
21. Roghani, M., Becherer, J. D., Moss, M. L., Atherton, R. E., Erdjument-Bromage, H., Arribas, J., Blackburn, R. E., Westkap, G., Tempest, P., and Blobel, C. P. (1999) J. Biol. Chem. 274, 3531–3540
22. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., and Steinman, R. M. (1992) J. Exp. Med. 176, 1693–1702
23. Buxbaum, J. D., Liu, K. N., Luo, Y., Slack, J. L., Stocking, R. L., Peschon, J. J., Johnson, R. S., Castner, B. J., Cerretti, D. P., and Black, R. A. (1999) J. Biol. Chem. 273, 27765–27767
24. Mullberg, J., Rauch, C. T., Wolfson, M. F., Castner, B., Fitzner, J. N., Otten-evans, C., Mohler, K. M., Cosman, D., and Black, R. A. (1999) FEBS Lett. 401, 235–238
25. Arribas, J., Coodly, L., Vollmer, P., Kishimoto, T. K., Rose-John, S., and Massague, J. (1996) J. Biol. Chem. 271, 11376–11382
26. Werb, Z. (1997) Cell 91, 439–442
27. Rosenstahl, M. S., Ko, S. C., Long, D. L., Brewer, M. T., Rosenzweig, B., Hedl, E., Anderson, L., Pyle, S. M., Moreland, J., Meyers, M. A., Kohno, T., Lyons, D., and Lichenstein, H. S. (1997) J. Biol. Chem. 272, 24588–24593
28. Blobel, C. P. (1997) Cell 90, 589–592
29. Black, R. A., and White, J. M. (1998) Curr. Opin. Cell Biol. 10, 654–659