Acid-induced Conformational Changes in Phosphoglucone Isomerase Result in Its Increased Cell Surface Association and Deposition on Fibronectin Fibrils*

Mohammad Amraei, Zongjian Jia, Pascal Reboul‡, and Ivan R. Nabi§

From the Département de pathologie et biologie cellulaire, Université de Montréal, Montréal, Québec H3C 3J7, Canada and the Osteoarthritis Research Unit, Hôpital Notre-Dame, Centre Hospitalier de l’Université de Montréal, Montréal, Québec H2L 4M1, Canada

Phosphoglucone isomerase (PGI) is a glycolytic enzyme that exhibits extracellular cytokine activity as autocrine motility factor, neuroleukin, and maturation factor and that has been recently implicated as an autoantigen in rheumatoid arthritis. In contrast to its receptor-mediated endocytosis at neutral pH, addition of 25 μg/ml of either Alexa 568- or FITC-conjugated PGI to NIH-3T3 cells at progressively acid pH results in its quantitatively increased association with cell surface fibrillar structures that is particularly evident at pH 5. A similar pH-dependent cell surface association of PGI is observed for first passage human chondrocytes obtained from osteoarthritic joints. At acid pH, PGI colocalizes with fibronectin fibrils, and this association occurs directly upon addition of PGI to the cells. In contrast to the receptor-mediated endocytosis of PGI, fibril association of 25 μg/ml PGI at pH 5 is not competed with an excess (2 mg/ml) of unlabeled PGI. PGI binding at acid pH is therefore neither saturable nor mediated by its receptor. PGI is enzymatically active as a dimer and we show here by non-denaturing gel electrophoresis as well as by glutaraldehyde cross-linking that it exists at neutral pH in a tetrameric form. Increasingly acid pH results in the appearance of PGI monomers that correlates directly with its enhanced cell surface association. However, glutaraldehyde cross-linked PGI is endocytosed at neutral pH and still exhibits enhanced cell surface binding at pH 5. Circular dichroism analysis revealed pH-dependent changes in the near but not the far UV spectra indicating that the tertiary structure of the protein is specifically altered at pH 5. Conformational changes of PGI and exposure of the monomer-monomer interface under acidic conditions, such as those encountered in the synovial fluid of arthritic joints, could therefore result in its deposition on the surface of joints and the induction of an autoimmune response.

Glucose-6-phosphate isomerase or phosphoglucone isomerase (PGI)1 is a glycolytic enzyme essential for neoglucogenesis that is equivalent to the autocrine motility factor (AMF)/neuroleukin/maturation factor (MF) cytokine (1–4). PGI is therefore a cytosolic enzyme that upon release from the cell acquires a de novo function as a neurokine, lymphokine, and tumor cell cytokine (4–8). While the mechanism of release of PGI remains uncertain, enhanced secretion of PGI following overexpression of PGI by stable transfection of NIH-3T3 cells induces cellular transformation and tumorigenicity (9). Serum PGI activity has long been reported and is associated with tumor expression (10, 11) indicating that this protein is actively released from both normal and tumor cells.

PGI exists as a dimer and enzyme dimerization is necessary for its enzymatic activity (12–15). The active site of the enzyme has been characterized by x-ray crystallography and is localized to the cleft between the two PGI monomers (16–19). The motifs responsible for PGI cytokine activity remain to be determined. Inhibitors of PGI isomerase activity block its cytokine activity (1, 20, 21). Reports that the bacterial form of PGI, whose sequence homology with the mammalian enzyme is limited to residues in the enzymatic active site, presents cytokine activity further supported a role for the active site in PGI cytokine activity (17, 22). However, neither cytokine activity or receptor binding of the bacterial or yeast forms of the enzyme were detected in NIH-3T3 cells (23) and punctual mutations in the PGI sequence that disrupt its enzymatic activity do not affect its cytokine function (24). The latter studies argue that motifs implicated in receptor binding include regions of the protein that present differences between the mammalian and bacterial forms of the enzyme including the N-terminal, C-terminal, and internal hook domains (18).

The PGI receptor, gp78 or AMF-R, is a seven-transmembrane domain G protein-coupled receptor (25). AMF-R expression is significantly increased in neoplastic tissue and its expression is correlated with tumor malignancy and poor survival and prognosis of patients with gastric, colorectal, bladder and esophageal carcinomas, cutaneous malignant melanoma, and pulmonary adenocarcinoma (26–32). In normal brain, AMF-R expression is increased during development and associated with learning and development implicating PGI cytokine activity in normal cellular activity (33, 34). AMF-R is expressed both at the cell surface where it associates with caveolae as well as within a smooth domain of the endoplasmic reticulum (35–39). AMF-R internalizes its ligand via both caveolae/raft-dependent endocytosis to the smooth ER and clathrin-dependent endocytosis to multivesicular bodies (35, 40–43). The latter pathway

1 The abbreviations used are: PGI, phosphoglucone isomerase; MES, 4-morpholineethanesulfonic acid; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; AMF, autocrine motility factor; ER, endoplasmic reticulum; CD, circular dichroism.

* This study was supported by a grant from the Canadian Institutes of Health Research. 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.

§ An Investigator of the Canadian Institutes of Health Research. To whom correspondence should be addressed: Département de pathologie et biologie cellulaire, Université de Montréal, C. P. 6128, succursale A, Montréal, Quebec H3C 3J7, Canada. Tel.: 514-343-6291; Fax: 514-343-2459; E-mail: ivan.robert.nabi@umontreal.ca.

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
Denaturation and Fibril Deposition of PGI at Acid pH

is associated with the recycling of AMP/PGI to cell surface fibronectin fibrils (40).

Interestingly, PGI has recently been identified as an autoantigen implicated in rheumatoid arthritis (RA) in the K/BxN mouse model as well as in humans (44, 45). PGI and anti-PIG are specifically localized to the articular surface of joints (45–47). However, the basis for the selective binding of this protein to the surface of the synovial lining as well as for the generation of an immune response against this ubiquitous self-antigen remains a paradox (46, 48). Indeed, the extent to which PGI autoantibodies are prevalent in the sera of RA patients remains controversial. While earlier reports indicated that 64% of sera from RA patients contain antibodies to PGI (45), more recent studies have questioned the prevalence and specificity of the PGI autoimmune response in RA (49–51). Localization of the autoimmune response to PGI to lymph nodes adjacent to the affected joints in the K/BxN mouse led the authors to suggest that PGI in the joint is different in form or quantity than that circulating in other regions of the body (52). We demonstrate here the dramatically increased binding of PGI to fibronectin fibrils at acid pH that corresponds directly to PGI denaturation and, more specifically, to changes in PGI tertiary structure. Localized acidosis in synovial fluid could therefore enable denaturation and consequent binding of circulating PGI to synovial cell extracellular matrix permitting the generation of an autoimmune response against exposed non-native PGI epitopes.

**EXPERIMENTAL PROCEDURES**

Cells and Materials—NIH-3T3 fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, vitamins, non-essential amino acids, glutamine, and penicillin-streptomycin antibiotics (Canadian Life Technologies) at 37 °C in a humidified atmosphere of 5% CO₂/95% air. Chondrocytes were obtained from articular cartilage (femoral condyles and tibial plateaus) of patients (aged 58 ± 8, mean ± S.D.) who had undergone total knee arthroplasty. All osteoarthritis (OA) patients were evaluated by a certified rheumatologist and were diagnosed as having OA based on the criteria developed by the American College of Rheumatology Diagnostic Subcommittee for OA (53). Chondrocytes were obtained from the articular cartilage by sequential enzymatic digestion at 37 °C as previously described (54), and cultured in DMEM supplemented with 10% fetal bovine serum (Canadian Life Technologies) and penicillin-streptomycin antibiotics (Canadian Life Technologies) at 37 °C in a humidified atmosphere of 5% CO₂/95% air incubator as previously described (35). Human chondrocytes were obtained from articular cartilage (femoral condyles and tibial plateaux) of patients (aged 58 ± 8, mean ± S.D.) who had undergone total knee arthroplasty. All osteoarthritis (OA) patients were evaluated by a certified rheumatologist and were diagnosed as having OA based on the criteria developed by the American College of Rheumatology Diagnostic Subcommittee for OA (53). Chondrocytes were released from the articular cartilage by sequential enzymatic digestion at 37 °C as previously described (54), and cultured in DMEM supplemented with 10% fetal bovine serum (Canadian Life Technologies) and penicillin-streptomycin antibiotics (Canadian Life Technologies) at 37 °C in a humidified atmosphere of 5% CO₂/95% air incubator as previously described (35). Human chondrocytes were obtained from articular cartilage (femoral condyles and tibial plateaux) of patients (aged 58 ± 8, mean ± S.D.) who had undergone total knee arthroplasty. All osteoarthritis (OA) patients were evaluated by a certified rheumatologist and were diagnosed as having OA based on the criteria developed by the American College of Rheumatology Diagnostic Subcommittee for OA (53). Chondrocytes were released from the articular cartilage by sequential enzymatic digestion at 37 °C as previously described (54), and cultured in DMEM supplemented with 10% fetal bovine serum (Canadian Life Technologies) and penicillin-streptomycin antibiotics (Canadian Life Technologies) at 37 °C in a humidified atmosphere of 5% CO₂/95% air incubator as previously described (35). Human chondrocytes were obtained from articular cartilage (femoral condyles and tibial plateaux) of patients (aged 58 ± 8, mean ± S.D.) who had undergone total knee arthroplasty. All osteoarthritis (OA) patients were evaluated by a certified rheumatologist and were diagnosed as having OA based on the criteria developed by the American College of Rheumatology Diagnostic Subcommittee for OA (53).

Rabbit PGI (P9544) was purchased from Sigma Chemical Co. (Oakville, Ontario). Mouse anti-fibronectin was purchased from Transduction Laboratories (Mississauga, Ontario) and Alexa 488-conjugated anti-mouse secondary antibody from Molecular Probes (Eugene, OR). Nondenatured protein molecular weight markers were purchased from Sigma and Kaleidoscope SDS molecular weight markers from BioRad. Antibody assays were performed using 1:100 dilution of rabbit anti-mouse antibodies and Alexa 488-conjugated anti-mouse secondary antibodies from Molecular Probes (Eugene, OR).

The Alexa Fluor 568 protein labeling kit and the FluoReporter® FITC protein labeling kit were purchased from Molecular Probes, and Alexa 488-conjugated anti-mouse secondary antibody from Molecular Probes (Eugene, OR). The Alexa Fluor 568 protein labeling kit and the FluoReporter® FITC protein labeling kit were purchased from Molecular Probes, and Alexa 488-conjugated anti-mouse secondary antibody from Molecular Probes (Eugene, OR). The Alexa Fluor 568 protein labeling kit and the FluoReporter® FITC protein labeling kit were purchased from Molecular Probes, and Alexa 488-conjugated anti-mouse secondary antibody from Molecular Probes (Eugene, OR). The Alexa Fluor 568 protein labeling kit and the FluoReporter® FITC protein labeling kit were purchased from Molecular Probes, and Alexa 488-conjugated anti-mouse secondary antibody from Molecular Probes (Eugene, OR). The Alexa Fluor 568 protein labeling kit and the FluoReporter® FITC protein labeling kit were purchased from Molecular Probes, and Alexa 488-conjugated anti-mouse secondary antibody from Molecular Probes (Eugene, OR).

**FIG. 1. Acid pH results in progressively increased fibrillar labeling of PGI.** NIH-3T3 cells were incubated for 30 min with 25 μg/ml Alexa 568-PGI in regular medium (control) or in bicarbonate-free medium containing 100 mM HEPES at pH 7.0 or pH 6.5 or 100 mM MES at pH 6.0, 5.5, or 5.0, as indicated. After fixation with 3% paraformaldehyde, and several washes with PBS-CM, cell-associated Alexa568-PGI was visualized directly by confocal microscopy. All images were acquired with the same confocal settings.

**Non-denaturing Gel Electrophoresis—**Lyophilized rabbit PGI was dissolved in water and protein concentration determined with the BCA protein assay (Pierce). 1.5 μg of mammalian PGI was loaded on non-denaturing 8% acrylamide gels prepared with an upper gel using Laemmli buffers without SDS (55). To a solution of 1.5 μg of PGI, HEPES or MES buffers at the indicated pH were added to a final concentration of 250 mM and then sample buffer consisting of glycerol and bromphenol blue was added. Protein bands and non-denatured protein markers (Jack bean urease: 272 kDa; bovine serum albumin: 132 and 66 kDa; chicken egg albumin: 45 kDa; carbonic anhydrase: 29 kDa) were revealed by silver staining.

**PGI Cross-linking—**Cross-linking of rabbit PGI with glutaraldehyde (MECALAB LTD. Montreal, Quebec, Canada) was carried out as previously described (56). Briefly, 0.5 mg/ml PGI was incubated with 0.1% (v/v) glutaraldehyde in 75 μl of PBS for different times at room temperature. The reaction was stopped by addition of SDS sample buffer. Samples and SDS molecular weight markers were boiled and reduced, separated in 5% SDS-polyacrylamide gels, and protein bands revealed by staining with Coomassie Blue. Alternatively, Alexa 568 and FITC-PGI were cross-linked, diluted with 2 ml of 100 mM glycine for at least 30 min to quench the reaction and then concentrated using Amicon filter units and added to cells at 25 μg/ml for fluorescent visualization as described previously.

**Circular Dichroism—**Circular dichroism (CD) analysis of PGI was performed using a Jasco 3-710 spectropolarimeter (Dept. of Chemistry and Biochemistry, Concordia University, Montreal, Quebec). Spectra were recorded in a 0.1-cm quartz cuvette at room temperature in MES- or HEPES-based buffers at pH 7.5, 6.5, and 5.5 and background signal obtained from parallel scans of the buffer alone were subtracted from the measurements. Far UV spectra of 0.1 μg/ml PGI were recorded from 260 to 190 nm at a speed of 100 nm/min in 0.2-nm steps and with a
signal averaging time of 0.25 s. Near UV spectra of 0.5 g/ml PGI were recorded from 320 to 250 nm at a speed of 20 nm/min in 0.2-nm steps and with a signal averaging time of 2 s. The presented spectra are representative of at least three separate scans.

RESULTS

Increased Cell Surface Fibrillar Binding of PGI Binding at Acid pH—PGI is an intracellular glycolytic enzyme however its ability to associate with the articular surface of joints (45–47) implicates the binding of exogenous PGI to the surface of cells. Incubation of NIH-3T3 cells with Alexa 568-conjugated PGI and fixation with paraformaldehyde results in the predominant visualization of its expression in multivesicular bodies (MVBs) as well as to more faintly labeled fibrils (40), a distribution still observed upon pH reduction of the medium to pH 6.5 (Fig. 1). At pH 6.0, fewer MVBs are labeled, likely due to inhibition of clathrin-dependent endocytosis at low pH (57). Further reduction to pH 5.5 and 5.0 results in the progressive accumulation of fibril-associated PGI such that at pH 5.0 the extent of cell-associated PGI is dramatically increased (Fig. 1). At pH 5.0, Alexa 568-PGI also exhibits significant association with cell-free regions of the substrate. Identical results were obtained using FITC-conjugated PGI although increased recycling of FITC-PGI to cell surface fibrils was observed at neutral pH compared with Alexa 568-PGI (not shown). Quantification of the binding of both Alexa 568 and FITC-PGI as a function of pH shows a dramatic increase in fluorescence at pH 5 (Fig. 2). In particular, a significant increase in binding is seen between labeling at pH 5 and pH 5.5. A similar pH-dependent increase in binding of Alexa 568-PGI to human chondrocytes obtained from patients with osteoarthritis was also observed (Fig. 3). As for NIH-3T3 cells, a dramatic increase in binding at pH 5 was observed compared with pH 5.5.

PGI labeling of NIH-3T3 cells at pH 5.0 colocalizes with fibronectin fibrils and is also observed to associate with non-fibrillar cell surface domains and the cell-free substrate (Fig. 4). Fibrillar association of PGI at pH 5.0 can be observed as early as 5 min after incubation with PGI (Fig. 4) indicating that PGI association with cell surface fibronectin occurs directly and not following PGI endocytosis and recycling (40). The enhanced binding of PGI to the cells at acid pH was not due to loss of cell integrity as subsequent incubation of the cells at neutral pH resulted in the internalization of cell associated PGI (Fig. 5, A and B). While 2 mg/ml unlabeled PGI inhibits the endocytosis of 25 µg/ml Alexa 568-PGI at pH 7.5 (Fig. 5, C–F), PGI association with cell surface fibronectin fibrils at pH 5.0 is not saturable, potentially nonspecific, and apparently not mediated by the PGI receptor, AMF-R.

Acid pH Induces Changes in PGI Conformation—PGI is active as a dimer of two monomers of ~66 kDa each (12–15). However, in non-denaturing gels, PGI equilibrated at pH 7.5 before loading migrates at 270 ± 14 kDa (Fig. 6A). In the presence of buffers at pH 6 and below, a band of 73 ± 4 kDa is detected corresponding to the monomeric form of the protein. While this band represents only a minor form at pH 6, increasing amounts of this band are detected at pH 5.5 and pH 5 such that at pH 5.0, only the monomeric form of the protein is present (Fig. 6A). The extent of formation of the monomeric
form correlates directly with the extent of fibrillar PGI deposition at pH 6.0, 5.5, and 5.0 (Figs. 1–3).

Cross-linking of PGI with glutaraldehyde for different times prior to SDS-PAGE (56) was used to further characterize the high molecular weight form of PGI (Fig. 6B). In the absence of cross-linking, PGI was detected exclusively as a monomer of 60 ± 5 kDa but with increasing time of cross-linking distinct protein bands appeared progressively at 124 ± 7 kDa, corresponding to PGI dimers, and at 242 ± 9 kDa (Fig. 6B). Taking into consideration differences in migration in non-denaturing gels and SDS-PAGE, the latter band is equivalent to the high molecular weight 270-kDa band observed at neutral pH by non-denaturing gel electrophoresis (Fig. 6A). A semi-log plot of the migration of the 3 bands detected in the cross-linking experiment confirmed that they correspond to PGI monomer, dimer and tetramer (Fig. 6C). The transition from dimer to tetramer observed in the cross-linking experiments suggests that the tetrameric form of PGI reported here corresponds to the stable interaction of two dimers.

To determine whether PGI monomerization is required for its interaction with cell surface fibrils at acid pH, the glutaraldehyde cross-linked form of the protein was added to cells at pH 7.5, 5.5, or 5.0 (Fig. 6B). The transition from dimer to tetramer observed in the cross-linking experiments suggests that the tetrameric form of PGI reported here corresponds to the stable interaction of two dimers.

At pH 5, PGI binding to cell surface fibrils was detected under all cross-linking conditions although reduced labeling was observed for PGI cross-linked predominantly in its dimeric form for 10 min. The cell surface association of the various cross-linked forms of PGI was quantified and, as observed for native PGI, at all times of cross-linking PGI still exhibited increased cell surface binding at pH 5 relative to pH 5.5 (Fig. 8). In particular, binding at 30 min of cross-linking was equivalent to that observed for native PGI indicating that dissociation of PGI monomers is not required for fibril association.

The fact that cross-linked PGI was still able to bind to cell surface fibrils at pH 5 indicated that changes in quaternary structure of the protein were not responsible for the enhanced binding. We therefore analyzed PGI structure by circular dichroism (CD). CD spectra of rabbit PGI at pH 7.5 (Fig. 9) correspond to those previously reported for human PGI (59) and essentially identical spectra were observed at pH 6 and 5.5 in both the far and near UV ranges (not shown). At pH 5, the spectra obtained in the far UV was equivalent to that obtained at neutral pH indicating that pH does induce changes in the secondary structure of PGI (Fig. 9, left panel). However, in the near UV range (Fig. 9, right panel), while the positive band at 296 was not affected, the negative band at 286 and the positive band at 256 were absent at pH 5. Acidification to pH 5 is therefore associated with specific effects on the tertiary folding of PGI.

**DISCUSSION**

**Conformation-dependent Association of PGI with Fibronectin Fibrils**—The ability of PGI to associate with fibronectin at acid pH adds further complexity to the behavior of this multifunctional protein. We have reported the association of PGI and of
its receptor, AMF-R, with cell surface fibronectin fibrils following endocytosis and passage through multivesicular bodies (40). This study confirms this association and further suggests that it is facilitated by PGI denaturation at acid pH. PGI isomerase activity is pH-dependent (15) and, similarly, we detected a 70% reduction in isomerase activity at pH 6.5 and complete inhibition of activity at pH 5.0 (data not shown). Inhibition of the majority of PGI isomerase activity at pH 6.5 is not associated with detectable monomer formation or increased fibril deposition of PGI (see Figs. 1, 2, and 6). pH induced conformational changes able to inhibit PGI isomerase activity do not therefore result in fibril association of the enzyme. Rather, the dramatic increase in fibronectin binding observed at pH 5.0 is a consequence of more drastic conformational changes able to disrupt monomer-monomer interactions. The fact that glutaraldehyde cross-linking does not abrogate fibril binding indicates that this association does not require monomer dissociation per se (Figs. 7 and 8). pH-dependent changes in the near UV were detected by CD confirming that changes in PGI conformation are induced by acid. Acid-induced changes in PGI tertiary structure, most specifically at pH 5, therefore result in conformational changes that are associated with both the disruption of monomer-monomer interactions in the native protein and exposure of protein domains that enable binding to fibronectin fibrils.

Earlier studies using gel chromatography defined PGI as a dimer and showed clearly that dimerization is essential for the isomerase activity of the enzyme (12–14). Detection of a higher molecular weight form corresponding to tetramers may reflect the presence of lower affinity interaction between PGI dimers.
that are stabilized in non-denaturing gel electrophoresis but not following dilution in gel chromatography. Furthermore, while x-ray crystallographic studies have clearly identified PGI as a dimer (17, 19, 60), these studies do not exclude the possibility of dimer-dimer interactions. The functional significance of the existence of PGI tetramers remains to be determined; however, the fact that PGI cross-linked as a tetramer is internalized to MVBs at neutral pH indicates that this oligomeric form of the protein is able to interact with its receptor (Fig. 7). Furthermore, dimer-dimer interactions could contribute to the formation of a glycolytic matrix (61).

Implications for the Role of PGI in Rheumatoid Arthritis—Binding of monomeric PGI directly and in large amounts to cell surface fibrils at acid pH provides a possible explanation for the postulated role of PGI in RA. The ability of monomeric PGI to associate with the cell surface is consistent with the fact that the immune reaction in autoimmune arthritis is associated with surface-bound antigen and not soluble circulating PGI immune complexes (46, 48). It further provides an explanation for the generation of a localized autoimmune response in lymph nodes draining the affected joints (52). Stable association of distinct conformational forms of PGI with the joint surface would result in exposure of anti-

**Fig. 7.** Cross-linked PGI still binds to cell surface fibrils at acid pH. Alexa 568-PGI was left un-cross-linked (No glut), incubated with glutaraldehyde and immediately quenched with glycine (+ glut 0 min), or cross-linked for 10 (+ glut 10 min) or 30 min (+ glut 30 min) before quenching (see “Experimental Procedures”). The variously cross-linked forms of Alexa 568-PGI were then incubated at 25 μg/ml at either pH 7.5 or pH 5 for 30 min before visualization by confocal microscopy. The images at pH 5 for cells under the various conditions were all acquired with the same confocal settings.

**Fig. 8.** Quantification of the acid-dependent cellular association of differentially cross-linked PGI. Cell surface binding at pH 7.5 (black bars), 5.5 (gray bars), and 5.0 (white bars) of the variously cross-linked forms of PGI, as described in the legend to Fig. 7, was quantified using a fluorescence plate reader and the appropriate filters. The values represent the average and S.E. of three independent experiments. Significance of differential binding at pH 5: Glut 10 min with No Glut and Glut 30 min: p < 0.1; Glut 10 min with Glut 0 min: p < 0.05.

**Fig. 9.** Circular dichroism spectra of PGI at neutral and acid pH. CD spectra of PGI at pH 7.5 (solid line) and pH 5 (dashed line), as indicated, were obtained in the far (left panel) and near UV (right panel) range. No significant pH-related differences were observed in the far UV range however in the near UV range incubation at pH 5 was associated with reduction in the positive band centered at 256 nm and the negative band centered at 286 nm.
Denaturation and Fibril Deposition of PGI at Acid pH

J. Mol. Biol. 309, 447–463

Funakoshi, T., Haga, R., Az, A., and Nagase, H. (2001) Biochem. Biophys. Res. Commun. 285, 118–128

Zhi, J., Sommerfeld, D. W., Ruben, C. T., and Haddadry, U. (2001) J. Bone Miner. Res. 16, 1994–2000

Chou, C.-C., Sun, Y.-J., Meng, M., and Hsiao, C.-D. (2000) J. Biol. Chem. 275, 21514–21519

Arakawa, M., and Nabi, I. R. (2002) FEBS Lett. 525, 151–155

Tsutsumi, S., Gupta, S. K., Hagan, V., Tanaka, N., Nakamura, K. T., Nabi, I. R., and Raz, A. (2003) FEBS Lett. 534, 49–53

Shintani, K., Tani, M., Watanabe, H., Nagamachi, Y., Nizamii, Y., Shiribishi, T., Ohwada, S., Raz, A., and Yokota, J. (1999) FEBS Lett. 456, 295–300

Hirano, Y., Fusuhisa, S., Yonemura, Y., Yamamoto, H., Watanabe, H., and Raz, A. (1999) J. Biol. Chem. 274, 20003–20007

Nakamori, S., Watanabe, H., Kameyama, M., Imakura, S., Furukawa, H., Ishi- kawa, O., Sasaki, Y., Kubato, T., and Raz, A. (1994) Cancer 74, 1855–1862

Otto, T., Richard, W., Schmidt, U., Hinke, A., Schipper, J., Ruben, H., and Raz, A. (1994) Cancer Res. 54, 3120–3123

Maruyama, K., Watanabe, H., Hatiso, T., Takayama, T., Gofuku, J., Yano, H., Inoue, M., Tamura, S., Raz, A., and Monden, M. (1995) Int. J. Cancer 64, 316–321

Nagai, Y., Ishikawa, O., Miyachi, Y., and Watanabe, H. (1996) Dermatology 192, 8–11

Takamani, I., Takeuchi, K., Nara, M., Koshira, S., Tanaka, F., Watanabe, H., and Raz, A. (1998) Tumor Biol. 19, 384–389

Taniguchi, Y., Yonemura, Y., Nojima, N., Hirano, Y., Fusuhisa, S., Fujimura, T., Miwa, K., Endo, Y., Yamamoto, H., and Watanabe, H. (1998) Cancer 82, 2112–2122

Leclerc, N., Vallée, A., and Nabi, I. R. (2000) J. Neurosci. Res. 60, 602–612

Luo, Y., Long, J. M., Lu, C., Chan, S. L., Spangler, E. L., Mascaluce, P., Raz, A., Longo, D. L., Mattarotto, M. P., Ingram, D. K., and Weng, N. P. (2002) J. Neurosci. 20, 351–361

Benlamine, N., Le, P. U., and Nabi, I. R. (1998) Mol. Biol. Cell 9, 1773–1786

Benlamine, N., Simard, D., and Nabi, I. R. (1996) J. Cell Biol. 129, 455–471

Wang, H.-J., Guay, P., Gogan, L., Saurve, N., and Nabi, I. R. (2000) J. Cell. Biol. 150, 1489–1498

Wang, H.-J., Benlamine, N., and Nabi, I. R. (1997) J. Cell Sci. 110, 3043–3053

Acola, M. A., Huang, B., Al Masri, A., and McNiven, M. A. (2002) J. Biol. Chem. 277, 3227–3234

Le, P. U., Benlamine, N., Laguna, A., Raz, A., and Nabi, I. R. (2000) J. Cell. Sci. 113, 2227–2234

Le, P. U., Guay, G., Altschuler, Y., and Nabi, I. R. (2002) J. Biol. Chem. 277, 3371–3379

Le, P. U., and Guay, G., Altschuler, Y., and Nabi, I. R. (2002) J. Biol. Chem. 277, 1059–1071

Nabi, I. R., and Le, P. U. (2003) J. Cell Biol. 161, 673–677

Matsumoto, I., Staub, A., Benoit, C., and Mathis, D. (1999) Science 286, 1722–1725

Schaller, M., Burton, D. R., and Ditzel, H. J. (2001) Nat. Immunol. 2, 746–753

Matsumoto, I., Maccioni, M., Lee, D. M., Maurice, M., Simoons, B., Brenner, M., Mathis, D., and Benoit, C. (2002) Nat. Immunol. 3, 360–365

Wigge, B. T., Wang, Z., Kim, J., McCarthy, T. J., and Allen, P. P. (2002) Nat. Immunol. 3, 366–372

Maccioni, M., Zeder-Lutz, G., Heublein, E., Ebel, C., Gerber, P., Hergueux, J., Marchal, P., Duchatelle, C., Degutis, C., van Begem, M., Benoit, C., and Mathis, D. (2002) J. Exp. Med. 195, 1071–1077

Herve, C. A., Wait, R., and Venables, P. J. (2003) Rheumatology (Oxford) 42, 368–485

Nakamura, I., Lee, D. M., Goldbach-Mansky, R., Sumida, T., Hitchen, C. A., Scher, P. H., Anderson, R. J., Coblyn, J. S., Weinblatt, M. E., Brenner, M., Duusos, B., Pauwels, J. L., El-Gabalawy, H., Mathis, D., and Benoit, C. (2003) Arthritis Rheum. 48, 944–954

Kassahn, D., Kolb, C., Solomon, S., Bothler, P., and Illeges, H. (2002) Nat. Immunol. 3, 411–412; discussion 413–414

Mandlik-Nayak, L., Wigge, B. T., Shih, F. F., Unnare, E., and Allen, P. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 14368–14373

Altman, R., Asch, E., Bloch, D., Bole, G., Borenstein, D., Brandt, K. Christy, W. Cooke, T. D., Greenland, R., Hochberg, M., et al. (1986) Arthritis Rheum. 29, 1039–1049

Tardif, G., Pelletier, J. P., Dupuis, M., Geng, C., Cloutier, J. M., and Martel-Pelletier, J. (1999) Arthritis Rheum. 42, 1147–1158

Laemmli, U. (1970) Nature 227, 685–688

Daruwala, S., Tsafatsidou, Y., and Daniel, E. (1987) Biochem. J. 242, 689–694

Hirano, Y., and Raz, A. (1999) J. Cell Biol. 143, 884–891

Nizamii, Y., Haga, A., Negishi, A., Yamazoe, H., Raz, A., and Amagasa, T. (2002) Oral Oncol. 38, 49–55

Lu, H. S., Talent, J. M., and Grady, R. W. (1981) J. Biol. Chem. 256, 785–792

Jeffreys, C. J., Hardie, R., and Salmon, L. (2001) Biochem. J. 360, 153–158

Qian, J., and Nollett, E. A. (1993) Biochem. J. 286, 568–574

Nakamura, N., and Nollett, E. A. (1993) Biochem. J. 287, 568–567

Bruch, P., Nollett, K. D., and Grady, W. R. (1976) J. Biol. Chem. 251, 605–618

Dyson, J. E., and Nollett, E. A. (1968) J. Biol. Chem. 243, 1401–1414

ArnesenEA.,Hardre,R.,Salmon,L., and Jeffreys, C. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 99, 5872–5877

Sakurai, T., Su, Y. C., Chen, W. S., Wu, R. T., Meng, M., and Hsiao, C. D. (1996) Biochem. J. 318, 541–547

Jeffrey, C. J., Bahnm, B. J., Chien, W., Ringe, D., and Petsko, G. A. (2000) Biochem. 39, 955–964

Read, J., Pearce, J., Li, X., Muirhead, H., Chirgwin, J., and Davies, C. (2001)