Localization and significance of pp55, a gastric mucosal membrane protein with tyrosine kinase activity

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Localization and significance of pp55, a gastric mucosal membrane protein with tyrosine kinase activity. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G863–G870, 1998.—In Fischer 344 rats, induction of gastric mucosal proliferative activity, whether the result of aging or injury or occurring after administration of epidermal growth factor, gastrin, or bombesin, is associated with a rise in tyrosine kinase activity and tyrosine phosphorylation of several mucosal proteins, including a protein with a molecular mass of 53–55 kDa. We hypothesized that this phosphorylating membrane protein (referred to as pp55) may play a role in regulating gastric mucosal cell proliferation and differentiation. Purification and subsequent immunoprecipitation studies now show that pp55 is a tyrosine kinase. In addition, the enzyme activity in the gastric mucosa is found to be fourfold higher in aged rats than in young rats. Incubation of gastric mucosal membranes with transforming growth factor-α (2 × 10−8 M) stimulates tyrosine kinase activity of pp55. Immunolocalization studies reveal that pp55 immunoreactivity is predominantly present in mucous cells that are located just above the proliferative zone and spasmolytic peptide-immunoreactive mucus neck cells. Tyrosine kinase activity as well as expression of pp55 are also greatly increased in the gastric mucosa after hypertonic saline-induced injury, a condition that results in stimulation of surface mucosal cell proliferation and differentiation. Our current data suggest that pp55 is a tyrosine kinase, likely localized to pre-surface cells. The presence of pp55 in pre-surface mucous cells and the expression and tyrosine kinase activity of this protein, which can be stimulated during mucosal cell proliferation and differentiation, strongly suggest a role for pp55 in differentiation of gastric surface mucous cells.

Majumdar, Adhip P. N., and James R. Goldenring. Localization and significance of pp55, a gastric mucosal membrane protein with tyrosine kinase activity. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G863–G870, 1998.—In Fischer 344 rats, induction of gastric mucosal proliferative activity, whether the result of aging or injury or occurring after administration of epidermal growth factor (EGF) or bombesin, were also accompanied by parallel alterations in gastric mucosal tyrosine kinase activity and tyrosine phosphorylation of a membrane protein with M, of ~55 kDa (20, 23–25). Similar changes were also observed in isolated gastric mucosal cells from rats as well as in rat small intestinal epithelial cell line (IEC) in response to gastrin (27, 31), an antral hormone that stimulates mucosal cell proliferation in much of the gastrointestinal tract, including the stomach and small intestine (12). These observations prompted us to speculate that the 55-kDa phosphorylating membrane protein (hereafter referred to as pp55) may play a role in modulating gastrointestinal mucosal cell proliferation and differentiation. To characterize and study the functional properties of pp55, we have raised polyclonal antibodies to this protein. In this study, we describe its localization and functional properties in the gastric mucosa.

METHODS

Animals. Fischer 344 male rats, aged 4–5 and 23–25 mo, obtained from the National Institute on Aging (Bethesda, MD) were used. New Zealand White male rabbits were used to raise antibodies. In some experiments, groups (n = 4) of overnight fasted 4- to 5-mo-old rats were given either 2 M NaCl or an equivalent volume of water (controls) via intragastric tube. The animals were killed 24 h later. The oxyntic gland area was dissected out, and two small portions of the tissue were fixed in Hollande’s fixative for immunocytochemistry as stated below. Mucosal scrapings were obtained from the rest of the oxyntic gland area (hereafter referred to as gastric mucosa) and stored at ~90°C in small aliquots for measurement of tyrosine kinase activity as stated below.

Production of polyclonal antisera. In an effort to characterize pp55, polyclonal antibodies were raised in rabbits against this protein. Briefly, gastric mucosal membranes (30,000 g), prepared from 4- to 5-mo-old rats, were subjected to SDSPAGE in a 30-cm-long electrophoresis apparatus, as described previously (21, 24). Each lane contained 0.15–0.2 mg membrane protein. After electrophoresis, the gel was subjected to indirect staining according to the manufacturer’s instructions (Diversified Biotech, Newton Center, MA), and the band corresponding to 55 kDa was excised. Ten to twelve gel pieces containing the protein were squashed, emulsified in 2 ml PBS and complete Freund’s adjuvant (1:1, vol/vol) and injected subcutaneously in 10–12 sites on each of three rabbits. The rabbits were boosted at 3- to 4-wk intervals with the same dose of antigen in incomplete Freund’s adjuvant. After five booster shots, blood was withdrawn from the ear vein and IgG prepared from the serum by ammonium sulfate fractionation. After dialysis against PBS, IgG was analyzed.

Although the mucosa of the gastrointestinal tract possesses one of the most rapid cell renewal rates of any tissue in the body (19), intracellular events that regulate proliferation and differentiation of mucosal cells have not been fully elucidated. It is now recognized that tyrosine kinases, which catalyze phosphorylation of tyrosine residues in proteins, play an important role in regulating proliferation, differentiation, and transformation of cells (6, 11, 37). Previously, we demonstrated that tyrosine kinase activity in a highly proliferative tissue, such as the gastric mucosa, was ~20- to 40-fold higher than in the liver and pancreas (21). This was also associated with increased tyrosine phosphorylation of a membrane protein with relative molecular mass (M,) of 53–55 kDa (21). In subsequent studies, we observed that in Fischer 344 rats, changes in gastric mucosal proliferative activity, whether the result of aging or injury or occurring after administration of epidermal growth factor (EGF) or bombesin, were also accompanied by parallel alterations in gastric mucosal tyrosine kinase activity and tyrosine phosphorylation of a membrane protein with M, of ~55 kDa (20, 23–25). Similar changes were also observed in isolated gastric mucosal cells from rats as well as in rat small intestinal epithelial cell line (IEC) in response to gastrin (27, 31), an antral hormone that stimulates mucosal cell proliferation in much of the gastrointestinal tract, including the stomach and small intestine (12). These observations prompted us to speculate that the 55-kDa phosphorylating membrane protein (hereafter referred to as pp55) may play a role in modulating gastrointestinal mucosal cell proliferation and differentiation. To characterize and study the functional properties of pp55, we have raised polyclonal antibodies to this protein. In this study, we describe its localization and functional properties in the gastric mucosa.
for pp55 immunoreactivity by Western immunoblot as described below.

Detection of pp55 by Western immunoblot. Aliquots of gastric mucosal membrane fraction (30,000 g pellet) containing 0.1 mg protein were subjected to SDS-PAGE. The electrophoresed proteins were transferred to a nitrocellulose membrane over a period of 40 min with the use of the Genie electrophoretic blotter (Research Products International, Mount Prospect, IL) in a transfer buffer containing 3.03 g Tris base, 14.4 g glycine, and 200 ml methanol per liter and were left overnight in a blocking buffer (PBS containing 7% nonfat dry milk, 0.5% BSA, and 0.1% Tween 20) and then washed three times (10 min each) at room temperature in PBS-0.1% Tween. The membranes were then incubated at room temperature for 2 h with the polyclonal antibodies raised against the 55-kDa gastric mucosal membrane protein (referred to as pp55 antibody) at a final dilution of 1:5,000 in PBS containing 0.5% BSA and 0.1% Tween 20 and were washed again the same way as stated above. The membranes were then incubated with a secondary goat anti-rabbit immunoglobulin Fc fragment containing horseradish peroxidase (ICN, Costa Mesa, CA) in PBS with 0.5% BSA and 0.1% Tween 20 for 2 h at room temperature at a final dilution of 1:4,000 and washed again as stated above. Protein bands were visualized with the use of an enhanced electrochemiluminescence detection system from Amersham, according to the manufacturer's instructions.

Purification of pp55. To purify pp55, gastric mucosal membrane preparations were solubilized in radioimmunoprecipitation assay (RIPA) buffer (29) and subsequently passed (4 times) through a small column (0.5 × 5 cm) containing pp55 antiserum coupled to Sepharose 4B (Sigma Chemical, St. Louis, MO). After the column was washed extensively with 20 mM HEPES containing 0.1% Triton X-100 (pH 7.4), pp55 was eluted with 3 M thiocyanate sodium in HEPES buffer. The eluted material was dialyzed against 20 mM HEPES buffer, pH 7.4, and then concentrated and finally dissolved in 20 mM HEPES buffer. A small amount of immunofluorescence-purified material was subjected to SDS-PAGE.

Autokinase and tyrosine kinase activity of pp55. To determine whether pp55 might possess protein kinase activity, autophosphorylation of pp55 was determined. Briefly, gastric mucosal membrane membrane preparations containing the same amount of protein (0.1 mg) in resuspension buffer (10 mM HEPES, pH 7.2, 150 mM NaCl, 1 mM sodium orthovanadate, 10 µg/ml leupeptin, 1 µg/ml apro tinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM 1,10-phenanthroline, and 0.02% Triton X-100) were subjected to autophosphorylation reaction as described previously (21, 25). Briefly, the reaction mixture contained, in a final volume of 50 µl, 2.5 µmol HEPES, pH 7.8, 2.5 µmol MgCl2, 0.5 nmol Na3VO4, 0.5 nmol [γ-32P]ATP (4 × 106 dpm), and 0.02% Triton X-100. The reaction at 0–2°C for 30 min was initiated with 20 µl of membrane fraction (50 µg) and was terminated by adding an equal volume of RIPA buffer (29) and 5 µg of pp55 antisera. The samples were incubated at 4°C for 4 h, and the immune complex was precipitated with protein G bound to agarose by incubating the mixture for 2 h at 4°C. The immunoprecipitates were suspended in gel stopping buffer (24), heated at 100°C for 10 min, and centrifuged again. The supernatant was then subjected to SDS-PAGE. After electrophoresis, the gel was fixed, equilibrated, dried, and finally exposed to X-Omat AR film. The molecular mass of the labeled band(s) was calculated from protein marker run concurrently.

Tyrosine kinase activity of pp55 was determined by assaying the extent of phosphorylation of acid-denatured enolase by pp55 essentially according to the method of Cartwright et al. (1). Briefly, aliquots of gastric mucosa were homogenized in lysis buffer (20 mM sodium phosphate, pH 7.4, 0.5% Triton X-100, 0.5% Nonidet P-40, 5 mM EDTA, 1 mM PMSF, 1 mM Na3VO4, 10 µg/ml leupeptin, 1 µg/ml apro tinin, and 1 mM 1,10-phenanthroline). After stirring at 4°C for 30 min, the homogenate was centrifuged at 10,000 g for 10 min at 4°C and the supernatant was used as source for the enzyme. To aliquots of 10,000 g supernatant containing 0.35 mg protein, an equal volume of RIPA buffer (29) was added, and pp55 was immunoprecipitated with pp55 antibody as stated above. After the immunoprecipitates were washed several times with RIPA and homogenizing buffer (1:1), they were resuspended in 25 µl of assay buffer (100 mM HEPES, pH 7.5, 10 mM MnCl2, 80 mM KCl and 40 mM 2-mercaptoethanol). The incubation at 30°C for 8 min was initiated by adding 25 µl of the assay cocktail, which contained 20 µM ATP, 10 µCi [γ-32P]ATP, and 10 µg acid-denatured enolase (Sigma Chemical); enolase was denatured by adding an equal volume of 0.1 M acetic acid and incubating the mixture for 8 min at 30°C. The reaction was terminated by adding 40 µl gel-loading buffer and subsequently subjected to SDS-PAGE (21). After electrophoresis, the gel was fixed, washed, dried and finally exposed to X-Omat AR film (21). In some experiments, tyrosine kinase activity of pp55 was also measured using the synthetic polymer of L-Glu-L-Tyr (4:1) as substrate as described previously (21).

In all experiments, protein content was determined using the Bio-Rad protein assay kit (Richmond, CA).

Immunogold electron microscopy. A small portion of the oxyntic gland area of the stomach was fixed for 4 h in 4% paraformaldehyde, then dehydrated through a series of graded ethanol and finally embedded in LR white resin with osmic acid. Sixty-nanometer sections were cut and placed on nickel grids. The sections were rehydrated in 0.1 M PBS, pH 7.4, and then treated with 10% normal goat serum for 15 min to reduce background staining. The primary antibody (pp55 antisera) was applied at a dilution of 1:100 in PBS for 1 h, and diluted normal rabbit serum was used on control grids. The grids were rinsed three times (5 min each in PBS, followed by incubation with goat-rabbit IgG bound to 10-nm gold particles (1:50 dilution in PBS for 1 h). The grids were rinsed again in PBS, blotted, and air dried. The sections were poststained with 4% aqueous uranyl acetate.

Immunohistochemistry. Formalin-fixed tissue sections were analyzed for proliferating cell nuclear antigen (PCNA), pp55, and spasmolytic peptide (SP) immunoreactivities by light and confocal microscopy. For all analyses, the paraffin-embedded tissues were serially sectioned at 5-µm intervals. After deparaffinization and blocking with either goat or donkey serum, sections were either incubated with anti-PCNA (1:50; monoclonal antibody, DAKO, Glostrup, Denmark), anti-pp55 (1:200), or affinity-purified mouse anti-SP (1:10; a generous gift from Dr. N. Wright, Hammersmith Hospital, London, UK) at ambient temperature for 60–120 min. To detect PCNA and pp55 by light microscopy, the avidin-biotin technique was then performed with matched components (LSAB kit; DAKO), according to the manufacturer's instructions. 3-Amino-9-ethylcarbazole was used as chromagen to localize immunoreactivity. For immunofluorescence detection of pp55 and SP by confocal microscopy, tissue sections were incubated simultaneously with the appropriate antibodies for 120 min, followed by incubation with Cy3-donkey anti-rabbit IgG and FITC-donkey anti-mouse IgM for 60 min at 22°C. Sections were mounted with Prolong antifade solution (Molecular Probes) and examined on a scanning confocal fluorescence microscope (Molecular Dynamics, Sunnyvale, CA). Double-labeled images were prepared from 0.5-µm optical sections.
Statistical analysis. When applicable, results were statistically evaluated with Student’s t-test for unpaired values, taking \( P < 0.05 \) as the level of significance.

RESULTS

Polyclonal antibodies were raised against SDS-PAGE gel-isolated 55-kDa gastric mucosal membrane protein. To evaluate the specificity of the polyclonal antibodies (referred to as pp55 antibody) raised in rabbits against the 55-kDa protein, gastric mucosal membrane preparations were subjected to Western immunoblot analysis with pp55 antibody. The antibody, at a final dilution of 1:5,000, reacted primarily with one protein band of \( M_r \) 55 kDa (referred to as pp55) (Fig. 1B).

The pp55 antisera was then used to purify and characterize pp55. When the detergent-solubilized gastric mucosal membrane preparations were subjected to purification in a pp55 immunoaffinity column and the eluted materials analyzed on SDS-PAGE, we observed a single protein band with \( M_r \) of 55 kDa (Fig. 1A), indicating a high specificity of the antibody for pp55.

Utilizing the pp55 antibody, we then performed immunoprecipitation studies to determine whether pp55 might possess protein kinase activity, and if so, whether a relationship exists between the enzyme activity and gastric mucosal proliferative activity. Indeed, pp55 was found to possess protein kinase activity as evidenced by autophosphorylation of the 55-kDa protein band (Fig. 1C). In addition, protein kinase activity of pp55 was found to be \( \approx \) 250% higher in gastric mucosal membranes from aged rats than from young rats (Fig. 1C). Early studies from our laboratory (20, 21, 23) have demonstrated that aging is associated with increased gastric mucosal proliferative activity.

To determine further whether pp55 might also possess tyrosine kinase activity, gastric mucosal membrane preparations from young and aged rats were subjected to immunoprecipitation with pp55 antibody and the immunoprecipitates were assayed for tyrosine kinase activity with polymer of L-Glu-L-Tyr (4:1), a substrate that has been shown to be highly specific for the enzyme (38). We observed that pp55 not only possessed intrinsic tyrosine kinase activity, but the enzyme activity was \( \approx \) 300% higher in aged rats than in young rats (young vs. aged, 2.6 ± 0.3 vs. 10.5 ± 1.5 pmol \(^{32}\)P incorporated/mg protein, respectively; \( P < 0.001 \)). Taken together, the results show a relationship between gastric mucosal proliferative activity and protein kinase activity of pp55.

Earlier, Singh et al. (31) reported that in rat small intestinal cell line (IEC-6), gastrin stimulates phosphorylation of an Src-like 54-kDa membrane protein. Majumdar and Wahby (27) have demonstrated that in isolated gastric mucosal cells, gastrin stimulates phosphorylation of a 55-kDa membrane protein. These observations raised the possibility that pp55 possesses some structural and functional similarities with the Src family of enzymes. To examine this possibility, aliquots of immunoaffinity column-purified pp55 were subjected to immunoprecipitation with a monoclonal antibody (MAb 327; Oncogene Science, Manhasset, NY) that crossreacts with rat pp60src (26). The immunoprecipitates were assayed for tyrosine kinase activity, using polymer of L-Glu-L-Tyr as a substrate. Results revealed that the MAb 327 immunoprecipitated \( \approx \) 15% of purified pp55 (basal vs. immunoprecipitates, 8.75 ± 1.12 vs. 1.62 ± 0.22 pmol \(^{32}\)P incorporated/5 µg column-purified pp55; \( P < 0.001 \), \( n = 5 \)).

In the next set of experiments, we studied the cellular localization of pp55 in the gastric mucosa. Dual-staining confocal microscopy was performed to compare the cellular distribution and localization of pp55 with that of SP, a trefoil peptide located in mucous neck cells of the gastric mucosa (3, 7, 9). The mucosa was examined at 24 h after hypertonic saline-induced injury. We have earlier demonstrated (5, 22) that mucosal proliferative activity in rats, as assessed by PCNA or bromodeoxyuridine (BrdU) immunoreactivity, is greatly increased 24 h after injury, compared with

![Fig. 1. A: SDS-PAGE of gastric mucosal membrane proteins from 4-mo-old rats, purified by pp55 antibody bound to activated Sepharose 4B column. Affinity purified materials containing 25 µg protein were electrophoresed and subsequently subjected to indirect protein staining (Diversified Biochemicals). Right lane 1 predominant band corresponding to M, 55 kDa was observed. Left lane molecular weight markers. B: Western immunoblot showing pp55 in gastric mucosal membranes from 4-mo-old rats. Gastric mucosal membranes containing 100 µg protein were subjected to Western immunoblot with pp55 antibody at a final concentration of 1.5,000. C: kinase specific activity of pp55 in gastric mucosal membranes from young (4-mo-old) and aged (24-mo-old) rats. pp55 from detergent-solubilized mucosal membrane fraction containing 50 µg protein was immunoprecipitated with pp55 antibody, and the immunoprecipitates were subjected to autophosphorylation reaction followed by SDS-PAGE. Gel containing \(^{32}\)P-labeled proteins was washed, dried, and autoradiographed.](http://ajpgi.physiology.org/ by 10.220.33.4 on June 25, 2017)
the water-fed controls. Data from water-fed controls revealed that whereas SP immunoreactivity was located in mucous neck cells below the proliferative zone of the gastric gland (about one-third below the surface), the pp55-immunoreactive cells were present just above this region (Fig. 2). At 24 h after injury, the relative abundance of pp55-immunoreactive cells was greatly increased compared with the controls (Fig. 2). The pp55 immunoreactivity was present throughout the regenerating surface cell region.

In another set of experiments, we compared the changes in expression of pp55 with that of PCNA at 24 h after hypertonic saline-induced injury, a condition that has been shown to stimulate the expression of PCNA (22). Indeed, the number of PCNA-positive cells per gland increased by ~60%, from 8.7 ± 0.9 in water-fed controls to 14.2 ± 1.2 (P < 0.001) in injured rats (Fig. 3). This was accompanied by increased pp55 staining of mucous surface cells (Fig. 3).

Immunogold electron microscopy was performed to evaluate subcellular localization of pp55. Most of the gold particles were found in association with the membranes of large clear vesicles in a subapical and perinuclear position within the labeled cells (Fig. 4).

Stimulation of gastric mucosal proliferation at 24 h after injury is associated with a marked rise in overall and EGF-receptor tyrosine kinase activity (5, 22). To determine whether a similar phenomenon would also persist for pp55, tyrosine kinase activity of this protein was also determined in both groups of rats. At 24 h after injury, tyrosine kinase activity of pp55 in the gastric mucosa, as assessed by the extent of enolase phosphor-

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Fig. 2. Photomicrographs showing the changes in pp55 and spasmolytic peptide (SP) immunoreactivities in the gastric mucosa of 4-mo-old rats at 24 h after orogastric administration of 2 M NaCl (injury) or water (controls). Sections (5 µm) of gastric fundic mucosa from control (A–C) and injured (D–F) rats were dual immunostained for pp55 (A, D; red) and SP (B, E; green). Images represent 0.5-µm confocal fluorescence optical sections. Dual-label overlays are shown in C and F. Injury causes an increase in pp55 immunoreactivity in the surface cell foveolar region, while staining for SP is unchanged in the mid-gland region. Bar, 20 µm.

Fig. 3. Representative photomicrograph showing changes in proliferating cell nuclear antigen (PCNA) and pp55 immunoreactivities in the gastric mucosa of 4-mo-old rats at 24 h after orogastric administration of 2 M NaCl (injury; Inj) or water (control; Con). Magnification, ×500.
Fig. 4. Electron photomicrograph showing immunogold localization of pp55 in gastric mucosal cells. Most of the gold particles were associated with the membrane-bound vesicles. Control sections exhibited no such accumulation of gold particles, but merely a generalized random distribution of a few individual gold particles (data not shown). Magnification, ×46,000.

Fig. 5. Autoradiograph showing pp55 tyrosine kinase activity in the gastric mucosa of 4-mo-old rats at 24 h after orogastric administration of 2 M NaCl (injury) or water (controls). Detergent-solubilized gastric mucosal membrane fraction containing 100 µg protein was incubated with pp55 antibody, and the immunoprecipitates were assayed for protein kinase activity with enolase as a substrate. At termination, 32P-labeled proteins were separated by SDS-PAGE and processed for autoradiography.
tyrosine kinase activity of pp55 (Fig. 6).

The exact functional role of pp55 also remains to be determined. However, our observations that in intact animals EGF stimulates tyrosine phosphorylation of the 55-kDa gastric mucosal membrane protein (20) and that, in mucosal membrane preparations, TGF-α also induces tyrosine kinase activity of pp55 suggest a role for pp55 in the EGF-receptor signaling process. This postulation is strengthened by the observation that gastric mucosal injury, which activates EGF-receptor tyrosine kinase by tyrphostin during the proliferative phase of the gastric mucosa after injury greatly attenuates mucosal regeneration (22), implicating a role for this receptor tyrosine kinase in the mucosal reparative process.

It is conceivable that there are also other tyrosine kinases that may play a role in regulating mucosal cell proliferation and/or differentiation in various tissues of the gastrointestinal tract, including the stomach. Since isolation of the first tyrosine kinase, pp60src, in 1979 (4), there has been an explosion in the number of identified protein tyrosine kinases, resulting largely from molecular cloning (10). Although at least 30 tyrosine kinases have been identified, mammalian tissues may contain many more yet unidentified protein tyrosine kinases (10). Evidence is also accumulating that suggests there are also tissue- or cell-specific tyrosine kinases. With respect to the gastrointestinal tract, Tyner and associates (32, 36) have recently cloned from the mouse small intestine an intracellular (cytoplasmic) tyrosine kinase (sik) with Mr of ~50 kDa whose expression is restricted to differentiating cells in the small intestine. Sunitha and Avigon (33) have also identified a novel tyrosine kinase (GASK) from rat small intestine that is expressed at high levels in intestinal cells but only at low levels in other epithelial and nonepithelial cells.

Our current data suggest that pp55 is another tyrosine kinase that may be involved in proliferation and/or differentiation of gastric mucosal cells. Results of our immunogold electron microscopy studies indicate that this protein is membrane associated. Moreover, the observation that ~15% of purified pp55 can be immunoprecipitated with pp60src antibodies suggests that pp55 may possess some structural similarity with pp60src. However, whether pp55 belongs to the Src family of enzymes cannot be conclusively stated at this time and must await the complete amino acid sequence analysis of the peptide.

DISCUSSION

Mucosal cells of the gastrointestinal tract undergo a constant process of renewal, which in normal adults reflects a balance between proliferation of precursor cells and exfoliation of surface cells (19). In the oxyntic gland area of the stomach, the progenitor cells are located in the upper one-third of the gastric gland from which the newly formed cells migrate upward or downward, providing a continuous supply of cells to replace the shed cells. As the newly formed cells move along the glandular axis they differentiate and give rise to phenotypically distinct epithelial cell lineages, which include surface mucous, mucous neck, parietal, chief, and enteroendocrine cells (13–18). Although the intracellular events that regulate gastric mucosal proliferation and differentiation are not well understood, accumulating evidence suggests a role for tyrosine kinases in these processes (6, 11, 37). However, since tyrosine kinases are associated with receptors of several growth factors and products of many protooncogenes (6, 11, 37), studies have also been performed to evaluate the role of different tyrosine kinases in regulating gastric mucosal cell proliferation and differentiation. Our recent observation that the age-related rise in gastric mucosal proliferative activity is accompanied by increased tyrosine kinase activity and expression of EGF receptor and pp60src (26, 35) suggests that they may be involved in modulating gastric mucosal cell proliferation and differentiation. We have also observed that inhibition of EGF-receptor tyrosine kinase activity by tyrphostin during the proliferative phase of the gastric mucosa after injury greatly attenuates mucosal regeneration (22), implicating a role for this receptor tyrosine kinase in the mucosal reparative process.

Fig. 6. Autoradiograph showing pp55 tyrosine kinase activity in gastric mucosal membranes from 4-mo-old rats after 15 min incubation (at 24°C) in the absence (basal) or presence of 2 × 10−8 M transforming growth factor-α (TGF-α). Assay condition was the same as in Fig. 5 legend.
sin, each of which stimulates gastric mucosal proliferative activity (12), enhances tyrosine phosphorylation of a mucosal membrane protein with $M_r$ of 55 kDa (25, 27). It remains to be determined whether this 55-kDa phosphotyrosine protein represents pp55, and if so, whether this protein plays a role in regulating mucosal proliferation in response to other gastrointestinal hormones/peptides, including gastrin and bombesin.

Increases in the number of gastric surface mucous cells are common to models of both aging and acute mucosal injury. Ligands for the EGF/TGF-α receptor, especially TGF-α, have been implicated as major stimulators of the maturation of surface cells from the progenitor zone. Overexpression of TGF-α in the gastric mucosa elicits a marked expansion in surface cell number with concomitant loss of parietal cells (2, 8, 30, 34). This expansion of the surface cell compartment in metallocrin-TGF-α mice results from a change in the progenitor zone differentiation program biased toward the maturation of surface mucous cells (8). The pp55-immunoreactive cells are present just above the mucous neck cell zone, an area that is predominantly occupied by pre-surface mucous cells, which give rise to mature surface mucous cells. The electron microscopic appearance of immunogold-labeled cells also is consistent with pre-surface cells. This, together with the observation that at 24 h after injury, pp55 but not SP or PCNA immunoreactivity extended up to the mucosal surface, suggests a role for pp55 in pre-surface cell differentiation. The association of pp55 with secretory granule membranes suggests that this protein may also play a role in maturation of granules or secretion. Because pp55 appears to be present in pre-surface cells or recently maturing surface cells, the pp55 protein may be an important tyrosine-phosphorylated protein mediating the influence of EGF and TGF-α on lineage differentiation from the gastric progenitor zone.

In conclusion, our data suggest that pp55, a gastric mucosal tyrosine-phosphorylated membrane protein, is a tyrosine kinase whose activity can be stimulated by TGF-α. The observation that the tyrosine kinase activity of pp55 and expression of this protein are increased markedly during mucosal cell proliferation and differentiation suggests that pp55 may play a role in regulating these processes. Moreover, the likely localization of pp55 predominantly in pre-mucous surface cells in the gastric mucosa strongly suggests its role in differentiation of surface mucous cells in the gastric mucosa.

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