J. Biochem. **126**, 266-270 (1999)

Direct Evidence for *In Vivo* Reversible Tyrosine Phosphorylation of the N-Terminal Domain of the H/K-ATPase α-Subunit in Mammalian Stomach Cells[1]

Motoi Kanagawa,* Shunji Kaya,* Hideki Umezu,* Shunsuke Watanabe,* Katsuhiko Togawa,* Akira Shimada,* Toshiaki Imagawa,* Sven Mårdh,† and Kazuya Taniguchi*,[2]

**Biological Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810; and* †*Department of Biomedicine and Surgery, Faculty of Health Science, Linkoping University, 58185, Sweden*

Received June 1, 1999; accepted June 17, 1999

***In vivo* reversible phosphorylation of Tyr-7 and Tyr-10 of the pig stomach H/K-ATPase α-chain was initially demonstrated in mammals, rat, rabbit, and pig, in the presence of vanadate+H_2O_2. *In vitro* phosphorylation has also been unequivocally demonstrated *via* the use of protease inhibitors during membrane H/K-ATPase preparation. An amphoretic detergent permitted each intrinsic kinase to phosphorylate each fusion protein containing the requisite Tyr residues, along with a reduction in α-chain phosphorylation. These and other data suggest that some important enzyme systems are present in the apical membrane and that they are in sufficient proximity to participate in the reversible phosphorylation of the amino terminal soluble domain of the α-chain with an unknown physiological function in the membrane embedded H/K-ATPase.**

Key words: H/K-ATPase, phosphotyrosine, reversible phosphorylation, Tyr kinase.

It has been reported that gastric acid secretion is regulated through several receptor-mediated signal transduction pathways. Histamine stimulates acid secretion *via* the H2 receptor-mediated activation of cAMP-dependent protein kinases (*1-3*). Acetylcholine also stimulates acid secretion by increasing protein kinase C activity, *via* an increase in the intracellular calcium concentration (*4, 5*). These protein kinase-dependent acid secretions are coupled with intracellular protein phosphorylation (*6-12*). Recent studies suggested that acid secretion requires both cAMP and calcium at a certain level (*13, 14*). However, the molecular mechanisms of acid secretion, including that of the fusion of tubulovesicles to apical membranes and the activation of specific ion channels, remained to be elucidated. Our previous studies demonstrated that Tyr-7, Tyr-10, and Ser-27 of the N-terminal domain of H/K-ATPase were reversibly phosphorylated by intrinsic kinases and phosphatases, which are endogenous in partially purified membrane H/K-ATPase preparations, namely the G1 fraction, from pig stomach (*15, 16*). Further studies showed that Ser-27 is also phosphorylated by an intrinsic Ca^{2+}-dependent kinase, and that Ser-27 of the SDS-purified H/K-ATPase α-chain is also phosphorylated by cAMP-dependent protein kinase and protein kinase C (*16*). Although these specific Tyr and Ser residues are conserved in all mammalian species, phosphorylation of the H/K-ATPase has been observed only in the pig preparation *in vitro* (*17*). Furthermore, no evidence is available that such reversible Tyr phosphorylation occurs *in vivo*. In this paper, we show that reversible Tyr phosphorylation of H/K-ATPases occurs *in vivo* and *in vitro* in the cases of the rat and rabbit in a manner similar to that in the pig (*15*).

Vesicles containing stomach H/K-ATPase (G1 fraction) from pigs, rats (Wistar, male, 6 weeks), and rabbits (Japanese White, male, 6 weeks) were prepared according to the method of Sachs *et al.* (*18*). The H/K-ATPase activity and protein concentrations of enzymes were determined by previously described methods (*19, 20*). [γ-^{32}P]-ATP and GSH-Sepharose were purchased from Amersham Pharmacia Biotech. POLY(Glu:Tyr) was obtained from Sigma Co. and PVDF membrane (Immobilon 0.45 μm) was obtained from Millipore. Antibodies, anti-phosphotyrosine (anti-PY) and horseradish peroxidase-conjugated secondary antibodies, were obtained from WAKO and Jackson Immune Research Laboratories, respectively. An anti-PY antibody column was purchased from UBI. Anti-H/K-ATPase α-chain antibodies (anti-HK) were prepared as described previously (*21*).

Both Tyr-7 and Tyr-10 of the pig H,K-ATPase α-chain were shown to be reversibly phosphorylated by the intrinsic protein kinase and phosphatase *in vitro* (*15*). Although both residues are conserved in the rat, no detectable amount of phosphotyrosine (PY) was detected (*17*). This might be due to the presence of protease and/or phosphatase, respectively, which could break the structure

[1] This work was supported, in part, by Grants for Scientific Research and the International Scientific Research Program from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Fax/Tel: +81-11-736-2074, E-mail: ktan@ccms1.hucc.hokudai.ac.jp

Abbreviations: PVDF, polyvinylidene difluoride; CBB, Coomassie Brilliant Blue; PMSF, phenylmethylsulfonyl fluoride; NP-40, Nonidet P-40; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonic acid; GST, glutathione S-transferase; TCA, trichloroacetic acid; TPCK, tosylphenylalanyl chloromethyl ketone.

necessary for the N-terminal Tyr phosphorylation down and/or that PY was formed immediately. In fact, amino acid sequence analysis of rat α-chains, purified by SDS-PAGE (*22*), showed that nearly all the rat α-chains of the G1 fraction, which was prepared using a buffer containing 0.5 mM EGTA-Tris, pH 7.4, 1 mM PMSF, 1 μg/ml pepstatin A, and 0.1% BSA, started from Ser-10. This finding suggests that proteolysis had occurred. To more effectively inhibit proteolysis, G1 fractions were prepared using a buffer containing both 2 mM EGTA-Tris and PMSF, 2.5 μg/ml pepstatin A, 0.2% BSA, 10 mM EDTA-Tris, 1 mM benzamidine, 5 μg/ml leupeptin, and 5 μg/ml aprotinin. N-terminal sequence analysis of the α-chains from G1 fractions of rat, rabbit and pig, which were prepared with this procedure, showed that up to 90% of these peptides started from Gly-2 (not shown).

Figure 1 clearly shows the presence of an anti-phosphotyrosine (anti-PY) reactive band with an anti-HK band in rat and rabbit as well as pig for the G1 fraction thus obtained and preincubated with ATP. Mild TPCK-trypsin treatment completely abolished PY with no observed change in the mobility of the α-chains (not shown). These data suggest that *in vitro* Tyr phosphorylation occurs at Tyr-7 and Tyr-10 of the N-terminal α-chain in rat and rabbit as in the case of the pig H/K-ATPase (*15*). The time courses of PY formation in the G1 fractions of rat and rabbit with or without vanadate indicated the presence of vanadate-sensitive Tyr phosphatase in each G1, as in the case of pig (not shown).

The reversible Tyr phosphorylation *in vitro* provides convincing evidence that it also likely occurs *in vivo*. However, little Tyr phosphorylation was detected in the rat parietal primary culture system (*17*). It has been reported that the treatment of cells with vanadate + H_2O_2 (PV-treatment) permitted the detection of Tyr phosphorylation through the irreversible inhibition of cellular Tyr phosphatase activity (*23, 24*). Minced rat and rabbit stomach tissues (*25*) were incubated with 1 mM vanadate + 3 mM H_2O_2 for 20 min. Microsome fractions were then prepared (*15*) using lysis buffer (Fig. 2). Western blotting analysis with an anti-PY antibody clearly showed that the PV treatment permitted the detection of several protein bands containing PY (Fig. 2, A–C). A protein band corresponding to a molecular weight of 100 kDa also contained PY in each species. The data showed that the PY detected had been formed inside the cells, since PY bands were only detected after pretreatment with vanadate + H_2O_2 (data not shown). If this phosphorylation had occurred in the cell homogenate, PY formation would have been detectable in the presence of vanadate and the absence of H_2O_2.

To determine whether or not this protein band corresponds to the α-chain, G1 fractions were prepared from tissues with or without PV treatment. The G1 fractions were then solubilized with NP-40 and the resulting supernatants were applied to an anti-PY antibody–conjugated column. Tyr phosphorylated proteins were eluted with phenylphosphate, pH 7.4, and with a glycine-HCl buffer,

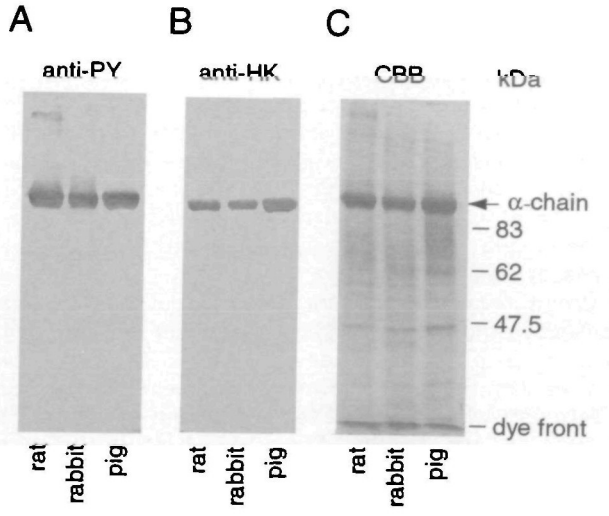

Fig. 1. **Tyr phosphorylation of the H/K-ATPase α-chain *in vitro*.** G1 fractions (0.4 mg protein/ml) were incubated with 20 μl of a phosphorylation mixture containing 20 mM HEPES-Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM Na_3VO_4, 2 mM DTT, and 2 mM ATP at 30°C for 20 min. The reaction was stopped by the addition of an equal volume of SDS-sample buffer. After SDS-PAGE, the gel was stained with CBB (C) or blotted onto a PVDF membrane, followed by Western blotting using anti-PY (A) or anti-HK (B) antibodies. The position of the α-chain is indicated by an arrow. Molecular weight markers are shown at the right.

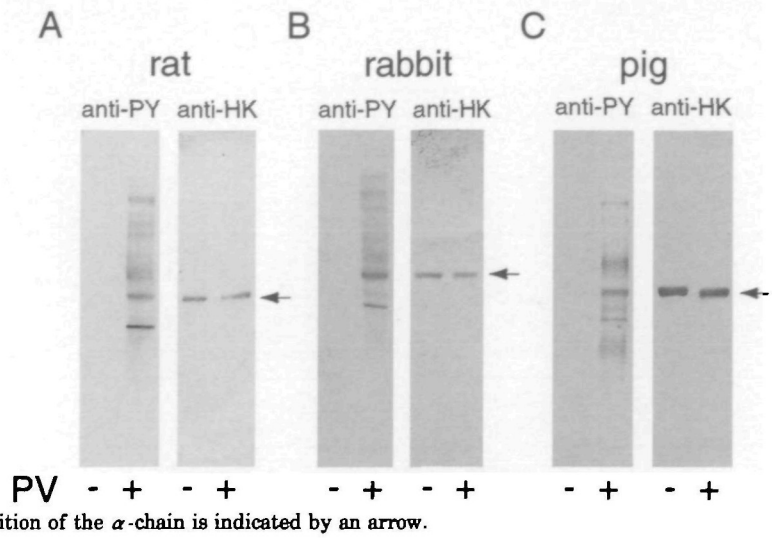

Fig. 2. **Tyr phosphorylation of the H/K-ATPase α-chain *in vivo*.** Gastric tissues were obtained as reported previously (*25*). One gram of dissected tissue was treated with 3 mM H_2O_2 and 1 mM Na_3VO_4 in 5 ml of buffer at 37°C for 20 min under an O_2 atmosphere. The buffer contained 25 mM HEPES-NaOH, pH 7.6, 137 mM NaCl, 5.4 mM KCl, 0.9 mM $MgCl_2$, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 11 mM glucose, 2 mM isoleucine, 0.5 mM $CaCl_2$, and 0.2% BSA. After this (PV) treatment, the tissue pieces were washed, suspended in 5 ml of lysis buffer and then homogenized with a Polytron homogenizer (type PT10/35, set 5, 10 s × 4) on ice to prepare microsomal fractions. The lysis buffer contained 20 mM Tris-HCl, pH 7.4, 10 mM EDTA-Tris, 2 mM EGTA-Tris, 2 mM Na_3VO_4, 50 mM NaF, 10 mM pyrophosphate, 0.2% BSA, 1 mM benzamidine, 2 mM PMSF, 5 μg/ml leupeptin, 2.5 μg/ml pepstatin A, and 5 μg/ml aprotinin. G1 fractions were obtained as reported (*18*). The microsomal fractions of the rat (A), rabbit (B), and pig (C) G1 fractions were subjected to SDS-PAGE. Tyr phosphorylation was detected by Western blotting. The position of the α-chain is indicated by an arrow.

pH 2.3, and then subjected to SDS-PAGE. The blots were developed with anti-PY and anti-HK antibodies. Tyr phosphorylated proteins, including the 100 kDa protein, the α-chain, were recovered on both elutions (Fig. 3). Treatment with a one-hundredth weight of TPCK-trypsin removed the PY from the α-chains, prepared from the G1 fractions of PV-treated rat, rabbit and pig tissues (not shown). The data constitute the first direct evidence for the Tyr phosphorylation of the N-terminal H/K-ATPase α-chain *in vivo*. The α-chain prepared from tissues without PV treatment passed through the column, suggesting that the PY formed was immediately split in the absence of PV. This may be one reason for the absence of detectable PY. Another reason could be the presence of protease, which breaks the N-terminal domain down, which is required for phosphorylation or liberation of PY-containing peptides from the α-chain due to incomplete inhibition of the proteases (*17*).

The data are clearly consistent with the reversible Tyr phosphorylation of the H/K-ATPase α-chain in these animals both *in vitro* and *in vivo*. Further studies will be required to clarify the relationship between the reversible phosphorylation of both Tyr-7 and Tyr-10, and the regulation of acid secretion, which may be related to Tyr kinase in parietal cells, as has been suggested with the use of Tyr kinase modifiers (*26, 27*).

Preliminary experiments showed that a Tyr kinase present in the pig G1 fraction was rather specific for the N-terminal phosphorylation of the H/K-ATPase α-chain (not shown). However, the kinase could be removed by SDS treatment, which resulted in a ~1.5 fold increase in the specific activity of H/K-ATPase (*15*). CHAPS (0.5%) was shown to solubilize Tyr kinase from the G1 fraction of pig by ultracentrifugation at 100,000 rpm for 10 min at 2°C. To determine whether or not solubilized Tyr kinase is capable of phosphorylating the α-chain, the SDS-treated G1 fraction was incubated in the presence of ATP without or with the CHAPS supernatant. Western blotting of samples clearly showed the presence of PY in the α-chains of the G1 fraction preincubated with ATP and SDS-treated G1 preincubated with CHAPS supernatant and ATP, but it was not present in the absence of the supernatant. Treatment with a one-hundredth weight of TPCK-trypsin removed the PY from the α-chain (not shown), as has already been reported (*15*).

To further investigate the effect of solubilization of the kinase on the phosphorylation of the α-chain, the pig G1 fraction preincubated without or with CHAPS was further incubated with [γ-^{32}P]ATP, Mg^{2+}, and vanadate. Samples were subjected to SDS-PAGE. CHAPS showed decreased ^{32}P incorporation into the α-chain (0.8-fold) in contrast to a 1.3-fold increase in ^{32}P incorporation into the 3.3% TCA-precipitable protein of the G1 fraction. CHAPS induced the appearance of anti-PY antibody reactive bands and new low molecular weight ^{32}P bands without detectable changes in Coomassie Blue stained bands, as already reported in the case of pig (*28*). An increase in the phosphorylatability of other proteins was also detectable with an increase in the development time in rat and rabbit as well as pig (Fig. 4, A–C, lanes 1 and 2).

These data suggest that the membrane-bound Tyr kinase preferentially phosphorylates the N-terminal domain of the H/K-ATPase in the membrane-bound form. The solubilization and/or presence of CHAPS reduced the accessibility of the kinase to the N-terminal domain of the ATPase, but increased it in the cases of other proteins. It also appears that the kinase phosphorylates synthetic soluble substrates more easily in the presence of CHAPS, as is shown below.

Each G1 fraction, from rat, rabbit, and pig, was incubated with [γ-^{32}P]ATP and a random copolymer of poly-(Glu/Tyr) with or without CHAPS pretreatment. The incorporation of ^{32}P into the 3.3% TCA insoluble fraction by these G1 preparations increased by factors of ~2 and 4–8 fold in the presence of CHAPS and the copolymer, respectively. The incorporation increased by 10–20-fold in the presence of both CHAPS and copolymers. SDS-PAGE of these samples showed that the phosphorylation levels in the presence of the copolymer were due to the incorporation into the polymers themselves (not shown).

Figure 4 shows the results of similar experiments involving soluble fusion proteins, GST-HKs. The data clearly show that the presence of CHAPS greatly increased the Tyr phosphorylation of each fusion protein, as well as that of some other proteins, which were more clearly detectable. The reduction of the level of PY of the α-chain accompanied

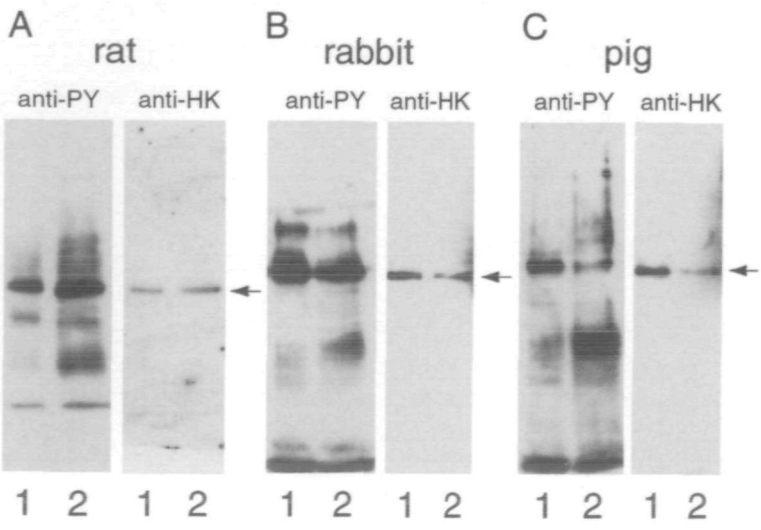

Fig. 3. Isolation of Tyr phosphorylated H/K-ATPase α-chains. The G1 fractions (0.5 mg/ml) of PV-treated rat (A), rabbit (B), and pig (C) gastric tissues were solubilized with 160 μl of 2% NP-40-containing lysis buffer except that NaF and BSA were omitted and the concentration of Na_3VO_4 was 1 mM. Samples were centrifuged at 100,000 rpm for 10 min. The supernatants were applied on an anti-PY antibody column (100 μl), which had been equilibrated with the binding buffer (20 mM Tris-HCl, pH 7.4, 10 mM EDTA-Tris, 100 mM NaCl, 0.2 mM Na_3VO_4, and 1% NP-40). After washing with 10 column volumes of the binding buffer, the bound Tyr phosphorylated proteins were eluted with 5 column volumes of the binding buffer containing 100 mM phenylphosphate (lane 1) and 2 column volumes of 50 mM glycine-HCl, pH 2.3 (lane 2). The eluted materials were analyzed by Western blotting using anti-PY and anti-HK antibodies. The position of the α-chain is indicated by an arrow.

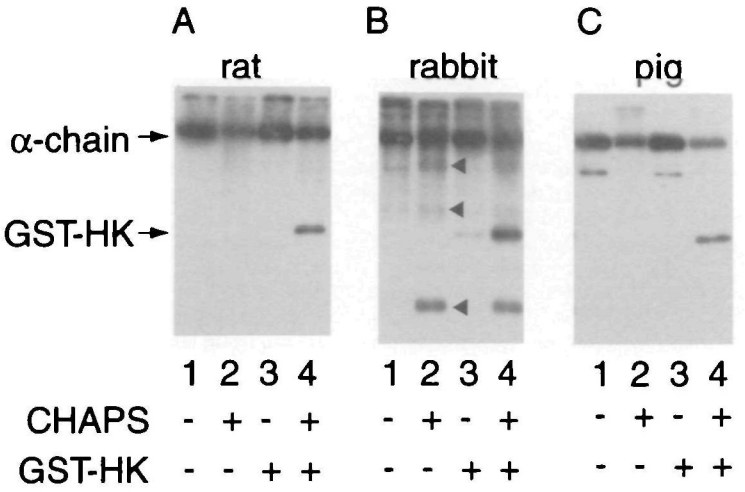

Fig. 4. **Tyr phosphorylation of GST-fusion proteins.** The cDNA corresponding to the N-terminal 111 amino acids of the H/K-ATPase α-chain was amplified by PCR. The amplified cDNA fragments were ligated to a pGEX vector and expressed in *E. coli*. The recombinant proteins (GST-HK) were purified by GSH-Sepharose chromatography according to standard procedures. Each G1 fraction (1 mg/ml) was pretreated with or without 1% CHAPS for 30 min with stirring at 0°C. The reactions were carried out in 50 μl of buffer containing 20 mM HEPES-Tris, pH 7.4, 2 mM EDTA-Tris, 2 mM EGTA-Tris, 5 μg/ml leupeptin, 2.5 μg/ml pepstatin A, and 5 μg/ml aprotin. Rat (A), rabbit (B), and pig (C) G1 protein (0.1 mg/ml) pretreated with (lanes 1 and 3) or without (lanes 2 and 4) CHAPS was, respectively, incubated in 20 μl of the phosphorylation mixture with (lanes 3 and 4) or without (lanes 1 and 2) GST-fusion proteins (0.05 mg/ml). The positions of the α-chain and GST-HK are indicated by arrows. CHAPS-induced Tyr phosphorylated membrane proteins in rabbit (B) other than the α-chain are indicated by arrowheads.

by an increase in the PY of GST-HK caused by CHAPS is clearly shown in the case of pig (Fig. 4C, lane 4). When Tyr residues (corresponding to Tyr-7 and Tyr-10 in the H/K-ATPase α-chain) were mutated to Phe, phosphorylation dramatically decreased (data not shown).

The data described above constitute the first direct evidence that both Tyr-7 and Tyr-10 of the α-chain are reversibly phosphorylated *in vivo*. They also show that the Tyr kinase is located in the apical membrane domain of parietal cells in sufficiently close proximity to permit specific phosphorylation of these residues in the N-terminal domain of the H/K-ATPase α-chain. However, treatment with CHAPS caused the solubilization of the kinase from the domain, thus permitting the phosphorylation of other membrane-bound or soluble proteins, including synthetic peptide and fusion proteins.

The question arises as to the amount of PY in the α-chain *in vivo*. The value in each animal tissue pretreated with PV was compared with that on *in vitro* phosphorylation of G1 fraction in the presence of vanadate but not treated with PV as a control. The control gave rather constant values in the case of pig, 0.7 mol of PY/mol of phosphoenzyme intermediate (*15*). However, the relative amount of Tyr phosphorylated α-chains *in vivo* was in the range of 30-200% of the control level and was dependent on the individual rat, rabbit, and pig stomach used. These data suggest the presence of some other factor(s) in parietal cells which regulate the reversible phosphorylation of the α-chain, and which were not controlled under our experimental conditions.

It has been reported that Tyr phosphorylation of plasma membrane Ca^{2+}-ATPase results in 75% inhibition of the ATPase activity (*29*). No significant change in H/K-ATPase activity was detected before or after Tyr phosphorylation of the α-chain *in vitro* (*15*). The PV treatment of stomach tissues induced irreversible inhibition of H/K-ATPase activity in isolated G1 fractions. Further studies are required to clarify the physiological role of the reversible phosphorylation.

REFERENCES

1. Chew, C.S., Hersey, S.J., Sachs, G., and Berglindh, T. (1980) Histamine responsiveness of isolated gastric glands. *Am. J. Physiol.* **238**, G312-320
2. Hersey, S.J., Owirodu, A., and Miller, M. (1983) Forskolin stimulation of acid and pepsinogen secretion by gastric glands. *Biochim. Biophys. Acta* **755**, 293-299
3. Soll, A.H. and Berglindh, T. (1987) Physiology of isolated gastric gland and parietal cells: receptors and effectors regulating function in *Physiology of the Gastrointestinal Tract* (Johnson, L.R., ed.) pp. 839-909, Raven, New York
4. Chiba, T., Fisher, S.K., Park, J., Seguin, E.B., Agranoff, B.W., and Yamada, T. (1988) Carbamoylcholine and gastrin induce inositol lipid turnover in canine gastric parietal cells. *Am. J. Physiol.* **255**, G99-105
5. Chew, C.S. and Brown, M.R. (1986) Release of intracellular Ca^{2+} and elevation of inositol trisphosphate by secretagogues in parietal and chief cells isolated from rabbit gastric mucosa. *Biochim. Biophys. Acta* **888**, 116-125
6. Chew, C.S. and Brown, M.R. (1987) Histamine increases phosphorylation of 27- and 70-kDa parietal cell proteins. *Am. J. Physiol.* **253**, G823-829
7. Modlin, I.M., Oddsdottir, M., Adrian, T.E., Zdon, M.J., Zucker, K.A., and Goldenring, J.R. (1987) A specific histamine-stimulated phosphoprotein in isolated parietal cells. *J. Surg. Res.* **42**, 348-353
8. Urushidani, T., Hanzel, D.K., and Forte, J.G. (1987) Protein phosphorylation associated with stimulation of rabbit gastric glands. *Biochim. Biophys. Acta* **930**, 209-219
9. Urushidani, T., Hanzel, D.K., and Forte, J.G. (1989) Characterization of an 80-kDa phosphoprotein involved in parietal cell stimulation. *Am. J. Physiol.* **256**, G1070-1081
10. Malinowska, D.H., Sachs, G., and Cuppoletti, J. (1988) Gastric H^+ secretion: histamine (cAMP-mediated) activation of protein phosphorylation. *Biochim. Biophys. Acta* **972**, 95-109
11. Brown, M.R. and Chew, C.S. (1989) Carbachol-induced protein phosphorylation in parietal cells: regulation by $[Ca^{2+}]_i$. *Am. J. Physiol.* **257**, G99-110
12. Hanzel, D.K., Urushidani, T., Usinger, W.R., Smolka, A., and Forte, J.G. (1989) Immunological localization of an 80-kDa phosphoprotein to the apical membrane of gastric parietal cells. *Am. J. Physiol.* **256**, G1082-1089
13. Li, Z.Q., Cabero, J.L., and Mårdh, S. (1995) Gastrin and carbachol require cAMP to elicit aminopyrine accumulation in isolated pig and rat parietal cells. *Am. J. Physiol.* **268**, G82-89
14. Li, Z.Q. and Mårdh, S. (1996) Interactions between Ca^{2+}- and cAMP-dependent stimulatory pathways in parietal cells. *Biochim. Biophys. Acta* **1311**, 133-142
15. Togawa, K., Ishiguro, T., Kaya, S., Shimada, A., Imagawa, T., and Taniguchi, K. (1995) Reversible phosphorylation of both Tyr^7 and Tyr^{10} in the α-chain of pig stomach H^+,K^+-ATPase by a membrane-bound kinase and a phosphatase. *J. Biol. Chem.* **270**, 15475-15478

16. Togawa, K., Kaya, S., Shimada, A., Imagawa, T., Mårdh, S., Corbin, J., Kikkawa, U., and Taniguchi, K. (1996) Ser-27, Tyr-10 and Tyr-7 in the α-chain of pig stomach H^+,K^+-ATPase as Ca^{2+}-dependent phosphorylatable sites by intrinsic and extrinsic protein kinases. *Biochem. Biophys. Res. Commun.* **227**, 810-815
17. Kaya, S. and Mårdh, S. (1998) Protein kinase-dependent phosphorylation of H,K ATPase-containing membranes from rat and pig stomachs. *Acta Physiol. Scand.* **162**, 57-62
18. Sachs, G., Chang, H.H., Rabon, E., Schackman, R., Lewin, M., and Saccomani, G. (1976) A nonelectrogenic H^+ pump in plasma membranes of hog stomach. *J. Biol. Chem.* **251**, 7690-7698
19. Eguchi, H., Kaya, S., and Taniguchi, K. (1993) Phosphorylation of half and all sites in H^+,K^+-ATPase results in opposite changes in tryptophan fluorescence. *Biochem. Biophys. Res. Commun.* **196**, 294-300
20. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254
21. Tømmerås, K., Chen, Y., Rhedin, M., Cabero, J.L., and Mårdh, S. (1997) Proliferation and differentiation of cells from explants of fetal rat stomach. *Acta Physiol. Scand.* **159**, 155-161
22. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
23. Heffetz, D., Bushkin, I., Dror, R., and Zick, Y. (1990) The insulinomimetic agents H_2O_2 and vanadate stimulate protein tyrosine phosphorylation in intact cells. *J. Biol. Chem.* **265**, 2896-2902
24. Huyer, G., Liu, S., Kelly, J., Moffat, J., Payette, P., Kennedy, B., Tsaprailis, G., Gresser, M.J., and Ramachandran, C. (1997) Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. *J. Biol. Chem.* **272**, 843-851
25. Mårdh, S., Norberg, L., Ljungstrom, M., Humble, L., Borg, T., and Carlsson, C. (1984) Preparation of cells from pig gastric mucosa. Isolation of parietal cells by isopycnic centrifugation on linear density gradients of Percoll. *Acta Physiol. Scand.* **122**, 607-613
26. Tsunoda, Y., Modlin, I.M., and Goldenring, J.R. (1993) Tyrosine kinase activities in the modulation of stimulated parietal cell acid secretion. *Am. J. Physiol.* **264**, G351-356
27. Chew, C.S., Nakamura, K., and Petropoulos, A.C. (1994) Multiple actions of epidermal growth factor and TGF-α on rabbit gastric parietal cell function. *Am. J. Physiol.* **267**, G818-826
28. Togawa, K., Kaya, S., Mori, M., Shimada, A., Imagawa, T., Taniguchi, K., Mårdh, S., Corbin, J., and Kikkawa, U. (1997) Phosphorylation of Tyr^7, Tyr^{10}, and Ser^{27} of α-chain in H^+,K^+-ATPase by intrinsic and extrinsic kinases in *Na/K-ATPase and Related Transport ATPases* (Beauge, L.A., Gadsby, D.C., and Garrahan, P.J., eds.) Vol. 834, pp. 582-584, Annals of the New York Academy of Sciences, New York
29. Dean, W.L., Chen, D., Brandt, P.C., and Vanaman, T.C. (1997) Regulation of platelet plasma membrane Ca^{2+}-ATPase by cAMP-dependent and tyrosine phosphorylation. *J. Biol. Chem.* **272**, 15113-15119