J. Biochem. 121, 922-929 (1997)

# Transcriptional Activation of $H^+/K^+$-ATPase Genes by Gastric GATA Binding Proteins[1]

**Tsuyoshi Nishi,*[,2] Kentaro Kubo,* Masahisa Hasebe,* Masatomo Maeda,*[,†] and Masamitsu Futai*[,3]**

*Department of Biological Science, The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567; and †Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565*

Received for publication, December 13, 1996

**$H^+/K^+$-ATPase (composed of $\alpha$ and $\beta$ subunits) and histamine $H_2$ receptor are specifically expressed in gastric parietal cells. The GATA binding proteins (GATA-GT1 and GATA-GT2, also called GATA-6 and GATA-4, respectively) originally found in the gastric mucosa recognized a sequence motif [gastric motif, (G/C)PuPu(G/C)NGAT(A/T)PuPy] in the upstream regions of the ATPase genes [Tamura, S., Wang, X.-H., Maeda, M., and Futai, M. (1993) *Proc. Natl. Acad. Sci. USA* 90, 10876-10880]. These proteins activated the transcription of the reporter gene ligated downstream of the control region of the rat ATPase $\alpha$ or $\beta$ subunit gene but had no effect on the same reporter ligated downstream of the $H_2$ receptor gene. Deletion analyses suggested that the upstream 249 ($\alpha$ gene) and 323 ($\beta$ gene) base pair sequences from the first letter of the initiation codon are sufficient for activation by the GATA proteins. Interestingly, two and three gastric motifs are located near the TATA-boxes of the $\alpha$ and $\beta$ genes, respectively. Mutagenesis studies demonstrated that the two motifs proximal to the TATA-box sequences of the ATPase $\alpha$ and $\beta$ subunit genes were essential for the activation. These results suggest that both the $\alpha$ and $\beta$ subunit genes are regulated similarly by the GATA binding proteins. The expression system established in this study is a useful system for analyzing the roles of GATA proteins in transcriptional regulation of the $H^+/K^+$-ATPase gene.**

**Key words: GATA binding protein, $H^+/K^+$-ATPase, promoter, stomach, transcription.**

$H^+/K^+$-ATPase belongs to the P-type transport ATPases and is responsible for acid secretion into the stomach lumen (*1, 2*). The primary structures of the $\alpha$ and $\beta$ subunits and their gene organization are highly similar to those of the corresponding subunits of $Na^+/K^+$-ATPase (*3-6*). However, in contrast to the ubiquitous presence of $Na^+/K^+$-ATPase (*7*), $H^+/K^+$-ATPase is specifically expressed in gastric parietal cells (*8-12*), suggesting that the transcription of $H^+/K^+$-ATPase genes should be strictly regulated. Furthermore, the $\alpha$ and $\beta$ subunit genes may be expressed through the same mechanism because the amounts of the two subunits increased simultaneously during development of the mouse (*13*).

Both subunit genes have the upstream sequence motif [gastric motif, (G/C)PuPu(G/C)NGAT(A/T)PuPy] recognized specifically by nuclear protein(s) from gastric mucosa (*5, 14*). This motif includes the (A/T)GATA(G/A) sequence (GATA sequence) (*15-17*) recognized by GATA binding proteins. It was reasonable to assume that the nuclear protein(s) from gastric mucosa is similar to GATA binding protein. Based on this idea, we cloned cDNA for two additional members of the GATA binding protein family from a gastric cDNA library (*18*): they are GATA-GT1 and GATA-GT2, later also named GATA-6 and GATA-4, respectively (*19*). The two gastric GATA binding proteins could recognize the sequence motifs described above, and were detected in pig gastric mucosa (*18*) and rat parietal cells (*20*).

In this study, we demonstrated that gastric GATA binding proteins activate transcription of the $H^+/K^+$-ATPase $\alpha$ and $\beta$ subunit genes using a reporter gene expression system. Mutational analysis of the 5′-upstream regions of the subunit genes indicated that the two sequence motifs proximal to the TATA-box are important for gene activation. However, transcription of the histamine $H_2$ receptor gene (*21*) did not respond to gastric GATA proteins, although the receptor is present in parietal cells and the upstream region contains a sequence similar to the gastric motif.

## MATERIALS AND METHODS

*Construction of Plasmids*—GATA-GT1 and GATA-GT2 (*18*) expression plasmids were constructed using pSR$\alpha$Eco

[1] This work was supported by grants from the Japanese Ministry of Education, Science, Sports and Culture, and the Human Frontier Science Program.
[2] T.N. was supported by Research Fellowship from the Japan Society for the Promotion of Science for Young Scientists.
[3] To whom correspondence should be addressed. Tel: +81-6-879-8480, Fax: +81-6-875-5724, E-mail: m-futai@sanken.osaka-u.ac.jp
Abbreviations: PBS, phosphate-buffered saline [10 mM sodium phosphate buffer (pH 7.2), 137 mM NaCl, 3 mM KCl]; bp, base pairs; kb, kilobase pairs.

(kindly provided by Dr. K. Maruyama, Institute of Medical Sciences, University of Tokyo) as a vector: the *Ase*I-*Kpn*I fragment of rat GATA-GT1 cDNA was ligated into the *Eco*RI and *Kpn*I sites, and the *Eco*RI-*Eco*RI fragment of rat GATA-GT2 cDNA into the *Eco*RI site. The 4 kb fragment (*Eco*RI fragment of the genomic clone, λHKBR3) of the rat $H^+/K^+$-ATPase β subunit gene (*5*) was sequenced by the chain-termination method (*22*). The *Sma*I fragment (−2933 to +51 bp) of the rat β subunit gene was inserted between the *Sma*I sites of the PGV-B vector (Toyo Ink, Tokyo) carrying the luciferase gene without a promoter sequence. Portions of the insert were systematically deleted from this construct using appropriate restriction enzyme sites. The −2984 to +3 bp fragment of the rat α subunit gene was inserted between the *Nhe*I and *Hin*dIII sites of PGV-B, and deletion constructs were similarly obtained. pGVH2SA (*21*) carrying the human $H_2$ receptor gene control region (−1773 to −37 bp) ligated with the reporter gene was also used. To introduce mutations into the gastric and GATA motifs, oligonucleotides carrying TCT(A/T) instead of GAT(A/T) were synthesized and used as primers for the polymerase chain reaction.

*Transfection and Reporter Gene Assay*—HeLa cells (about $2\text{-}8\times10^6$ cells/10 cm diameter petri dish) were cultured overnight in Dulbecco's modified Eagle medium with 10% new-born calf serum. The monolayer was washed twice with PBS (phosphate-buffered saline) and then trypsinized. The cells were suspended in PBS and adjusted to $1\times10^7$ cells/ml. A portion (400 μl) of the cell suspension was transferred to a 0.4 cm cuvette (BioRad), mixed with 17 μl of a plasmid solution (2.5 μg DNA/μl), and then subjected to electroporation [500 μF, 200 V (Genepulser, BioRad)]. To normalize the transfection efficiency, 10 μg pact β-gal plasmid (Nippon Gene) was co-transfected. The cells were transferred to a 6 cm diameter dish and incubated for 48 h at 37°C, and then an extract of them was prepared, and subjected to assaying of luciferase and β-galactosidase (*21*). The PGV-C plasmid (Tokyo Ink) carrying the luciferase gene downstream of the SV40 promoter and enhancer was used as a positive control. The background level was determined using pBluescript SKII (+).

CHO-K1 cells were cultured in F-12 medium containing 10% new-born calf serum (*23*). The GATA-GT1 or GATA-GT2 expression plasmid was transfected into these cells by the calcium phosphate method. Stable transformants were selected and used for the gel mobility shift assay.

*Gel Mobility Shift Assay*—Gel mobility shift assays were carried out essentially as described previously (*5, 14*). A nuclear extract was prepared from CHO-K1 cells by the method of Andrew and Faller (*24*) with minor modification. The cells were washed with PBS, and then lysed in buffer A (10 mM HEPES-KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl) containing 0.5% (v/v) Nonidet P40. After centrifugation at $2{,}000\times g$ and washing with buffer A, the nuclei were

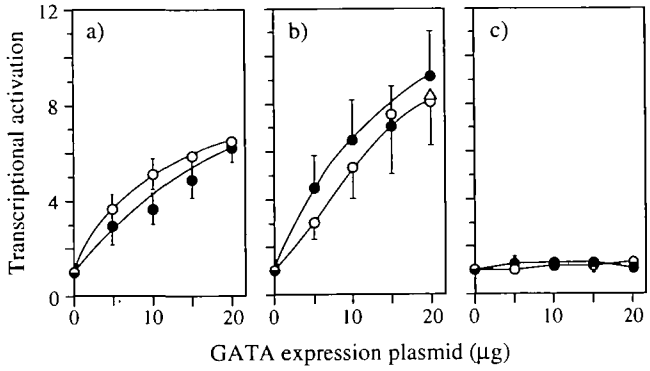


Fig. 2. **Transcriptional activation of gastric parietal cell-specific genes by GATA binding proteins.** A recombinant plasmid carrying the luciferase gene downstream of the control region of the $H^+/K^+$-ATPase α subunit gene (−2984 to −1 bp) (a), β subunit gene (−2933 to +51 bp) (b), or histamine $H_2$ receptor gene (−1773 to −37 bp) (c) was transfected into HeLa cells together with varying concentrations of an expression plasmid for GATA-GT1 (●) or GATA-GT2 (○). A mixture (1 : 1) of the two GATA binding proteins was also tested (b, triangle). The luciferase activities of the cell extracts were assayed and normalized as to the β-galactosidase activity due to pact β-gal plasmid. The transcriptional activation values with standard deviation shown are relative to a control without the GATA-GT1 or GATA-GT2 plasmid.

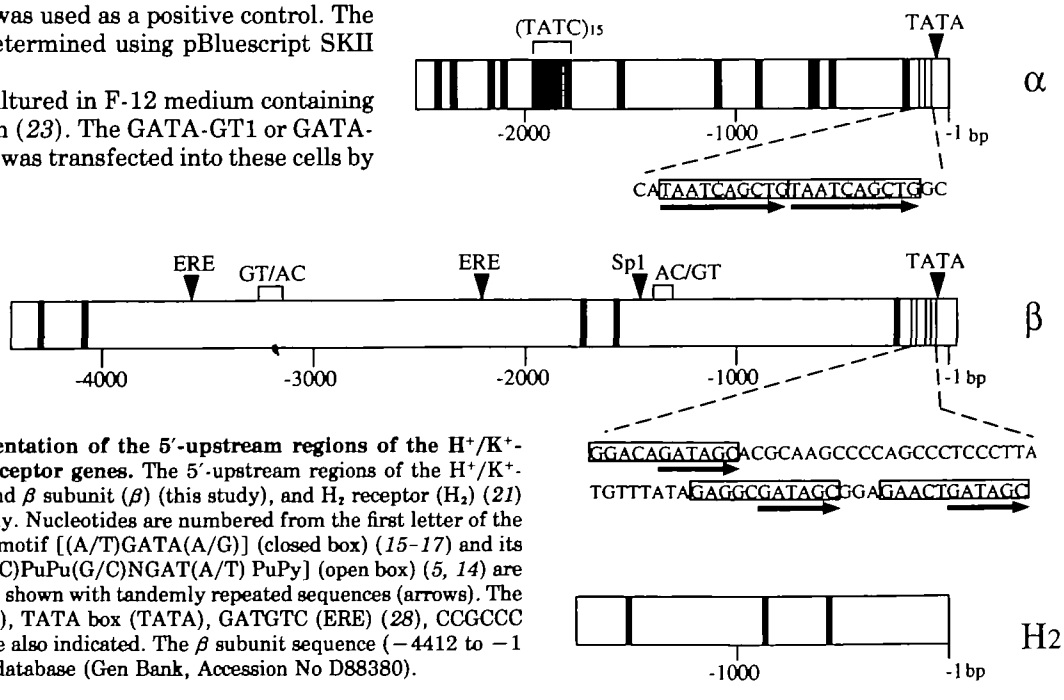


Fig. 1. **Schematic representation of the 5′-upstream regions of the $H^+/K^+$-ATPase subunit and $H_2$ receptor genes.** The 5′-upstream regions of the $H^+/K^+$-ATPase α subunit (α) (*8*) and β subunit (β) (this study), and $H_2$ receptor ($H_2$) (*21*) genes are shown schematically. Nucleotides are numbered from the first letter of the initiation codon. The GATA motif [(A/T)GATA(A/G)] (closed box) (*15-17*) and its derivative gastric motif [(G/C)PuPu(G/C)NGAT(A/T) PuPy] (open box) (*5, 14*) are shown. The gastric motifs are shown with tandemly repeated sequences (arrows). The AC repeat sequence (AC/GT), TATA box (TATA), GATGTC (ERE) (*28*), CCGCCC (Sp1) (*43*), and $(TATC)_{15}$ are also indicated. The β subunit sequence (−4412 to −1 bp) has been deposited in a database (Gen Bank, Accession No D88380).

resuspended in buffer C [20 mM HEPES-KOH, pH 7.9, 25% (v/v) glycerol, 420 mM, NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA] and kept on ice for 20 min. The extract was stored at −70°C until use. All buffers used contained 0.5 mM phenylmethylsulfonylfluoride, 2 μg/ml leupeptin, and 2 μg/ml pepstatin A (*24*). The *Pst*I–*Sty*I fragments (−185 to +10 bp) of the plasmid constructs for the wild and mutant β subunit genes were prepared, and their 3′-ends were labeled with [α-$^{32}$P]dCTP using the Klenow fragment. Antisera for GATA-GT1 (peptide between Thr-333 and Ala-391) and GATA-GT2 (between Leu-54 and Ala-130) were prepared from albino rabbits using glutathione-S-transferase fusion proteins.

*Chemicals*—Restriction enzymes, T4 DNA ligase, T4 polynucleotide kinase, calf intestine phosphatase, and the Klenow fragment were obtained from Takara Shuzo (Kyoto), Toyobo (Osaka), Nippon Gene (Toyama), or New England Biolabs (Beverly, MA). New-born calf serum and cell culture medium were purchased from Dainippon Pharmaceutical (Osaka). Sequenase was from United States Biochemical (Cleveland, OH). [α-$^{32}$P]dCTP (110 TBq/mmol) was from the Radiochemical Center, Amersham. All other chemicals used were of the highest grade commercially available.

## RESULTS

*Upstream Sequences of $H^+/K^+$-ATPase Genes*—Previously, we identified a conserved sequence [gastric motif, (G/C)PuPu(G/C)NGAT(A/T) PuPy] in the $H^+/K^+$-ATPase α and β subunit genes immediately upstream of the TATA box for major transcription start sites (*14*) (Fig. 1, open box). The motif includes the GATA sequences recognized by GATA-binding proteins (*15–17*). To identify GATA-related motifs in the upstream of $H^+/K^+$ ATPase genes, we sequenced the 5′-upstream of the β subunit gene

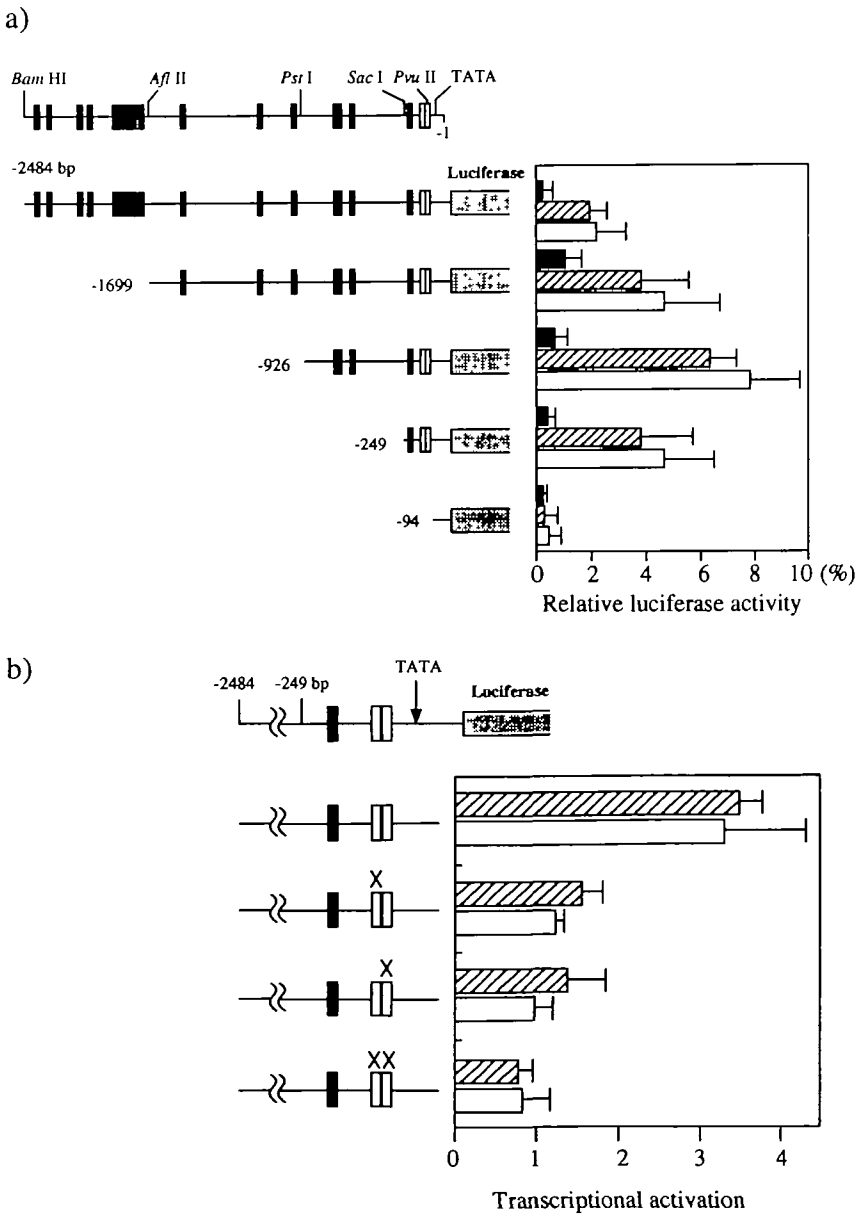


Fig. 3. **Gastric motifs in the 5′-upstream region of the $H^+/K^+$-ATPase α subunit gene are essential for transcription activation.** (a) A series of deletions of the 5′-upstream region of the $H^+/K^+$-ATPase α subunit gene were ligated in front of the luciferase gene and transfected with an expression plasmid for GATA-GT1 (shaded box) or GATA-GT2 (open box). The luciferase activities of the cell extracts were assayed and normalized as to the β-galactosidase activity. The values with standard deviation shown are relative to the luciferase activity of cells transfected with PGV-C (luciferase gene downstream of the SV40 promoters and enhancers), and are averages of at least three measurements of two different samples. An experiment without GATA expression plasmids (closed box) was also carried out as a control. (b) Mutations (X, GATT→TCTT) were introduced into the 5′-upstream region of the α subunit gene, which was then ligated with the reporter gene and transfected with the expression plasmid for GATA-GT1 (shaded box) or GATA-GT2 (open box). The values are relative to a control without GATA-GT1 or GATA-GT2.

between −4412 and −2221 bp further upstream of the reported sequence (*6*). The 5′-upstream region of the α subunit gene was cloned and sequenced previously up to −2485 bp (*8*). As shown schematically in Fig. 1, the GATA motifs were found in the further upstream region of both genes (Fig. 1, closed box). The GATA motifs were also found in the $H_2$ receptor gene (Fig. 1).

*$H^+/K^+$-ATPase Genes Are Responsive to Gastric GATA Proteins but the $H_2$ Receptor Gene Is Not*—Both $H^+/K^+$-ATPase (*20*) and histamine $H_2$ receptor (*25*) are expressed in gastric parietal cells. It was of interest to determine whether the expression of both genes is stimulated by gastric GATA proteins (GATA-GT1 or GATA-GT2). The upstream segments (α or β subunit gene, about 3 kb; $H_2$ receptor, 1.8 kb) of these genes were ligated with the 5′-end of the luciferase gene and then introduced into HeLa cells. The cells transfected with the α subunit gene expressed about 1% of the luciferase activity of the control plasmid (PGV-C, utilizing the SV40 promoter and enhancer). However, luciferase expression was enhanced significantly upon co-transfection of the recombinant plasmid expressing the GATA-GT1 or GATA-GT2 protein (Fig. 2a): several-fold stimulation was observed upon transfection of the GATA-GT1 or GATA-GT2 expression plasmid. These results suggest that the upstream 3 kb region of the α subunit gene contains a promoter that is activated by the gastric GATA proteins. Similarly, transcription of the β subunit reporter gene was stimulated about 10-fold upon co-transfection with the GATA-GT1 or GATA-GT2 expression plasmid, suggesting that the segment has a functional promoter responding to the GATA proteins (Fig. 2b). It is noteworthy that GATA-GT1 and GATA-GT2 gave essentially the same results. The addition of a mixture of the two plasmids (1 : 1 mixture, weight ratio) caused similar stimulation to that on the addition of either alone (Fig. 2b, triangle).

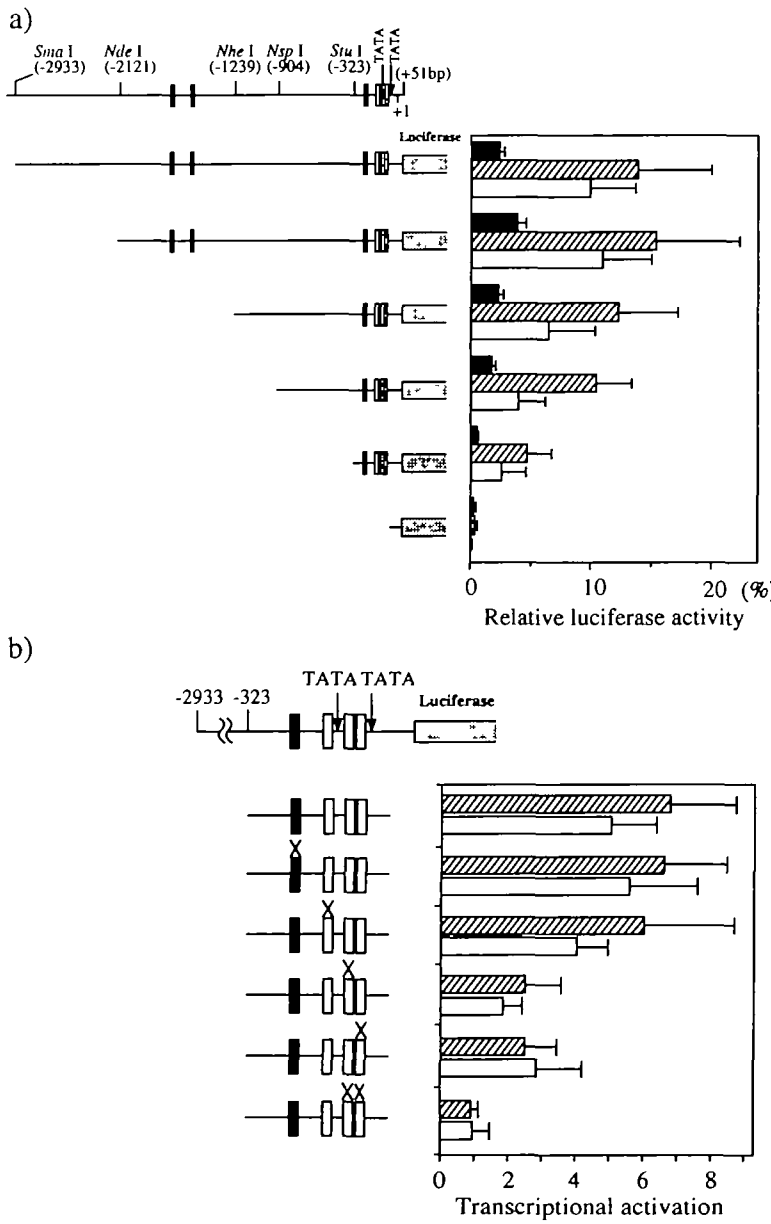


Fig. 4. **Gastric motifs in the β subunit gene are essential for transcription activation.** (a) A series of deletions of the 5′-upstream region of the $H^+/K^+$-ATPase β subunit gene were ligated in front of the luciferase gene, and then transfected together with an expression plasmid for GATA-GT1 (shaded box) or GATA-GT2 (open box). The luciferase activities of the extracts were assayed and normalized. The results shown are averages of at least three measurements of two different samples. Experiments without GATA expression plasmids were also carried out (closed box). (b) Mutations (X, GATA→TCTA) were introduced into the 5′-upstream region of the β subunit, which was then ligated with the reporter gene and transfected with the expression plasmid for GATA-GT1 (shaded box) or GATA-GT2 (open box). See the Fig. 3 legend and the text for more detail.

However, the promoter activity of the $H_2$ receptor gene did not change with the co-transfection of GATA expression plasmids (Fig. 2c), indicating that the $H_2$ receptor gene did not respond to the GATA proteins. Thus, expression of the receptor and ATPase genes is regulated differently. In this regard, a significant amount of the $H_2$ receptor mRNA is found in brain and stomach (*26*), whereas $H^+/K^+$-ATPase genes are only transcribed in stomach (*8-13*).

*Functional GATA Motifs Upstream of the α Subunit Gene*—To determine the upstream region essential for transcription activation of the α subunit gene, we constructed a series of deletions and ligated them with the reporter gene. The 5′-flanking sequence between −249 and −1 bp could still respond to GATA-GT1 or GATA-GT2, essentially similar to the −2.5 kb flanking sequence (Fig. 3a). However, essentially no enhancement was observed when the region upstream of the TATA box containing two gastric motifs was deleted. These results suggest that GATA sequences and $(TATC)_{15}$ found between −2.5 kb and 250 bp (Fig. 1) are not essential for transcription stimulation by gastric GATA proteins, and that at least one motif near the TATA box is essential for the stimulation.

It became of interest to determine which gastric motif is essential for the response to GATA-GT1 or GATA-GT2. We introduced a mutation (GATT→TCTT) into each sequence including the gastric motif. A mutation in either of the two proximal gastric motifs lowered the transcription drastically and ones in both motifs completely abolished the transcription activation (Fig. 3b). These results strongly suggest that the binding of gastric GATA proteins to the two gastric motifs is critical for the transcriptional activation.

*Functional GATA Motifs in the 5′-Upstream Region of the β Subunit Gene*—It was interesting to determine whether the β subunit gene transcription is similarly activated by the GATA proteins. This possibility seemed to be very high, because two gastric motifs are located proximal to the TATA-boxes of both the α and β subunit genes (*14*). Several GATA motifs are found in the 5′-upstream sequence in addition to these two gastric motifs. Analysis of a series of deletion constructs suggested that the two GATA motifs in addition to the proximal tandem gastric motifs may be responsible for the transactivation (Fig. 4a). These results suggest that repeats of AC (around −1.4 kb), ERE (epidermal growth factor responsive element) (−2.2 kb), and Sp1 (−1.5 kb) (Fig. 1) are not required for the transcription activation. Further analysis by site-directed mutagenesis (GATA→TCTA) demonstrated that the proximal tandem gastric motifs are essential for the response to the gastric GATA protein(s) (Fig. 4b). Thus the two motifs may play crucial roles in the transcription activation of both the α and β subunit genes by the GATA-GT1 or GATA-GT2 protein(s).

*GATA-Binding Proteins Could Bind to the Three Gastric Motifs of the β Subunit Gene*—To determine whether GATA-GT1 or GATA-GT2 can bind to the gastric motif in the 5′-upstream region of the $H^+/K^+$-ATPase genes, we carried out a gel mobility shift assay. Nuclear extracts containing GATA-GT1 and GATA-GT2 were prepared from CHO-K1 cells transfected with the respective expression plasmids. The extract proteins clearly bound to the β subunit upstream sequence (195 bp *Sty*I-*Pst*I segment) carrying three gastric motifs (Fig. 5a). These DNA-protein complexes formations were specific for the GATA sequences, since the complex formation was inhibited by the DNA segment with gastric motifs (GATA in Fig. 5a, TAGAGGCGATAGCGGAGA) but not by that without the motif (TCTA in Fig. 5a, TAGAGGCTCTAGCGGAGA). Furthermore, the proteins bound to the DNA segment were suggested to be GATA-GT1 and GATA-GT2, because all complexes remained at the origin on gel electrophoresis

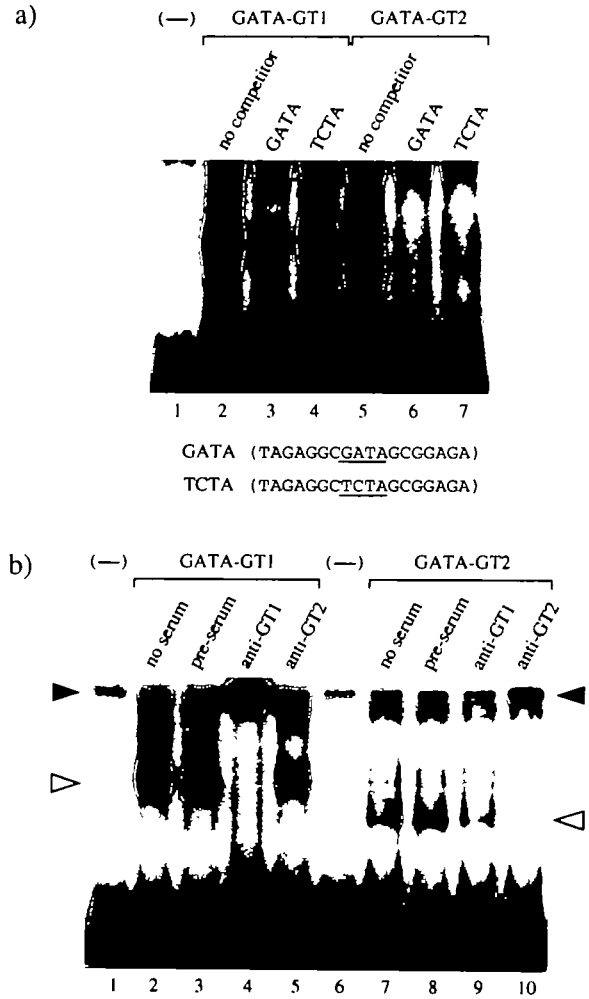


Fig. 5. **Binding of GATA-GT1 or GATA-GT2 to the GATA motifs in the β subunit gene.** (a) A nuclear extract of CHO-K1 cells containing GATA-GT1 (lanes 2-4) or GATA-GT2 (lanes 5-7) was incubated with the radio-labeled DNA fragments of the β subunit gene [the 195 bp *Sty*I-*Pst*I fragment (−185 to +10 bp, numbered from the translation initiation site)]. Non-labeled DNA fragments, GATA (TAGAGGCGATAGCGGAGA) and TCTA (TAGAGGCTCTAGCGGAGA), were used as competitors. A control nuclear extract of CHO-K1 cells (not transfected with the GATA-GT1 or GATA-GT2 expression plasmid) was also added (lane 1). (b) Effects of antisera against GATA-GT1 and GATA-GT2 on the mobilities of DNA-protein complex. Nuclear extracts containing GATA-GT1 (lanes 2-5) and GATA-GT2 (lanes 7-10) were incubated with the radio-labeled probe. Before the binding reaction, the nuclear extracts were incubated with preimmune serum (lanes 3 and 8), or antiserum against GATA-GT1 (lanes 4 and 9) or GATA-GT2 (lanes 5 and 10). The bands corresponding to the DNA complexes with the GATA-GT1 and GATA-GT2 proteins (open arrowheads) and super-shifted complexes (closed arrowheads) are shown. A nuclear extract without GATA-GT1 and GATA-GT2 was applied to lanes 1 and 6, respectively.

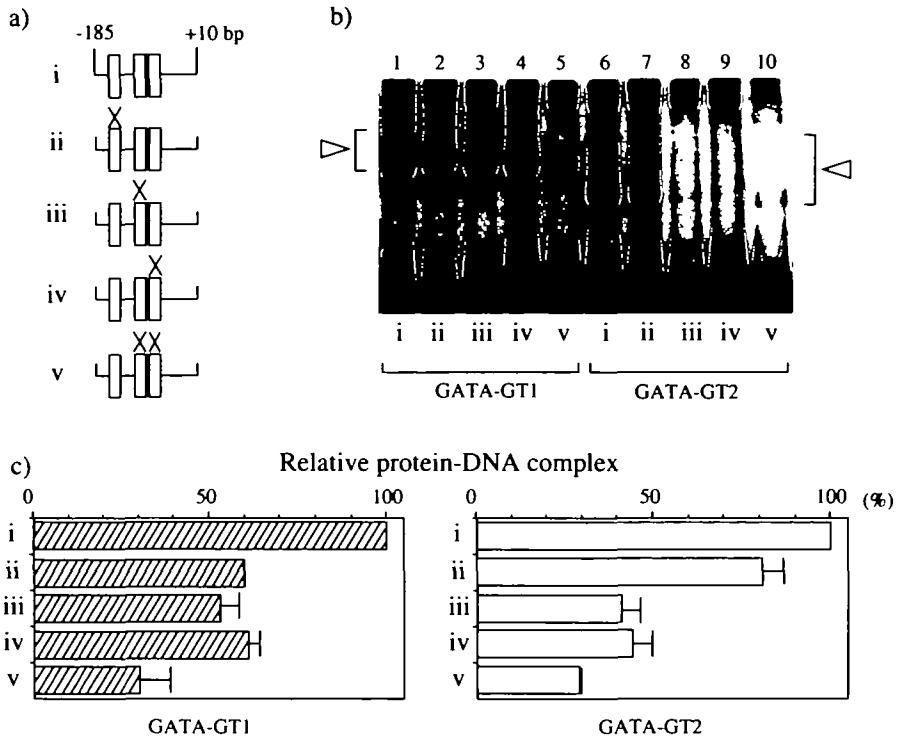


Fig. 6. **Binding of GATA-GT1 and GATA-GT2 to the β subunit gene with the mutant gastric (GATA) motif.** (a) Schematic illustrations of the wild and mutant fragments used as probes. The 195 bp *Sty*I-*Pst*I fragment (−185 to +10 bp from the translation initiation site) was used for the wild-type or mutant probe. The positions of mutations (x) are shown in the legend to Fig. 4b. The wild-type and mutant DNA segments (i-v) were labeled with the Klenow fragment. (b) Nuclear extracts were prepared from CHO-K1 cells transfected with GATA-GT1 (lanes 1-5) or GATA-GT2 (lanes 6-10), and then incubated with the radio-labeled DNA fragments (i-v) of the β subunit gene. DNA-protein complexes were visualized by gel-mobility shift assay. The positions of DNA-protein complexes are indicated (open arrowheads). (c) The radioactivities of the area indicated by open arrowheads were estimated with a BAS 1000 and their relative amounts are shown. Open arrowheads at right and left of the figure are for GATA-GT1 and GATA-GT2, respectively.

when antisera against GATA-GT1 and GATA-GT2, respectively, were added (Fig. 5b, lanes 4 and 10, respectively).

The results of reporter gene assays suggested that two gastric motifs proximal to the TATA box are important for transactivation by GATA proteins (Figs. 3 and 4). However, the third gastric motifs were found upstream of the TATA box (Fig. 6a), and were shown to bind gastric nuclear protein(s) (*5*). It was of interest to determine whether GATA proteins bind to the three gastric motifs. When a mutation was introduced into one of the three GATA sites, about 40% of the GATA-GT1-DNA complex disappeared and mutations in two gastric motifs further decreased the amount of the complexes (Fig. 6). These results suggest that GATA-GT1 bound to the three motifs similarly. On the other hand, about 20% of the GATA-GT2-DNA complex disappeared with the mutation in the most upstream motif, while 60% disappeared with a mutation in one of the tandem motifs. These results suggest that GATA-GT2 could bind preferentially to the tandem motifs. However, two tandem motifs proximal to the TATA-box are required for transactivation similarly by GATA-GT1 and GATA-GT2. We observed several shifted bands with GATA-GT1 or GATA-GT2 (Fig. 6b, open arrowhead). It was of interest to know whether these bands corresponded to the numbers of the gastric motifs in probes. However, number of the shifted bands did not change with the decrease of numbers of the motif, although the amount of each band was decreased. We made similar observation even with oligonucleotides with single gastric motif (*5*). Thus the GATA protein may form a oligomer or complex with other unknown nuclear proteins.

## DISCUSSION

$H^+/K^+$-ATPase is expressed specifically in gastric parietal cells (*1, 2*). Experiments with a transgenic mouse indicated that the reporter genes could be expressed in parietal cells when they were ligated with the 5′-flanking sequence up to −1035 bp of the β subunit gene (*27*). The sequence of the rat gene between −445 and −1 bp is highly homologous to that of mouse. Thus it was reasonable to assume that the 5′-flanking sequence up to −1.0 kb plays a pertinent role in the cell-specific transcription.

Immediately upstream of the TATA box, both the α and β subunit genes have the gastric motif (*14*), to which GATA-GT1 or GATA-GT2 (*18*) could bind. The motif is essentially a GATA sequence to which other GATA proteins (*2, 15-17*) can bind. The transcripts of the GATA-GT1 and GATA-GT2 genes are found in gastric mucosa (*18*) or parietal cells (*20*). These results suggest that GATA-GT1 or GATA-GT2 activates transcription of the α and β subunit genes. Yamada and coworkers showed the presence of epidermal growth factor (EGF)-responsive element (ERE) in the upstream region of the dog α subunit gene, and that the transcription of the gene was stimulated by EGF (*28*). However, the ERE was not found in the corresponding region of the rat α subunit gene, although two were found in the β subunit upstream region.

As no established cell line expressing $H^+/K^+$-ATPase is available at present, it is not easy to study transcription of the ATPase genes directly. The model system reported in this study induced specific transcription from the control region of $H^+/K^+$-ATPase genes dependent on gastric GATA proteins: GATA-GT1 or GATA-GT2 could activate transcription of the reporter gene downstream of the 5′-flanking sequences of the α and β subunit genes. Assays with systematic deletions of reporter genes indicated that upstream regions of the α and β subunit genes up to −249 and −323 bp, respectively, are sufficient for transcription stimulation by gastric GATA proteins. Directed mutation

studies indicated that the two gastric motifs proximal to the TATA box were essential for activation of both the $\alpha$ and $\beta$ subunit genes. These results suggest that the binding of the two molecules of the GATA-GT1 or GATA-GT2 protein to the proximal motifs could stimulate the transcription. It should be noted that similar motifs could not be found in the corresponding regions of $Na^+/K^+$-ATPase genes (*29, 30*), although the gene (intron/exon) organization, and amino acid sequences of $H^+/K^+$- and $Na^+/K^+$-ATPases are similar (*3, 5, 8*).

GATA-GT1 could bind similarly to the three gastric motifs, as shown by gel-mobility shift assay. However, mutations in the third gastric motif had no effect on gene expression. These results suggest that GATA binding proteins could bind to the third motif upstream of the proximal motif but that this binding was not directly related to the transcription activation. GATA-GT2 could bind to the two proximal motifs more preferentially. There results suggest that GATA-GT2 could bind to these motifs with high affinity, because they are tandemly repeated sequences (Fig. 1). In this regard, GATA-1 was shown to bind to the palindromic and tandemly-repeated GATA site with much higher affinity (*31*).

Since GATA-GT1 and GATA-GT2 recognize essentially the same sequence as other GATA proteins, it is reasonable to assume that other GATA proteins stimulate the transcription in parietal cells. As expected, GATA-1 (*32-34*) gave essentially the same degree of transcription activation in our system as GATA-GT1 or GATA-GT2 (data not shown). However, the presence of GATA-1 mRNA was shown to be very low in gastric mucosa by means of reverse transcriptase polymerase chain reaction and Northern hybridization (data not shown). Thus, the possibility of the regulation of $H^+/K^+$-ATPase genes by the GATA-1 protein may be low.

Recent studies showed that GATA-GT1 (GATA-6) and GATA-GT2 (GATA-4) activated cardiac-specific gene transcription, suggesting that they are responsible for the specific gene expression during cardiac development (*35-40*). Furthermore, the expression of GATA-GT1 and GATA-GT2 was shown in many tissues, including stomach and heart (*18, 35-39*). These results suggested that transcription of the tissue-specific genes is regulated not only by GATA-GT1 or GATA-GT2, but also other unknown factors. For erythroidal gene regulation, GATA-1 is necessary together with other factors, NF-E2, SP1 and EKLF (*41, 42*). Similarly, the $H^+/K^+$-ATPase genes may be regulated by GATA-GT1 or GATA-GT2 and other unknown proteins specifically present in parietal cells. All these proteins may not be present in the HeLa cells used in this study because they do not express $H^+/K^+$-ATPase. In this regard, the stimulation observed may be due to a part of the regulation actually operating in parietal cells, and further increase may be observed if we add other factors that interact specifically with GATA-GT1 or GATA-GT2 during the transcription of $H^+/K^+$-ATPase genes. Thus, the present study established a convenient system for searching such transcription factors.

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