ELSEVIER

FEBS Letters 340 (1994) 117–120

FEBS 13710

FEBS LETTERS

Genes for members of the GATA-binding protein family (GATA-GT1 and GATA-GT2) together with H^+/K^+-ATPase are specifically transcribed in gastric parietal cells

Sotaro Mushiake[a,*], Yuri Etani[a], Shoichi Shimada[b], Masaya Tohyama[b], Masahisa Hasebe[c], Masamitsu Futai[c], Masatomo Maeda[c]

Departments of [a]Pediatrics, and [b]Anatomy and Neuroscience, Osaka University Medical School, Suita, Osaka 565, Japan
[c]Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567, Japan

Received 24 December 1993; revised version received 17 January 1994

Abstract

mRNAs for novel DNA-binding proteins (GATA-GT1 and GATA-GT2) recognizing the (G/C)PuPu(G/C)NGAT(A/T)PuPy sequence and H^+/K^+-ATPase (proton pump) α subunit were detected in parietal cells of the rat gastric body mucosa by in situ hybridization. These results suggest that GATA-GT1 and GATA-GT2 together with H^+/K^+-ATPase are transcribed specifically in gastric parietal cells and that the two DNA-binding proteins may have important roles in cell specific gene regulation. Furthermore, we could detect parietal cells in different states of gene expression.

Key words: Parietal cell; Gastric specific transcription; GATA factor; DNA-binding protein; Hybridization (in situ); H^+/K^+-ATPase

1. Introduction

H^+/K^+-ATPase is specifically expressed in gastric parietal cells and is responsible for acid secretion into the stomach [1]. The primary structure and membrane topology of the pump ATPase composed of catalytic α and glycosylated β subunits has been determined by molecular biological studies [2]. The intron/exon organizations of the α and β subunit genes have been determined and the 5′-upstream sequences have been shown to be conserved between the human and rat α subunit genes [3,4], and between the rat and mouse β subunit genes [5,6]. The mechanisms of expression of the two subunit genes are of interest from the viewpoint of cell specific transcription, because both subunits are detected immunochemically in gastric parietal cells [7–9]. We recently demonstrated that a nuclear protein(s) from rat stomach recognizes a sequence motif [(G/C)PuPu(G/C)NGAT (A/T)PuPy] (Pu, purine nucleotide; Py, pyrimidine nucleotide) located in the 5′-upstream sequences of the α and β subunit genes [5,10]. This protein(s) is suggested to be important for transcriptional activation of these genes. Subsequently we found three novel zinc finger proteins in rat stomach and studied two of them (GATA-GT1 and GATA-GT2) in detail [11]. These two proteins are members of the GATA-binding protein family and their tissue distributions are distinct from those of other members of this family. GATA-GT1 and GATA-GT2 are expressed in gastric mucosa and bind to the above upstream motif of H^+/K^+-ATPase subunit genes. Although they are suggested to be candidates for the regulation of transcription of the H^+/K^+-ATPase subunit genes, it was necessary to demonstrate that they are actually expressed specifically in gastric parietal cells. In this study, we show direct evidence for the specific expressions of these protein messages in rat gastric parietal cells by in situ hybridization. Furthermore, we confirmed that the message for the gastric proton pump α subunit is also specifically expressed in the same cells. These results suggest that GATA-GT1 and GATA-GT2 are important in the specific transcription in parietal cells.

2. Materials and methods

2.1. Preparation of RNA probes

The *Nde*I–*Bam*HI fragment (2531–2921 bp) of H^+/K^+-ATPase α subunit cDNA (12), the *Bst*XI–*Bst*XI fragment (−17–1439 bp) of GATA- GT1 and the *Eco*RI–*Xba*I fragment (−562–2303 bp) of GATA-GT2 [11] were subcloned into *Bam*HI–*Eco*RV-, *Eco*RV- and *Eco*RV-digested pBluescript II SK(+) and named pHKA-BN, pGT1-Bst and pGT2-EX, respectively. These recombinant plasmids were linearized for hybridization assay: pHKA-BN was digested with *Nco*I (to prepare 243 base antisense and 147 base sense probes), pGT1-Bst was digested

*Corresponding author.

Abbreviations: bp, base pair; NaPB, 0.1 M sodium phosphate buffer pH 7.4.

SSDI 0014-5793(94)00094-C

with *Pst*I (for a 411 base antisense probe) or with *Nco*I (for a 231 base sense probe), and pGT2-EX was digested with *Bst*XI (for a 454 base antisense probe) or with *Nru*I (for a 445 base sense probe). Each DNA fragment (1 μg) was used as a template for in vitro transcription with digoxigenin-labeled dUTP by T3 or T7 RNA polymerase.

2.2. Tissue preparation

Five Wistar rats (200–230 g, male) were fasted overnight, anesthethized with sodium pentobarbital (55 mg/kg) and perfused transcardially with ice-cold 4% paraformaldehyde in NaPB (2 ml/g body weight). The stomach and duodenum were excised, immersed in the fixative for 2–3 h, and incubated in NaPB containing 30% (w/v) sucrose at 4°C until they sank. Then they were quickly frozen in dry-ice/2-methylbutane mixture, and 7 μm sections were cut on a cryostat and thaw-mounted on 3-aminopropyl-triethoxysilane-coated slides. The sections were rinsed with NaPB, and then treated with 10 μg/ml proteinase K (Boehringer Mannheim) in 50 mM Tris-HCl, 5 mM EDTA (pH 8.0) for 10 min. After further treatment with 4% paraformaldehyde, they were incubated in 0.2 N HCl for 10 min to inhibit the endogenous alkaline phosphatase activity. They were then rinsed with NaPB, acetylated with 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine, rinsed with NaPB, dehydrated in an ethanol series, and air-dried.

2.3. In situ hybridization procedures

The procedures used for hybridization and detection were basically as described previously [12]. Briefly, the sections were preincubated for 1 h at 50°C in hybridization buffer (10% sodium dextransulfate, 20 mM Tris-HCl (pH 8.0), 0.3 M NaCl, 0.2% sarcosyl, 0.02% salmon sperm DNA, 1x Denhardt solution and 50% formamide) and then incubated in the same buffer containing 0.2 μg/ml of each digoxigenin-labeled RNA probe in a humid chamber for 16 h at 50°C. They were rinsed in 5 × SSC (1 × SSC = 150 mM NaCl and 15 mM sodium citrate) and then in 50% formamide in 2 × SSC at 60°C, and equilibrated with RNase buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA and 0.5 M NaCl). They were then treated with RNase A (1 μg/ml) in the same buffer for 30 min, and rinsed in 50% (v/v) formamide and 2 × SSC for 30 min at 60°C. Hybridization products were detected colorimetrically according to procedure recommended in the manual of a DIG Nucleic Acid Detection Kit. Finally, the sections were briefly counterstained with by Carrazi's hematoxylin, rinsed in tap water and placed under coverslips in aqueous mounting medium.

2.4. Chemicals

Restriction enzymes and pBluescript II SK(+) were purchased from Takara Shuzo Co., (Kyoto Japan) and Toyoybo Inc., (Osaka Japan), respectively. A DIG RNA Labeling Kit, DIG Nucleic Acid Detection Kit, T3 and T7 RNA polymerases, RNase A, nitroblue tetrazolium and 5-bromo-4-chloro-3-indole phosphate were purchased from Boehringer Mannheim. All other chemicals used were of the highest grade commercially available.

3. Results and discussion

3.1. Specificities of the probes

To establish the specificities of the probes for *in situ* hybridization, we compared the nucleotide sequences of the cDNAs for GATA-GT1 and GATA-GT2 [11]. Harr plot analysis revealed essentially no similarities between GATA-GT1 and -GT2 except in the regions corresponding to their zinc finger domains (Fig. 1). This was also true for other GATA DNA-binding proteins (GATA-1, GATA-2 and GATA-3) carrying similar zinc finger domains [13] (not shown). Furthermore, we could not find similar nucleotide sequences in the Gen Bank or EMBL Data Bases (Rel. 76.0 April 1993 and Rel. 34.0 March 1993, respectively). The positions for the probes used in this study are shown in Fig. 1. The probes for the GATA-GT1 and H^+/K^+-ATPase α subunit have been used successfully for detecting their tissue specific messages by Northern blot hybridization [4,11].

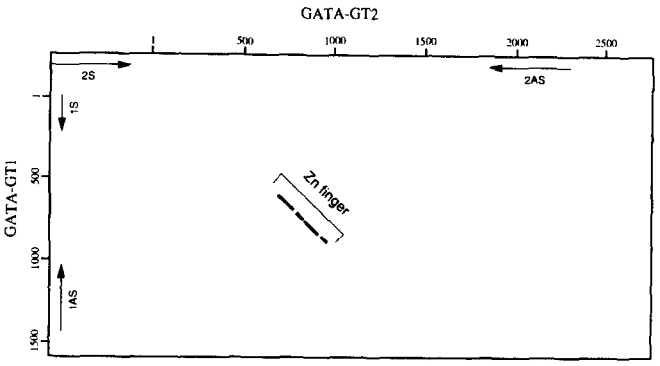

Fig. 1. Difference in nucleotide sequences of rat GATA-GT1 and GATA-GT2 cDNAs. The nucleotide sequences of rat GATA-GT1 and GATA-GT2 cDNAs [11] were compared by a Harr plot program (GENETYX, Software Development Co. Japan). Each dot represents more than 30 identical residues within a span of 40 residues. The highly homologous region corresponds to that of the zinc finger domain [13]. The antisense 1AS and 2AS and sense 1S and 2S probes shown by arrows were used for further experiments. The probes 1AS and 1S and 2AS and 2S were for GATA-GT1 and GATA-GT2, respectively.

3.2. Specificity control

The specificities of the hybridization signals were checked by comparing sections treated with the antisense and sense probes for the H^+/K^+-ATPase α subunit. Nonspecific immunostainings were detected on the extracellular mucous matrix on the surface mucus cell layer and in some intraluminal spaces of specimens treated with sense probes (Fig. 2A). This may be because the mucous matrix reacts with the reagents used in immunohistochemical techniques. However, no staining of epithelial cell cytoplasm, submucosa or muscular layers was observed. Furthermore, no hybridization signals were detected in sections of gastric antrum and duodenum treated with either antisense or sense probes (Fig. 2B and C, respectively).

3.3. Parietal cell specific transcriptions of GATA-GT1 and -GT2

In the rat gastric body mucosa, parietal cells are present from the isthmus to the base gland region with other types of cells (mucous, chief, and enteroendocrine cells). Within the mucosa, parietal cells differentiate in the neck of glands and migrate up or down the mucosal epithelium. They are present most densely, forming clusters, in the neck of glands [14,15]. At high magnification, parietal cells are easily identified morphologically as large, oval or pyramidal-shaped cells with coarse cytoplasm and appear to bulge into the surrounding lamina propria [15].

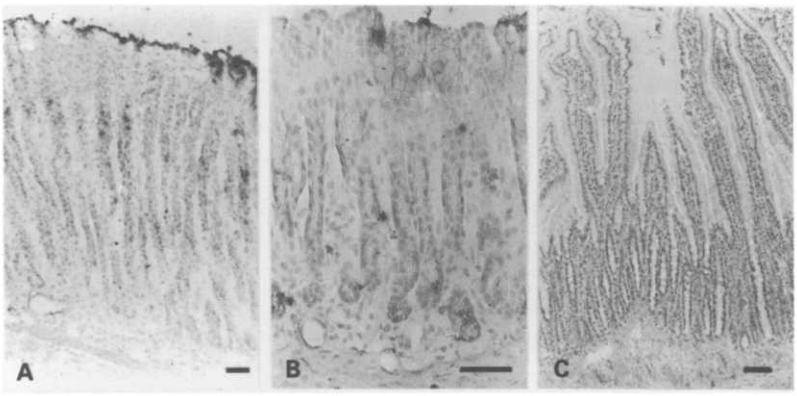

Fig. 2. Control staining of gastric body mucosa, gastric antrum and duodenum with sense and antisense probes. Hybridization patterns of sections of gastric body mucosa with sense probe (A), the gastric antrum with antisense probe (B) and the duodenum with antisense probe (C) for H^+/K^+-ATPase α subunit mRNA. Essentially the similar patterns were obtained with probes for GATA-GT1 and GATA-GT2. Each bar = 50 μm.

Using antisense probes for GATA-GT1 and GATA-GT2 mRNAs, hybridization signals were detected in the neck of glands, being visible as strongly stained band in the middle third of the mucosa that were faded away in the isthmus and basal gland regions (Fig. 3A, B). The distribution of hybridization signals of the antisense probe for H^+/K^+-ATPase α subunit mRNA was essentially similar to those for GATA-GT1 and GATA-GT2 (Fig. 3C). These results indicated that the mRNAs of GATA-GT1, GATA-GT2 and the H^+/K^+-ATPase α subunit are expressed in the limited region of the gastric body mucosa where parietal cells are clustered.

At higher magnification, hybridization signals of the three mRNAs were clearly located in parietal cells (Fig. 4), providing histological evidence that GATA-GT1 and GATA-GT2 together with H^+/K^+-ATPase are specifically transcribed in gastric parietal cells. These results further suggest that GATA-GT1 and GATA-GT2 participate in transcriptional regulation in these cells. The mechanism by which these proteins regulate expression of H^+/K^+-ATPase genes is of interest, because truncated GATA-GT1 and GATA-GT2 can both bind to gastric sequence motif in the control region of the ATPase genes (ref. [11] for GATA-GT2; Maeda, M., Wang, S.-H. and Futai, M. in preparation for GATA-GT1). An additional finding was that the signal strength clearly differed in the parietal cells (Fig. 4B). As shown by white arrowheads (Fig. 4A and B), the signals were observed diffusely but faintly in the cytoplasm of these parietal cells, indicating that the mRNA contents of GATA-GT1, GATA-GT2 and the H^+/K^+-ATPase α subunit were lower in the cytoplasm of these cells. Thus we could detect parietal cells in different states of gene expression by in situ hybridization.

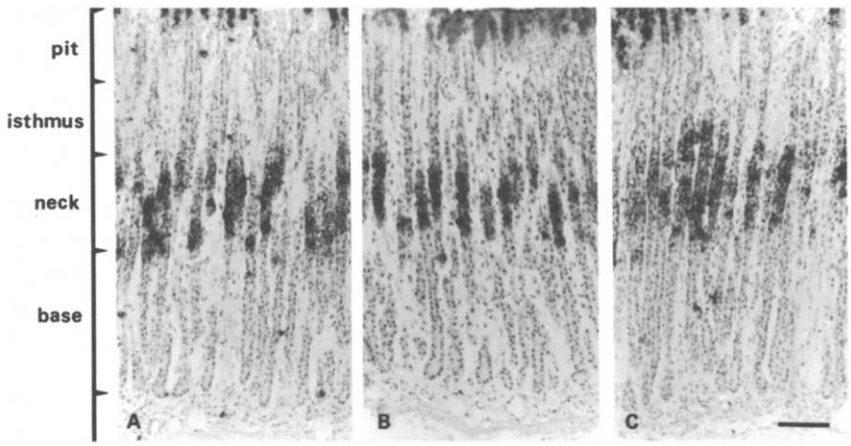

Fig. 3. Distributions of GATA-GT1, GATA-GT2 and H^+/K^+- ATPase α subunit mRNAs in the gastric body mucosa. Hybridization signals with antisense probes for GATA-GT1 (A), GATA-GT2 (B) and H^+/K^+-ATPase α subunit (C) mRNAs are observed as stained bands in the neck of gland regions. Sections were counter-stained faintly with hematoxylin. Bar = 100 μm.

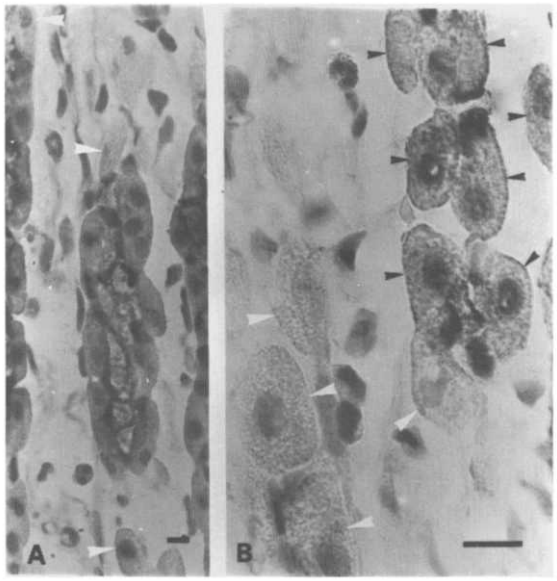

Fig. 4. High-power views of hybridization signals for GATA-GT2 and H^+/K^+-ATPase α subunit mRNAs in the neck of the gland. High-power views of hybridization signals for GATA-GT2 (A) and H^+/K^+-ATPase α subunit (B) mRNAs are shown. Specific labelings are seen in the cytoplasm of parietal cells clustered in the neck of glands (A, and black arrowheads in B). With a reduced aperture of the lens, parietal cells are clearly recognized by their characteristic morphology (B). Above and below the neck (isthmus or base), the signals in the parietal cells become fainter (white arrowheads; A, B). These findings were observed all with the three antisense probes including that for GATA-GT1 (not shown). Bar = 10 μm.

Acknowledgments: This research was supported by grants from the Ministry of Education, Science and Culture of Japan.

References

[1] Faller, L., Jackson, R., Malinowska, D., Mukidjam, E., Rabon, E., Saccomani, G., Sachs, G. and Smolka, A. (1982) Ann. N.Y. Acad. Sci. 402, 146–163.
[2] Maeda, M. (1994) J. Biochem. (Tokyo) 115, in press.
[3] Maeda, M., Oshiman, K., Tamura, S. and Futai, M. (1990) J. Biol. Chem. 265, 9027–9032.
[4] Oshiman, K., Motojima, K., Mahmood, S., Shimada, A., Tamura, S., Maeda, M. and Futai, M. (1991) FEBS Lett. 281, 250–254.
[5] Maeda, M., Oshiman, K., Tamura, S., Kaya, S., Mahmood, S., Reuben, M.A., Lasater, L.S., Sachs, G. and Futai, M. (1991) J. Biol. Chem. 266, 21584–21588.
[6] Canfield, V.A. and Levenson, R. (1991) Proc. Natl. Acad. Sci. USA 88, 8247–8251.
[7] Saccomani, G., Helander, H.F., Crago, S., Chang, H.H., Dailey, D.W. and Sachs, G. (1979) J. Cell Biol. 83, 271–283.
[8] Toh, B.-H., Gleeson, P.A., Simpson, R.J., Moritz, R.L., Callaghan J.M., Goldkorn, I., Jones, C., Martinelli, T.M., Mu, F.-T., Humphris, D.C., Pettitt, J.M., Mori, Y., Masuda, T., Sobieszczuk, P., Weinstock, J., Mantamadiotis, T. and Baldwin, G. (1990) Proc. Natl. Acad. Sci. USA 87, 6418–6422.
[9] Morley, G.P., Callaghan, J.M., Rose, B.R., Toh, B.H., Gleeson, P.A. and van Driel, I.R. (1992) J. Biol. Chem. 267, 1165–1174.
[10] Tamura, S., Oshiman, K., Nishi, T., Mori, M., Maeda, M. and Futai, M. (1992) FEBS Lett. 298, 137–141.
[11] Tamura, S., Wang, X.-H., Maeda, M. and Futai, M. (1993) Proc. Natl. Acad. Sci. USA 90, 10876–10880.
[12] Oh, J.D., Woolf, N.J., Roghani, A., Edwards, R.H., and Butcher L.L. (1992) Neuroscience 47, 807–822.
[13] Zon, L.I., Mather, C., Burgess, S., Bolce, M.E., Harland, R.M. and Orkin, S.H. (1991) Proc. Natl. Acad. Sci. USA 88, 10642–10646.
[14] Johnson, L.R. (1987) in: Physiology of the Gastrointestinal Tract (Johnson, L.R., Ed.) 2nd Edn., pp. 301–333, Raven Press, New York.
[15] Leeson, T.S., Leeson, C.R. and Paparo, A.A. (1988) Text/Atlas of Histology, W.B. Saunders, Philadelphia.