The highly differentiated gastric parietal cell has a characteristic morphology and is specialized for hydrochloric acid secretion into the stomach lumen. The major enzyme in this system is an ATP-driven, P-type proton pump (H⁺/K⁺-ATPase), which is responsible for proton translocation across the apical plasma membrane (Chow and Forte, 1995; Hirschowitz et al. 1995). The genes and cDNA for the ATPase α- and β-subunits have been cloned and sequenced (Shull and Lingrel, 1986; Maeda et al. 1988, 1990, 1991; Canfield et al. 1990; Newman et al. 1990; Reuben et al. 1990; Shull, 1990; Toh et al. 1990; Canfield and Levenson, 1991; Ma et al. 1991; Newman and Shull, 1991; Oshiman et al. 1991; Tamura et al. 1992). The amino acid sequences revealed that H⁺/K⁺-ATPase is closely related to Na⁺/K⁺-ATPase. Most of the positions of introns in the H⁺/K⁺-ATPase gene are essentially the same as those in the Na⁺/K⁺-ATPase gene (Maeda et al. 1990; Newman et al. 1990). Similar results were obtained for the β-subunits of the two enzymes (Canfield and Levenson, 1991; Maeda et al. 1991; Newman and Shull, 1991; Morley et al. 1992). The similarities in the intron/exon organization and primary structure suggest that the α- and β-subunit genes of the two ATPases were derived from respective common ancestors. In contrast, as expected from the gastric-specific expression, the 5’-upstream sequences of the H⁺/K⁺-ATPase genes were different from those of Na⁺/K⁺-ATPase. Our earlier studies (Maeda et al. 1991; Oshiman et al. 1991; Tamura et al. 1992) demonstrated that a DNA sequence motif, (G/C)PuPu(G/C)NGAT(A/T)PuPy, was present in both the α- and β-subunit genes from human and rat (Fig. 1). The motif was recognized by DNA-binding protein(s) from stomach but not by ones from other tissues. Interestingly, the gastric motif includes the (T/A)GATA(G/A) sequence recognized by the GATA-binding proteins (Orkin, 1990), a group of zinc-finger transcriptional factors implicated in the gene regulation of haematopoietic and several nonhaematopoietic cell types. The similarity of the DNA sequences recognized by the gastric-specific proteins and the GATA-binding proteins led us to postulate that the gastric proteins have similar zinc-finger domains with tandem CX₂C-X₁₇-CX₂C motifs.

**Novel gastric DNA-binding proteins with zinc-finger domains**

Degenerate primers were synthesized from the completely conserved sequences in the zinc-finger domains (119 amino acids)}
acid residues) (Zon et al. 1991) of the mammalian and chicken GATA-binding proteins, GATA-1, GATA-2 and GATA-3 (Table 1). The primers were combined with cDNA synthesized from pig (adult) gastric mucosa poly-A RNA (prepared from tissue being excised in a local slaughterhouse and immediately frozen) and then PCR (polymerase chain reaction) was carried out. Zinc-finger segments of the expected length were cloned and sequenced (Tamura et al. 1993). Surprisingly, three novel zinc-finger segments were identified in addition to GATA-2 and GATA-3: the translated sequences were very similar to those of the previously known GATA-binding proteins and were named GATA-GT1, GATA-GT2 and GATA-GT3 (GT, Gastrointestinal Tract) (Fig. 2). The primary structures of GATA-GT1 and GATA-GT2 were determined from the nucleotide sequences of full-length cDNAs, cloned from rat stomach cDNA library (5-week-old male Sprague-Dawley rat) using PCR-amplified sequences as probes. A clone for GATA-GT3 could not be obtained, possibly because of its low transcriptional level. A similar PCR method applied to yeast chromosomal DNA failed to amplify the sequence corresponding to the domain with tandem zinc-finger motifs, suggesting that yeast may only have GATA-binding proteins with a single zinc-finger motif (Cunningham and Cooper, 1991; Minehart and Magasanik, 1991).

**Structures of GATA-GT1 and GATA-GT2**

The gastric GATA proteins [GATA-GT1 (391 amino acid residues) and GATA-GT2 (440 amino acid residues)] (Tamura et al. 1993) had distinct zinc-finger motifs, CX2C-X17-CX2C, that were repeated twice in the middle of the primary structure (Fig. 3A,B). GATA-GT1 and GATA-GT2 constitute two new members of the ‘GATA-binding protein’ family (Table 1) and, like GATA-1, GATA-2 and GATA-3, they may regulate transcription through the DNA-binding ability of the zinc-finger domains. Currently six GATA-binding proteins have been reported in vertebrates (Table 1). Pairwise comparisons of the family members indicated that the zinc-finger domains were more than 70% conserved, but the remainder of the

<table>
<thead>
<tr>
<th>Name</th>
<th>Sites of expression</th>
<th>Target genes</th>
</tr>
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<tbody>
<tr>
<td>GATA-1</td>
<td>Erythroid cell, mast cell, megakaryocyte, eosinophil, testis (Sertoli cell)</td>
<td>Globins, erythropoietin receptor, haem biosynthetic enzymes, glycoprotein IIb, HOXB2</td>
</tr>
<tr>
<td>(=Eryf1, GF-1, NFE1a)</td>
<td></td>
<td>Preproendothelin-1</td>
</tr>
<tr>
<td>GATA-2</td>
<td>Endothelial cell, erythroid progenitor, mast cell, megakaryocyte, fibroblast, monocyte, kidney, embryo (brain, cardiac muscle and liver)</td>
<td>T-cell receptors</td>
</tr>
<tr>
<td>(=NFE1b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA-3</td>
<td>T-cell, mast cell, kidney, embryonic brain</td>
<td></td>
</tr>
<tr>
<td>(=NFE1c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA-GT1</td>
<td>Stomach (parietal cell), intestine, heart, ovary, lung, liver</td>
<td>Gastric proton pump (α- and β-subunits)</td>
</tr>
<tr>
<td>(= GATA-6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA-GT2</td>
<td>Cardiac myocyte, testis, ovary, intestine, stomach (parietal cell), primitive endoderm</td>
<td>Natriuretic peptide (A- and P-types), cardiac-muscle-specific troponin C, α-myosin heavy chain, gastric proton pump (α- and β-subunits)</td>
</tr>
<tr>
<td>(= GATA-4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA-5</td>
<td>Heart, testis, ovary, intestine, stomach, lung, spleen, liver, gall bladder</td>
<td></td>
</tr>
</tbody>
</table>

Various vertebrate GATA-binding proteins are listed. Their sites of expression and potential target genes are summarized. Cited from the following references: GATA-1, Yamamoto et al. (1990), Ito et al. (1993), Martin et al. (1993) and Vieille-Grosjean and Huber (1995); GATA-2, Yamamoto et al. (1990) and Lee et al. (1991); GATA-3, Yamamoto et al. (1990); GATA-GT1, Tamura et al. (1993) and Laverriere et al. (1994); GATA-GT2, Tamura et al. (1993), Arceci et al. (1993), Hon et al. (1994), Grépin et al. (1994), Molkentin et al. (1994) and Laverriere et al. (1994); GATA-5, Laverriere et al. (1994).
Gastric GATA factors

sequences exhibited lower similarity (less than about 30%, except for the 50% similarity between GATA-2 and GATA-3) (Fig. 4). This difference was clearly shown on HArr plot analyses, since straight lines were obtained only for zinc-finger motifs (Fig. 5). It should be noted that GATA-GT1 and GATA-GT2 correspond to GATA-6 and GATA-4, respectively, and Xenopus laevis GATA-4 was renamed GATA-5 (Laverriere et al. 1994).

Despite the differences in the sequences outside the zinc-finger domains, the amino acid compositions of all GATA-binding proteins have common features. They are rich in Ser and Thr residues (as high as 20% of the total) and in Pro (approximately 10%). The high content of α-helix breakers (Pro) and β-sheet breakers (Ser and Pro) suggests that the proteins are quite flexible. S(T)PXX, a motif typical for transcriptional regulatory proteins (Suzuki, 1989), was also scattered throughout the entire sequence. In contrast to other GATA-binding proteins, GATA-GT1 and GATA-GT2 had clusters of His and Ala residues, respectively (Fig. 3A,B). Furthermore, hydrophobic residues appeared at every seven residues of the carboxyl-terminal region of GATA-GT2, a distribution similar to that in Leu-zipper proteins (Landschultz et al. 1988). These characteristics of GATA-GT1 and GATA-GT2 may be important for their specific interaction with other regulator proteins and DNA motifs and for their unique role(s) in gastric tissue.

Tissue distribution of GATA-GT1 and GATA-GT2 mRNAs

Using a specific probe (outside the zinc-finger domains of GATA-GT1 and GATA-GT2), a strong hybridization signal was observed with stomach RNA but not with RNA from brain, heart, liver, kidney, spleen or lung (Tamura et al. 1993). A weak but significant signal was detected with intestinal RNA.

**Fig. 2. Alignment of the partial zinc-finger domains deduced from PCR-amplified cDNA sequences.** cDNA of pig (gastric mucosa) was added to the PCR reaction mixture together with degenerate primers corresponding to conserved amino acid sequences of the zinc-finger domains of the GATA-binding proteins. The amino acid sequences deduced from the amplified DNA are shown (except for the sequences covered by the primers) together with the corresponding sequences of human GATA-1, GATA-2 and GATA-3 (Tamura et al. 1993). Only residues different from GATA-1 are indicated.

**Fig. 3. Amino acid sequences of rat GATA-GT1 (A) and GATA-GT2 (B) (Tamura et al. 1993).** Zinc-finger domains are underlined. Zinc-finger motifs (CXC-X8-CXC) are coloured yellow. Green boxes indicate Ala-, Gly-, Pro- and Ser-rich sequences. The His cluster in GATA-GT1 and Ala clusters in GATA-GT2 are shown by pink boxes. S(T)PXX motifs are doubly underlined. Hydrophobic residues located at every seven residues of the GATA-GT2 carboxyl-terminal region are coloured blue.
RNA. In addition, a very weak signal for GATA-GT2 was detectable with RNA from testis. The GATA-GT1 and GATA-GT2 mRNAs were predominantly expressed in the gastric mucosa but not in the underlying muscular layer. To determine the cellular distribution of the mRNAs, rat stomach slices were subjected to *in situ* hybridization (Mushiake *et al.* 1994). The results indicated that GATA-GT1 and GATA-GT2, as well as the H*/K*/-ATPase *α*-subunit, are expressed specifically in gastric parietal cells. However, an intrinsic factor (vitamin B12 binding protein) transcript was found only in gastric chief cells (Maeda *et al.* 1995). The tissue distribution and potential target genes of the ‘GATA-binding protein’ family are summarized in Table 1. The proteins of this family showed separate or overlapping expression patterns. GATA-GT2 was also expressed in cardiac myocytes (Arceci *et al.* 1993; Grépin *et al.* 1994). The presence of the same GATA protein in different tissues suggests that other tissue-specific factor(s) may assist GATA protein function(s) (Merika and Orkin, 1995).

**Binding of GATA-GT1 and GATA-GT2 to the gastric DNA sequence motif**

In the GATA-1 protein, a zinc-finger motif located on the carboxyl side was determined to be responsible for DNA binding to the (T/A)GATA(G/A) motif (Yang and Evance, 1992). Do GATA-GT1 and GATA-GT2 bind to the gastric sequence motif, (G/C)PuPu(G/C)NGAT(A/T)PuPy, commonly located upstream of the H*/K*/-ATPase genes (Fig. 1)? In a previous experiment, only the binding of GATA-GT2 to this motif could be detected, and the negative results with GATA-GT1 may possibly be due to low expression and/or proteolytic degradation of the full-length protein in *Escherichia coli* (Tamura *et al.* 1992). Thus, we...
Transcriptional activation by gastric GATA DNA-binding proteins

Do upstream sequences of the H\(^+\)/K\(^+\)-ATPase subunit genes possess enhancers and promoter regions that are responsive to gastric GATA DNA-binding proteins? To test this possibility, the 3 kb upstream sequence of the rat \(\beta\)-subunit gene was ligated to the 5' side of the luciferase gene and then introduced into HeLa cells. The transfected cells expressed only a background level of luciferase activity. However, luciferase expression was enhanced significantly upon introduction of the expression plasmid of GATA-GT1 or GATA-GT2. These results suggest that the upstream region of the \(\beta\)-subunit gene contains a promoter that is activated by gastric GATA proteins (Fig. 7). We further demonstrated that the H2 receptor gene, which is expressed in parietal cells, is not controlled by GATA proteins (Nishi et al., 1995). Using this experimental system, the mechanism underlying the transcriptional activation, including the effects of protein phosphorylation, could be elucidated. Is the transcription of the \(\alpha\)- and \(\beta\)-subunit genes similarly regulated? The mechanism and regulation of subunit genes, respectively, competed for protein binding to the probe (Fig. 6A, competitors \(\alpha1\) and \(\beta2\)), but DNA segments having no motif, \(\alpha2\) and \(\beta1\), did not (Fig. 6A, competitors \(\alpha2\) and \(\beta1\)). These results strongly suggest that GATA-GT1 and GATA-GT2 are proteins that recognize the gastric sequence motif and regulate cell-specific expression of the H\(^+\)/K\(^+\)-ATPase genes.

Potential phosphorylation sites for kinase-mediated regulation of GATA-binding proteins

Protein phosphorylation may participate in the activation of acid secretion from parietal cells: histamine stimulates the secretion of acid through elevation of the cytoplasmic cyclic AMP level; acetylcholine and gastrin affect the Ca\(^{2+}\) level (Hirschowitz et al. 1995). It is reasonable to assume that elevation of the second messenger levels activates protein kinases, resulting in modulation of the functions of gastric GATA-GT1 and GATA-GT2. Interestingly, protein kinase A phosphorylated a GATA-GT2 fusion protein (Glu-128 to Gly-367) with glutathione S-transferase. The position of GATA-GT2 (Ser-234) is a potential site for phosphorylation (Yang et al., 1992), phosphorylation and dephosphorylation near the second finger of GATA-GT1 and GATA-GT2 may affect their DNA binding and/or transcriptional activation. The different distributions of phosphorylation sites in the GATA-binding proteins (Maeda, 1994) suggest that the specific function of each protein family member may be regulated differently.

constructed an expression system for a fusion gene comprising a partial sequence of GATA-GT1 (Ser-32 to Ala-391) or GATA-GT2 (Glu-128 to Gly-369) with glutathione \(S\)-transferase. Both fusion proteins were expressed in Escherichia coli cells and purified using a glutathione affinity column. For the binding assays, the upstream region of the H\(^+\)/K\(^+\)-ATPase \(\beta\)-subunit gene (Maeda et al. 1991) was radiolabelled as a probe (Fig. 6A). Mobility of the \(\beta\)-subunit gene fragment was clearly retarded after preincubation with the GATA-GT1 and GATA-GT2 fusion proteins [Fig. 6A, competitor (–) for GST-GT1 and GST-GT2], but not with the control glutathione \(S\)-transferase (Fig. 6A, GST), suggesting that both GATA protein sequences could bind to the probe DNA. DNA segments having the gastric sequence motif, \(\alpha1\) and \(\beta2\) (Fig. 6B), from the control regions of the H\(^+\)/K\(^+\)-ATPase \(\alpha\)- and \(\beta\)-subunit

Fig. 6. DNA-binding of fusion proteins carrying truncated GATA-GT1 and GATA-GT2. The DNA binding of fusion proteins carrying truncated GATA-GT1 (GST-GT1) and GATA-GT2 (GST-GT2) was examined by means of a gel retardation assay (Tamura et al. 1992). A large number of fusion proteins could be expressed in Escherichia coli and were easily prepared. (A) Affinity-purified fusion proteins (0.4 \(\mu\)g) were incubated with a radiolabelled DNA probe (100 Bq) encoding the gastric motif from the rat H\(^+\)/K\(^+\)-ATPase \(-\)subunit gene (Maeda et al. 1991). None, radioactive probe without protein; GST, the probe plus glutathione \(S\)-transferase; GST-GT1, the probe plus GATA-GT1 fusion protein; GST-GT2, the probe plus GATA-GT2 fusion protein. The shifted bands due to protein binding to the probe [competitor (–)] disappeared when competitors \(\alpha1\) and \(\beta2\) were added. Unrelated DNA sequence \(\alpha2\) and \(\beta1\) failed to bind to the fusion proteins. GST-GT2 gave several shifted bands possibly as a result of protein degradation. (B) The nucleotide sequences for \(\alpha1\) and \(\alpha2\) (sequences from rat \(\alpha\)-subunit gene) and \(\beta1\) and \(\beta2\) (sequences from rat \(\beta\)-subunit gene) (Tamura et al. 1992). Gastric motifs are underlined. GAT(A/T) sequences are double underlined.
Fig. 7. Schematic model of gastric-specific transcription. The potential role of gastric GATA-GT1 and GATA-GT2 in transcriptional activation of proton pump (H⁺/K⁺-ATPase) subunit genes is shown schematically. Gastric GATA-binding proteins may have important roles in transcriptional activation of proton pump subunit mRNA. This work was supported by grants from the Japanese Ministry of Education, Science and Culture, and the Japan Foundation for Applied Enzymology.

Conclusion

The PCR amplification of cDNA from pig gastric mucosa demonstrated the presence of novel zinc-finger proteins called GATA-GT1, GATA-GT2 and GATA-GT3; each had tandem zinc-finger motifs (CX_{2-3}C-X_{1-3}CX_{2-3}C), similar to those of previously characterized GATA-binding proteins. Subsequently, full-length cDNAs of GATA-GT1 and GATA-GT2 were obtained from rat stomach. Although the amino acid residues of their zinc-finger domains were as much as 70% identical to those from other GATA-binding proteins, regions outside the zinc fingers exhibited essentially no similarity. In addition to potential protein kinase phosphorylation sites, the characteristics of the primary structures of GATA-GT1 and GATA-GT2 have been described. They were expressed predominantly in the gastric mucosa (parietal cells) and at much lower levels in intestine and testis, a tissue distribution quite distinct from that of GATA-1, GATA-2 or GATA-3. GATA-GT1 and GATA-GT2 bound to the gastric DNA sequence motif (G/C)PuPu(G/C)NGAT(A/T)PuPy in the 5'-upstream regions of the H⁺/K⁺-ATPase α- and β-subunit genes. We further demonstrated that GATA-GT1 and GATA-GT2 stimulate the transcription of a reporter gene together with the 5'-upstream region of the β-subunit gene. These results suggest that gastric GATA DNA-binding proteins may have important roles in transcriptional activation of H⁺/K⁺-ATPase genes in stomach parietal cells.

References


pathway for cardiac but not skeletal muscle gene transcription. 


