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Analysis of the human guanylin gene and the processing and cellular localization of the peptide

(gut hormone/guanylyl cyclase C/regulatory peptide/enterochromaffin cells/diarrhea)

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ABSTRACT **The complete cell biological analysis of human guanylin, a recently discovered regulatory peptide, is offered in this investigation: (*i*) the nucleotide sequence of the gene, (*ii*) the isolation and characterization of its circulating molecular form, and (*iii*) its localization in enterochromaffin cells of the gut. As determined by molecular cloning, DNA sequencing, and comparison with the known cDNA sequence, the approximately 2.6-kbp large gene consists of three exons interrupted by two introns. The putative promoter region contains a TTTAAAA sequence motif and several potential binding sites for transcription factors such as AP-1, AP-2, Sp 1, and glucocorticoid receptors. The isolated hormonal form of guanylin is a 94-amino acid peptide with a molecular mass of 10.3 kDa. Western blot analysis of RP-HPLC fractions from blood plasma confirms this molecular form. Thus, guanylin is synthesized by gut enterochromaffin cells as a prohormone of 115 amino acids and is processed to the molecular form of 94 amino acids circulating in the blood.**

Guanylin is the first known endogenous peptide activating guanylyl cyclase C (GC-C), a receptor type earlier shown to be present in the apical epithelium of the small intestine (1). The original isolation of guanylin from rat jejunum resulted in the discovery of a small peptide containing 15 amino acids and two disulfide bonds (2). Previously known ligands of GC-C are several heat-stable enterotoxins of enterobacteriaceae causing diarrhea in humans and animals (1). The nucleotide sequences of the corresponding rat, mouse, and human guanylin cDNAs have been published (3–6), and translation of the cDNA sequences into amino acid sequences showed that the guanylin precursor of 115 amino acids contains the bioactive peptide isolated from rat intestine at its C terminus. However, it is not clear whether the isolated peptide is the product of a natural processing of the prohormone or of an artificial cleavage. Northern analysis confirms that guanylin is synthesized in the gut, especially in its distal segments (5). Guanylin is thought to modulate intestinal water/electrolyte transport in a paracrine mode (3, 4, 7, 8). This investigation reports data on the structure of the human gene¶ and the circulating form of its product as well as the identification of the guanylin-containing cells in the human intestine.

MATERIALS AND METHODS

Molecular Biological Methods. *PCRs.* PCRs (9) were carried out in a thermocycler 9600 (Perkin–Elmer), using *Thermus aquaticus* (*Taq*) DNA polymerase (Biomol, Hamburg, Germany) at 2.5 units/100 μl. Total RNA from human duodenum was isolated by means of an automatic nucleic acid extractor 340 (Applied Biosystems). The cDNA first strand was synthesized by using 5 μg of total RNA, Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL), and a synthetic oligo(dT) primer [UNIP-2, CCTGAATTCTAGAGCTCA$(T)_{17}$]. Aliquots (1/30) of the synthesis reaction mixtures were subjected to reverse transcription (RT)-PCR amplifications. For verification of the human guanylin gene promoter region by PCR, three sense primers located downstream (HGU-18, CACCCCTCTCTCGGGCACTG) and upstream (HGU-19, TTTAAAAGTCCCGCCGCTTC; HGU-20, CTCGAGCCTTATCTGATAAGG) from the region surrounding the putative transcription initiation site were constructed and used together with the antisense primer HGU-2 (CTAGCATCCGGTACAGGCAGCGTAGG; see Fig. 1) and human duodenum first-strand cDNA. *Genomic library screening.* A human genomic library in Lambda Fix II (Stratagene, catalog no. 9462039) was screened as described (10). The hybridization probe used was a partial rat guanylin cDNA PCR fragment (GenBank accession no. X67669) that had been [α-^{32}P]dCTP-labeled by using a random primed labeling kit (Boehringer Mannheim). *DNA sequencing.* The nucleotide sequence of the guanylin gene was determined on both strands according to the method of Sanger *et al.* (11), using a fluorescence sequencer 373 A (Applied Biosystems) (12) and a radioactive sequencing system (Macrophor, Pharmacia). *Southern blotting.* Restriction fragments of recombinant phage DNA and PCR products were checked for guanylin gene specificity by Southern hybridization (13), using the partial guanylin cDNA fragment mentioned or the oligonucleotide HGU-17 (CCAGGCCCCAAGGAGGCACAGTGC; see Fig. 1) as a probe.

Peptide Synthesis and Antisera. According to the published human proguanylin sequence (5, 6, 14) the peptides guanylin-(25–37), guanylin-(34–46), and [Lys^{100}]guanylin-(101–115) were synthesized as described previously (7). Polyclonal rabbit antisera K39 [recognizing guanylin-(25–37)], K42 [recognizing guanylin-(34–46)], and K605 [recognizing guanylin-(101–115)] were raised (for details, see refs. 7 and 15). In addition, antisera against serotonin and chromogranin A (7, 16, 17) were used for the identification of endocrine cells.

Characterization of the Circulating Form of Human Guanylin. *Purification.* Circulating human guanylin was purified from human hemofiltrate by using a method described in detail (14, 18). Crude polypeptide extracts were obtained from 10 liters of hemofiltrate and purified by two reverse phase (RP)-HPLC steps (15). In each HPLC step, the guanylin-containing fractions were identified by the T84-cell bioassay and by RIA. Aliquots from the fractions of the second HPLC

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Abbreviations: GC-C, guanylyl cyclase C; RT, reverse transcription; I_{sc}, short-circuit current.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. X74322).

step were used for Western blot analysis and for Ussing chamber studies. *T84-cell bioassay.* The cGMP generation by GC-C of human colon carcinoma cells (T84, passages 20–26, American Type Culture Collection) was used as a bioassay (14). *Guanylin RIA.* The guanylin concentration in HPLC fractions was determined by RIA (15). *Western blot analysis.* Samples of the HPLC fractions from the second purification step were analyzed by Tricine/SDS/PAGE (19) in 17.5% polyacrylamide gels and electroblotted onto hydrophobic polyvinylidene difluoride (PVDF)-based membranes (Pall) as described (7, 15). The membranes were incubated overnight at 4°C with the guanylin antisera K39, K42, or K605, each in a 1:1000 dilution. Immunoreactive polypeptides were visualized after incubation with alkaline phosphatase-conjugated goat antibody to rabbit IgG (1:8000; Sigma), using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as chromogens (Sigma). *Ussing chamber studies.* Mucosal sheets from rat proximal colon were dissected, mounted in Ussing chambers, and automatically voltage clamped (20). Short-circuit current (I_{sc}) was continuously recorded. The transepithelial conductance was determined every minute by bipolar voltage pulses of 1 mV. After an equilibrium period of 30 min, cumulative amounts of fraction aliquots of the second HPLC step and similarly cumulative concentrations of synthetic rat guanylin-(101–115) (10 pM to 1 μM) were tested in parallel experiments.

Immunohistochemistry. Specimens of human small intestine obtained from patients undergoing major gut surgery were fixed in Bouin fluid (without acetic acid) for 20 h. They were dehydrated with ethanol, cleared in xylene, and embedded in paraffin. Consecutive sections were cut at 4 μm and mounted on glass slides. After removal of paraffin by xylene and subsequent descending ethanol series, the sections were alternatively immunostained for guanylin (antiserum K42, diluted 1:1000; antiserum K605, diluted 1:1000), serotonin (diluted 1:20,000), and chromogranin A (diluted 1:8000) by the avidin–biotin–peroxidase complex (ABC) technique with incubation sequences as described (7). Specificity of immunostaining was verified by appropriate controls (7, 16, 17).

RESULTS

Characterization of the Guanylin Gene. We screened 400,000 clones of a human genomic library in λ under substringent conditions with the above-mentioned partial rat guanylin cDNA fragment as a probe [hybridization: 50°C, 12 h in 1 M NaCl/50 mM Tris·HCl, pH 7.5/0.1% SDS/10% (wt/vol) dextransulfate; final washing: 20 min in 2× SSC/0.1% SDS at hybridization temperature]. One clone (phHGU 35) was finally verified as true positive. *Sst* I restriction fragments of the insert were then subcloned in pBSK+, and two clones (pHGU 35-4, 4.0-kbp insert; pHGU 35-9, 0.5-kbp insert), which together contained the whole gene, were sequenced. An overlapping fragment was generated by PCR amplification of recombinant phage DNA and sequenced directly.

The gene spans ≈2.6 kbp and consists of three exons which are split by two introns (Fig. 1). The exon/intron splice junctions are in accordance with the exon [GTRAG–intron–AG] exon rule (31). Exon I codes for amino acids 1–25 of human guanylin, exon II, for amino acids 26–94, and exon III, for amino acids 96–115. The codon GAG for amino acid residue 95 (Glu) is interrupted by intron II (Fig. 1). With the exception of two nucleotides (see *Discussion*), exon sequences totally match the published cDNA sequence (5, 6).

The presumed promoter region exhibits no definite TATA-box motif. However, a TTTAAAA sequence is located 65 nucleotides upstream of the ATG translation start codon. No CCAAT-box motif could be found within the sequenced region.

Verification of the Promoter Region. RT-PCRs were performed with different sense primers located downstream (HGU-18) and upstream (HGU-19, HGU-20) from the putative cap site region and human duodenum first-strand cDNA. To be able to distinguish between amplified cDNA and genomic DNA, we used an antisense primer (HGU-2) located in the last exon (presumed exon III) of the gene. Prior to use, all four primers were tested for specificity and functionality. With the three primer combinations (HGU-18/2, HGU-19/2, HGU-20/2) 20, 25, 30, 35, and 40 PCR cycles were carried out. The products were separated in a 1.5% agarose gel, transferred to a nylon membrane, and hybridized with the internal oligonucleotide HGU-17. After autoradiography, only the primer combination HGU-18/2 gave signals in the size range expected for cDNA (Fig. 2). Neither the primer combination HGU-19/2 nor HGU-20/2 showed any signals. Supposing that at least the 3′-terminal halves of both primers HGU-18 and HGU-19, each consisting of 20 nucleotides, have to hybridize with the cDNA to make a PCR amplification possible, the results strongly indicate that the cap site is localized in the region between positions 346 and 385 of the gene. This confirms the supposed promoter region.

Characterization of the Circulating Guanylin. Circulating guanylin was purified from human hemofiltrate. Guanylin bioactivity (assayed by the T84-cell test) and immunoreactivity co-eluted in the identical HPLC fractions (fractions 29–31 contained higher concentrations, and fractions 28 and 32 contained low amounts of guanylin) (Fig. 3). The RIA recognizes the circulating 10.3-kDa form of human guanylin (15).

Western blot analysis was performed with aliquots of the HPLC fractions from the second purification step. The three antisera K39, K42, and K605 identified the major content of guanylin in the HPLC fractions 29–31 (Fig. 3), whereas lesser amounts were detected in fractions 28 and 32. In fractions 29–31 a strongly stained peptide band in the range of 10–11 kDa was identified. Further immunoreactive bands were observed above 20 kDa, appearing in a wide range of the HPLC fractions. The fractions showing only the high molecular mass substance contained no measurable bioactive guanylin, suggesting a nonspecific crossreaction.

In Ussing chamber experiments with rat intestinal mucosa, fractions 29–31 added to the bathing solution of the mucosal compartment evoked distinct successive increments in I_{sc} without changes in transepithelial conductance (Fig. 4). This effect occurred within minutes and was long lasting. As an example, the aliquot of fraction 30 added to the Ussing chamber could be calculated from the results of the RIA to correspond to a concentration of 45 nM guanylin. When this amount of fraction 30 was administered, I_{sc} was increased by 33 ± 5 μA/cm² ($n = 3$) (Fig. 4). In comparison, in the same experiments 10 nM synthetic rat guanylin-(101–115) increased I_{sc} by 35 ± 7 μA/cm². The effect of HPLC fractions 29–31 on I_{sc} was inhibited when 0.1 mM bumetanide ($Na^+/K^+/2Cl^-$ cotransport inhibitor) was added to the serosal compartment. This indicates that the increase in I_{sc} is due to stimulation of electrogenic chloride secretion (Fig. 4). Thus, the results obtained by the three methods generally concur.

Immunohistochemistry. When guanylin antisera K42 and K605 were used an intense immunostaining was detected in cells located in the epithelium of the small intestine (Fig. 5). Guanylin immunoreactivity was distributed in a population of cells that also react with chromogranin A antibodies. These cells constitute a subpopulation of the serotonin-containing enterochromaffin cells which are found in the mucosal epithelium of the crypt region and to a lesser extent in the villi. Strong guanylin immunoreactivity was present in the basal cytoplasmic region of these cells, where the secretory granules are predominantly clustered (Fig. 5). The apical pole, reaching mostly the gut lumen ("open type"), displayed scarce immunoreactivity. No other epithelial or nonepithelial cells showed

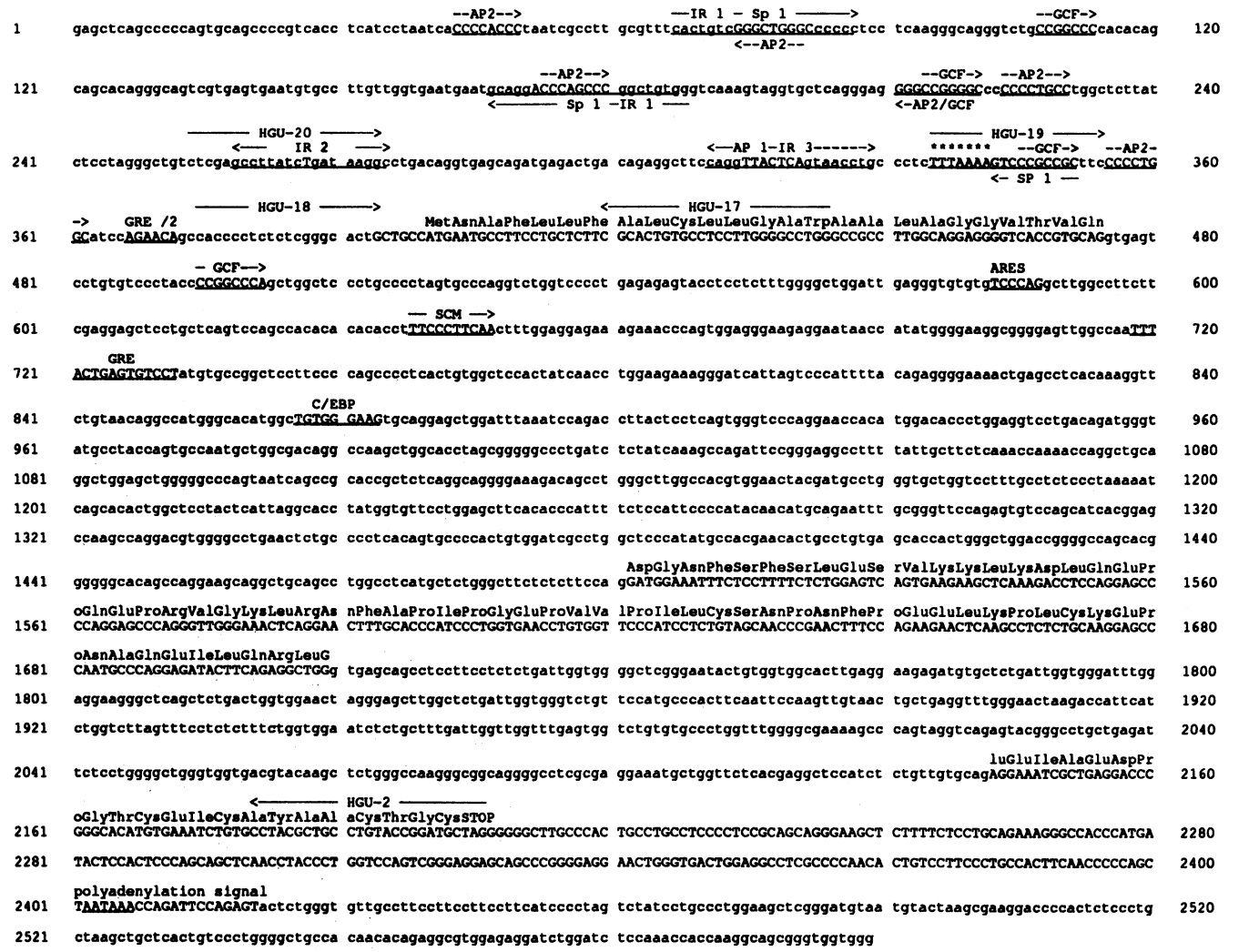

FIG. 1. Nucleotide sequence of the human guanylin gene. Nucleotides represented in the corresponding cDNA sequence (5, 6) are in capitals. Potential binding sites for transcription factors and the polyadenylylation signal are underlined and also in capitals. Positions and orientations of the primers used (HGU-2, HGU-17, HGU-18, HGU-19, and HGU-20) are indicated by arrows. A putative weak TATA box is marked with asterisks. The following abbreviations are used: AP1 (21), AP2 (22, 23), Sp 1 (24), GRE (25), GRE/2 (26), and GCF (27) for the corresponding transcription factors and elements; IR, inverted repeat; C/EBP, enhancer core sequence (28); ARES, putative acute response element (29); and SCM, SCM-inducible factor site (30). Arrows pointing right indicate an occurrence of the motif on the + strand, whereas arrows pointing left indicate an occurrence on the − strand.

any guanylin immunoreactivity. It is noteworthy that Paneth cells lacked any immunostaining with guanylin antisera.

DISCUSSION

The nucleotide sequence of the human guanylin gene was determined and analyzed in this study. Furthermore, we characterized the circulating form of guanylin and identified its cellular source in the small intestine.

The structural organization of the gene (three exons and two introns) could be deduced by comparison with the published cDNA sequence (5, 6). The splice sites are located at positions 83/84 and 291/292 of the cDNA sequence. For enumeration of the absolute positions we refer to the currently longest known cDNA sequence (TCGCTGCCATGAAT; residues 1–14) (5). Some genes, as for instance the mouse epithelin/granulin precursor gene (32), possess additional noncoding exons located upstream of the exon containing the ATG start codon. In the case of the human guanylin gene, this is unlikely because the 5′ nontranslated regions of both published cDNA sequences (5, 6) also appear in the postulated exon I of the gene. In addition, the presumed guanylin gene promoter region was confirmed by the RT-PCR results. The exact transcription initiation site is as yet unknown. Primer extension

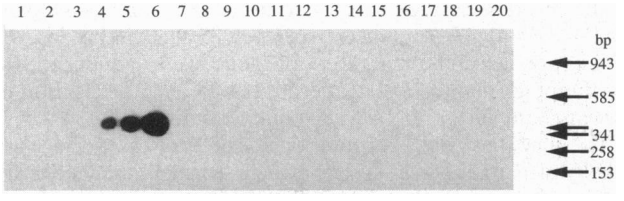

FIG. 2. Verification of the putative guanylin gene transcription initiation region by RT-PCR analysis. For description see text. Lanes 1–6, RT-PCR with the primer combination HGU-2/HGU-18: lane 1, 0-control (without cDNA); lanes 2–6, 20, 25, 30, 35, and 40 cycles of RT-PCR with human duodenum first-strand cDNA; lane 7, size marker; lanes 8–13, same as lanes 1–6 but primer combination HGU-2/HGU-19; lane 14, same as lane 7; lanes 15–20, same as lanes 1–6 but primer combination HGU-2/HGU-20. Cycle conditions: 94°C, 30 sec; 45°C, 30 sec; 72°C, 1 min. After blotting and hybridization with the internally localized guanylin-specific oligonucleotide HGU-17, only the primer combination HGU-2/HGU-18 shows signals which are in the size range expected for cDNA.

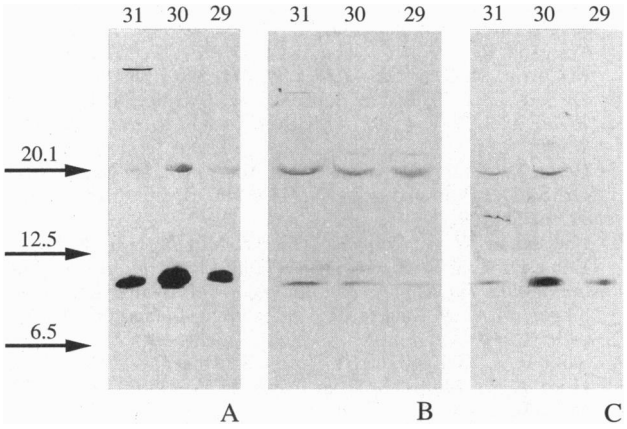

FIG. 3. Western blots after Tricine/SDS/PAGE of HPLC-fractionated human hemofiltrate. Positions of fraction aliquots are indicated on the top. Immunostaining was carried out with antiserum K39 against guanylin-(25–37) (*A*), antiserum K42 against guanylin-(34–46) (*B*), and antiserum K605 against guanylin-(101–115) (*C*) (all antisera, 1:1000). The migration positions of molecular mass markers (Boehringer Mannheim) are indicated (20.1, trypsin inhibitor; 12.5, cytochrome *c*; and 6.5, aprotinin). Note the prominent immunoreactive band below 12 kDa in the lanes corresponding to fractions 29, 30, and 31.

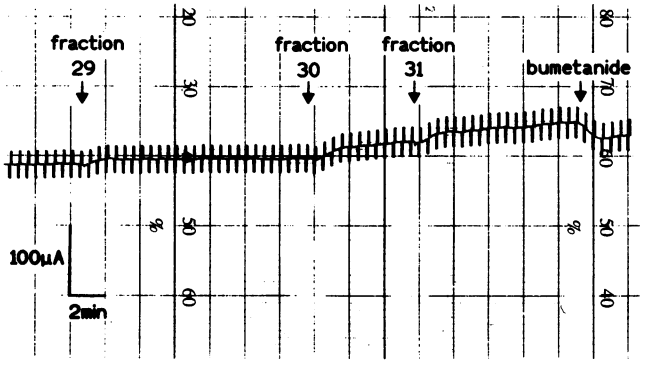

FIG. 4. Ussing chamber experiment with rat colonic mucosa, original tracing. Cumulative addition of HPLC fractions 29, 30, and 31 to the mucosal reservoir evoked successive increases in I_{sc}. The increase in I_{sc} was rapidly reversed by the subsequent addition of 0.1 mM bumetanide to the serosal solution.

assays with human duodenum and jejunum mRNA and two different primers showed a high background of nonspecific signals (data not shown) which did not allow an unambiguous interpretation. However, our RT-PCR results indicate a location of this site between positions 346 and 385 of the gene. As mentioned above, these data are based on the assumption that for a successful amplification at least the 3′-terminal halves of the primers have to hybridize with the cDNA. Related to the identified weak TATA-box motif (TTTAAAA), the adenosine in position 367 is the most probable cap site (Fig. 1).

In contrast to Wiegand and coworkers (5), we found that two nucleotides of the gene which correspond to the first two nucleotides (TC) of the cDNA sequence are arranged in reverse order (CT). This could be due to an error in the published cDNA sequence, which is supported by the fact that the CT order is also present in mouse and rat cDNA (3, 4, 6).

The presumed promoter region exhibits sequence motifs which could be assigned to well-characterized targets for transcription factors such as AP-1 and Sp 1 (Fig. 1). Furthermore, we could identify a potential glucocorticoid response element in intron I (GRE, position 718) and an equivalent half-site (position 367). Similar sequences that mediate transcription control by steroids have been detected in the promoter of the human metallothionein II_A gene (33) and the human growth hormone gene (34). In this context it is interesting to correlate blood guanylin levels to steroid-dependent diseases or to study cell lines producing guanylin. At position 582 we found the motif TCCCAG, which is homologous to the consensus sequence of elements located in hepatic genes responsible for interleukin-6-driven acute-phase reactions (29). Furthermore, a *fos*-gene-homologous motif is present in intron I, showing a postulated *sis*-conditioned medium-inducible element (30).

Besides the regions homologous with already-characterized regulatory elements, we identified several inverted repeats (Fig. 1). These sequence motifs could serve as potential target sites for transcription factors or exhibit prominent secondary structures. For example, the potential binding sites for Sp 1 and AP-1 are located within inverted repeat structures. Other striking sequences are some remarkable G+C-rich stretches (positions 106 and 210), and the importance of these motifs as regulatory elements has to be verified experimentally.

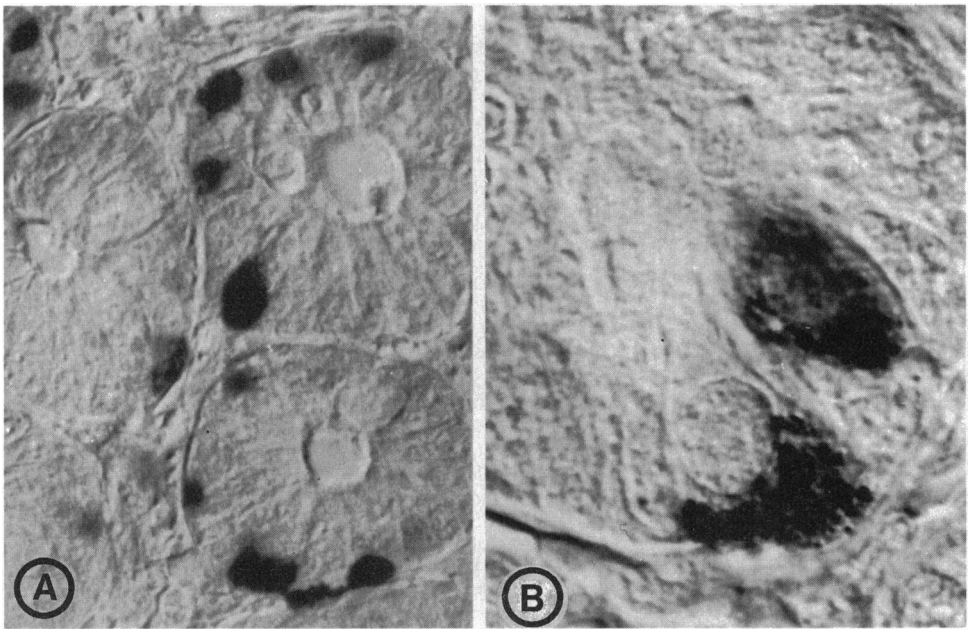

FIG. 5. Human small intestine immunostained for guanylin (antiserum K42). (*A*) Numerous enterochromaffin cells containing guanylin immunoreactivity are present in the epithelium of the Lieberkühn crypts. (*B*) At higher magnification it is seen that guanylin immunoreactivity is confined to secretory granules clustered in the basal part of these cells. The enterochromaffin cells contact the gut lumen and are of the open type. (Interference-contrast microscopy; *A*, ×280; *B*, ×880.)

Considering the present study with respect to functional and biochemical data, several intriguing questions must be raised. Here it is shown unambiguously that the guanylin system, in addition to its paracrine intestinal function, may constitute an endocrine system. This is supported by the fact that several organs are known to express GC-C (3, 35, 36), and the significance of this widespread distribution is not yet understood. Furthermore, there is still discrepancy about the existence of certain molecular forms of guanylin and their bioactivity. Expression of the human full-length guanylin cDNA in human embryonic kidney 293 cells resulted in a secreted polypeptide of 10,337 Da (6), the molecular form which corresponds to the naturally processed circulating guanylin (14). According to the results of our study, the 94-amino acid circulating guanylin is strongly bioactive. Some reported findings are in contrast because the recombinant large molecular form of 94 amino acids was found to be biologically inactive (6) or to exhibit low receptor affinity (37). The polypeptide may be activated by protease treatment, resulting in smaller C-terminal fragments—e.g., of 22 and 32 amino acids (6, 37), or by acid treatment (3), which activates the precursor-like polypeptide by an unknown mechanism. Further investigations are necessary to clarify the natural path of guanylin metabolism in both its paracrine and endocrine systems and to understand the precise mode of interaction with its receptor(s). The counterpart of the originally isolated rat guanylin of 15 amino acids (2), however, was not detected in our experiments with human hemofiltrate. This fragment may be artificial, but there is no doubt that the prerequisite structure of guanylin for bioactivity must include this C terminus exhibiting the two cysteine bridges.

Concerning the cellular source of intestinal guanylin, contradictory results have been published. By using *in situ* hybridization, guanylin expression was localized in Paneth cells of the crypts of Lieberkühn (6) and, contrariwise, in the enterocytes of the intestinal mucosa (38). Our previous findings showed that, in the guinea pig, guanylin immunoreactivity is localized in enterochromaffin cells (7), which is confirmed for humans in this work. The localization in Paneth cells by *in situ* hybridization as depicted by DeSauvage and coworkers (6) is, in our opinion, not conclusive: it may well be that the labeling shown in their paper stems from enterochromaffin cells adjacent to Paneth cells, not discriminated by the method applied in this investigation. The localization of guanylin in enterocytes (38) is barely understandable according to our results. Further studies using *in situ* hybridization are needed to clarify this point. In any case, the presence of guanylin in enterochromaffin cells of the gut is certainly in line with the high amounts of this peptide released into the bloodstream (15). On the basis of our morphological findings, that guanylin cells represent gut endocrine cells of the open type, we postulate that these cells release guanylin in two directions (8): (*i*) into the gut lumen to activate guanylyl cyclase C localized in the brush border membrane of enterocytes (38–40) and (*ii*) into the blood stream. The luminocrine role of guanylin in the regulation of intestinal fluid secretion represents a paracrine secretion. On the other hand, the role of the hormonal form of guanylin, released at the basal side of enterochromaffin cells, requires further investigation.

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1. Field, M., Graf, L. H., Laird, W. J. & Smith, P. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2800–2804.
2. Currie, M. G., Fok, K. F., Kato, J., Moore, R. J., Hamra, F. K., Duffin, K. L. & Smith, C. E. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 947–951.
3. Schulz, S., Chrisman, T. D. & Garbers, D. L. (1992) *J. Biol. Chem.* **267,** 16019–16021.
4. Wiegand, R. C., Kato, J. & Currie, M. G. (1992) *Biochem. Biophys. Res. Commun.* **185,** 812–817.
5. Wiegand, R. C., Kato, J., Huang, M. D., Fok, K. F., Kachur, J. F. & Currie, M. G. (1992) *FEBS Lett.* **311,** 150–154.
6. De Sauvage, F. J., Keshav, S., Kuang, W. J., Gillet, N., Henzel, W. & Goeddel, D. V. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9089–9093.
7. Cetin, Y., Kuhn, M., Kulaksiz, H., Adermann, K., Bargsten, G., Grube, D. & Forssmann, W. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2935–2939.
8. Forssmann, W. G., Cetin, C., Hill, O., Mägert, H. J., Kuhn, M., Kulaksiz, H. & Rechkemmer, G. (1995) in *Symposium on Gastrointestinal Tract and Endocrine System*, Falk Symposium 77, eds. Singer, M. V. & Ziegler, R. (MTP Press, Lancaster, U.K.), in press.
9. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
10. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
11. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
12. Smith, L. M., Sanders, J. Z., Kaiser, R. J., Hughes, P., Dodd, C., Connell, C. R., Heiner, C., Kent, S. B. H. & Hood, L. E. (1986) *Nature (London)* **321,** 674–679.
13. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
14. Kuhn, M., Raida, M., Adermann, K., Schulz-Knappe, P., Gerzer, R., Heim, J. M. & Forssmann, W. G. (1993) *FEBS Lett.* **318,** 205–209.
15. Kuhn, M., Kulaksiz, H., Adermann, K., Rechkemmer, G. & Forssmann, W. G. (1994) *FEBS Lett.* **341,** 218–222.
16. Cetin, Y., Bargsten, G. & Grube, D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2912–2916.
17. Cetin, Y. & Grube, D. (1991) *Cell Tissue Res.* **264,** 231–241.
18. Forssmann, W. G., Schulz-Knappe, P., Meyer, M., Adermann, K., Forssmann, K., Hock, D. & Aoki, A. (1993) in *Peptide Chemistry*, ed. Yanaihara, N. (ESCOM, Leiden, The Netherlands), pp. 553–557.
19. Schägger, H. & von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379.
20. Kuhn, M., Adermann, K., Jähne, J., Forssmann, W. G. & Rechkemmer, G. (1994) *J. Physiol. (London)* **479,** 433–440.
21. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49,** 729–739.
22. Mitchell, P. J., Wang, C. & Tjian, R. (1987) *Cell* **50,** 847–861.
23. Imagawa, M., Chiu, R. & Karin, M. (1987) *Cell* **51,** 251–260.
24. Kadonaga, J. T., Jones, K. A. & Tjian, R. (1986) *Trends Biochem. Sci.* **11,** 20–23.
25. Jantzen, H. M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R. & Schütz, G. (1987) *Cell* **9,** 29–38.
26. Klock, G., Strähle, U. & Schütz, G. (1987) *Nature (London)* **329,** 734–736.
27. Kageyama, R. & Pastan, I. (1989) *Cell* **59,** 815–825.
28. Johnson, P. F., Landschulz, W. H., Graves, B. J. & McKnight, S. L. (1987) *Genes Dev.* **1,** 133–146.
29. Majello, B., Arcone, R., Toniatti, C. & Ciliberto, G. (1990) *EMBO J.* **9,** 457–465.
30. Hayes, T. E., Kitchen, A. M. & Cochran, B. H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1272–1276.
31. Mount, S. M. (1982) *Nucleic Acids Res.* **10,** 459–472.
32. Baba, T., Nemoto, H., Watanabe, K., Arai, Y. & Gerton, G. L. (1993) *FEBS Lett.* **322,** 89–94.
33. Karin, M., Haslinger, A., Holtgreve, H., Cathala, G., Slater, E. & Baxter, J. D. (1984) *Cell* **36,** 371–379.
34. Slater, E. P., Rabenau, O., Karin, M., Baxter, J. D. & Beato, M. (1985) *Mol. Cell. Biol.* **5,** 2984–2992.
35. Laney, D. W., Mann, E. A., Dellon, S. C., Perkins, D. R., Gianell, R. A. & Cohen, M. B. (1992) *Am. J. Physiol.* **263,** G816–G821.
36. Laney, D. W., Bezerra, J. A., Kosiba, J. L., Degen, S. J. & Cohen, M. B. (1994) *Am. J. Physiol.* **266,** G899–G906.
37. Garcia, K. C., de Sauvage, F. J., Struble, M., Henzel, W., Reilly, D. & Goedell, D. V. (1993) *J. Biol. Chem.* **268,** 22397–22402.
38. Li, Z. & Goy, M. F. (1993) *Am. J. Physiol.* **265,** G349–G356.
39. Frantz, J. C., Jaso-Friedman, L. & Robertson, D. C. (1984) *Infect. Immunol.* **43,** 622–630.
40. Giannella, R. A., Luttrell, M. & Thompson, M. R. (1983) *Am. J. Physiol.* **245,** G492–G498.