

Function and Regulation of Gastrin in Transgenic Mice: A Review

TIMOTHY C. WANG, M.D., AND STEPHEN J. BRAND, M.D., Ph.D.

*Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital,
Boston, Massachusetts*

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The gastrin gene is expressed in fetal pancreatic islet cells, but in the adult is expressed mainly in the gastric antrum. To study the regulation of the gastrin promoter, we created several transgenes containing the human and rat gastrin 5' flanking regions joined to the coding sequences of the human gastrin gene. The human gastrin transgene contained 1,300 bp of 5' flanking DNA, while the rat gastrin transgene contained 450 bp of 5' flanking DNA. The human gastrin transgene was expressed in fetal islets, but was not expressed in adult gastric antrum. In contrast, the rat gastrin transgene was expressed in adult antral G cells, but no expression was observed in fetal islets. To study the possible role of gastrin as an islet growth factor, a chimeric insulin-gastrin (INS-GAS) transgene was created, in which the expression of the human gastrin gene is driven from the rat insulin I promoter. These INS-GAS mice were mated with mice overexpressing TGF α , transcribed from a mouse metallothionein-transforming growth factor α (MT-TGF α) transgene. While overexpression of gastrin or TGF α alone had no effect on islet mass, overexpression of both transgenes resulted in a twofold increase in islet mass. In conclusion, these data indicate that (1) gastrin can interact synergistically with TGF α to stimulate islet growth; (2) the human gastrin transgene contains the islet specific enhancer; (3) the rat gastrin transgene contains the antral specific enhancer.

The gastrin gene exhibits a complex pattern of tissue specificity and temporal regulation during development. In the adult rat organism, the major site of expression is in the gastric antrum, where gastrin functions as a regulatory peptide with at least several functions: (1) a hormone controlling acid secretion and (2) a growth factor stimulating mucosal proliferation [1,2]. The expression of gastrin mRNA in the gastric antrum does not begin until after birth, and then rises to achieve adult levels between postnatal days 10 and 20. In the antrum, gastrin is found localized to an endocrine cell population, known as G cells, where the gene is regulated by luminal contents, and by changes in gastric pH through modulating paracrine inhibition by somatostatin [3].

In contrast, in the rat fetus, the pancreas is the major site of gastrin gene expression. The highest levels of pancreatic gastrin mRNA are seen in the day 18 rat fetus [4]. After birth, pancreatic gastrin mRNA levels decline rapidly at the same time that other islet mRNAs (insulin, glucagon, somatostatin) achieve stable, maximal levels. By the third postnatal day, pancreatic gastrin mRNA levels are

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Abbreviations: AFP: alpha feto-protein (gene) hGAS: human gastrin HLH: helix-loop-helix INS-GAS: chimeric insulin-gastrin (transgene) MT-TGF α : metallothionein-transforming growth factor α NRE: negative regulatory element RAT-GAS: rat gastrin (transgene)

Address reprint requests to: Timothy C. Wang, M.D., Gastrointestinal Unit, Dept. of Medicine, Massachusetts General Hospital, Fruit Street, Boston, MA 02114

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undetectable. Until recently, however, the function of fetal pancreatic gastrin gene expression has remained unclear.

A number of previous observations suggest that gastrin may regulate the growth of pancreatic islets during development. The transient expression of gastrin in the neonatal pancreas corresponds to a period of rapid pancreatic growth. During this period, committed islet-cell precursors proliferate by budding from pancreatic ducts, and then differentiate into mature islet cells [5]. After birth, islet development dramatically decreases, and gastrin expression is also repressed. Furthermore, some clinical observations support such a role. Hypergastrinemia is associated with nesidioblastosis, and an abnormal persistence of gastrin has been documented in a case of infantile nesidioblastosis [6]. These reports did not, however, establish a causal relationship between nesidioblastosis and gastrin stimulation. Gastrin also acts as an autocrine growth factor for gastrin-expressing islet tumor cells growing in culture [Brand S: unpublished observations]. Nevertheless, studies demonstrating a direct effect of gastrin on islet growth *in vivo* have been lacking.

Furthermore, gastrin mRNA found in the antrum and the pancreas have identical initiation sites, indicating that they are transcribed from the same promoter [4]. This fact suggests that the developmental regulation of gastrin is controlled by a complex transcriptional mechanism. Studies examining the regulation of gastrin gene transcription were initially based on cell transfection systems, using human gastrin promoter-reporter gene constructs. Theill et al. examined human gastrin promoter activity in neuroblastoma cells, and showed that important cis-regulatory control elements lay downstream of the TATA box [7]. Merchant et al. reported that a human gastrin reporter gene construct containing only 40 bp of 5' flanking DNA was sufficient for basal transcription in GH4 pituitary cells [8].

More recently, through a series of deletions of the human gastrin promoter, we defined a cis-regulatory domain, located between -108 and -76, which controlled activity of the gastrin promoter in islet cells [9]. This domain in the gastrin promoter contained a negative element (ATTCCTCT), also found in the promoters of the beta interferon and the cytoactin genes, which inhibited transcription in islet cells. Adjacent to the negative element was a positive element (CATATGG), which is similar to cis-regulatory elements in the insulin promoter that determine islet-cell specific transcription of the insulin gene. This latter finding suggests that the gastrin gene may be activated by the same transcription factor that activates insulin transcription in islet cells. Insulin gene transcription is activated by helix-loop-helix (HLH) transcription factors binding to DNA sequences in the insulin promoter containing the CANNTG motif, also known as the E box. Gastrin transcription in islet cells is also regulated by HLH transcription factors, as demonstrated by experiments using antibodies to the cloned E2/PAN HLH factors [10], which inhibit binding of the islet nuclear proteins to the gastrin insulin enhancer-like element (CATATGG). Thus, the organization of the islet enhancer, with its tandem array of positive and negative elements, suggests a molecular switch, which might control the transient expression of the gastrin gene during pancreatic development.

The regulatory sequences identified by the transfection of cultured cells *in vitro* are not, however, always an accurate reflection of the mechanisms used in the whole animal [11]. Furthermore, the cis-acting elements targeting antral-specific expression of the gastrin gene have not been identified. This omission has been due in part to the lack of availability of a permanent gastrin-expressing antral G cell line.

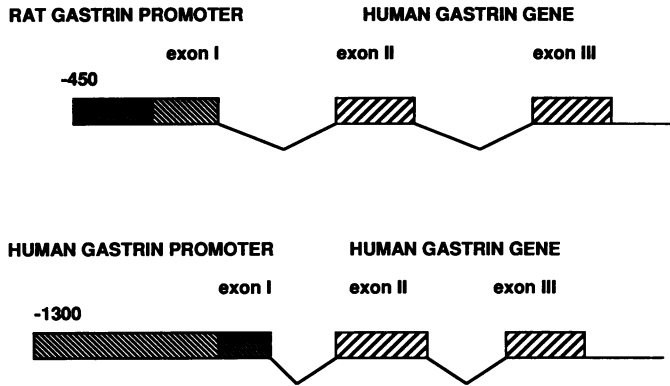


FIG. 1. Diagram of rat and human gastrin transgenes. The human gastrin gene contained approximately 1,300 bp of 5' flanking DNA plus the first non-coding exon, while the rat gastrin transgene contained 450 bp of 5' flanking DNA plus the first non-coding exon. Both transgenes contained the coding portion (exons 2 and 3 and introns) as the reporter gene.

Therefore, to determine whether the islet-cell specific regulatory domains in this promoter are fully functional *in vivo*, and to develop a murine model to study transcriptional regulation of gastrin in adult stomach, transgenic mice containing a variety of gastrin gene constructs were developed. This paper reviews a number of recent transgenic studies examining the regulation and the function of gastrin in transgenic mice. Further details of these studies will be published elsewhere [12,13].

EXPRESSION OF RAT AND HUMAN GASTRIN PROMOTER CONSTRUCTS IN TRANSGENIC MICE

Initially, we made a transgene containing 450 bp of the rat gastrin promoter, plus the first non-coding exon, joined to a reporter gene which consisted of the coding portions of the human gastrin gene (Fig. 1). This rat gastrin (RAT-GAS) transgene was cut with restriction enzymes to remove vector sequences, purified according to standard procedures [14], and microinjected into fertilized mouse eggs. Three founders were identified, two of which expressed the transgene at significant levels, as determined by Northern blot analysis [12]. Total RNA was isolated from the gastric antrum and several other tissues, size fractionated by electrophoresis in denaturing gels, transferred to nitrocellulose, and hybridized to a probe specific for exon II of the human gastrin gene. Significant levels of a 950 nt RNA, which co-migrated with the human gastrin-positive control, were observed in the gastric antrum of two of the lines examined: RAT-GAS 1 and RAT-GAS 2; no expression was seen in the third line, RAT-GAS 3. In the two expressing lines, no expression was seen in the gastric corpus or in tissues examined outside the gastrointestinal tract. Low levels of expression were, however, observed in the small intestine and colon.

The highest expressing line, RAT-GAS 1, was analyzed in more detail. To confirm that the expression of the RAT-GAS transgene seen in the gastric antrum was cell-specific for G cells, *in situ* hybridization was performed on formalin-fixed, paraffin-embedded sections, using a human gastrin exon II antisense riboprobe; in addition, the sections were tested with the rodent-specific (rat) gastrin exon III riboprobe in order to examine expression of the endogenous mouse gastrin gene [12]. Both the endogenous mouse gastrin gene and the transgene were expressed in G cells located at the base of the crypts in the antrum, and expression of both was extinguished sharply at the antral-corporum junction. While the endogenous mouse

gastrin gene was also sharply extinguished at the pylorus, however, the transgene demonstrated low levels of expression in the duodenum. The transgene therefore demonstrated correct cell-specific expression in the antral G cells.

A further developmental profile was carried out, using the technique of *in situ* hybridization [12]. To determine whether the RAT-GAS transgene was expressed in fetal tissue, 19-day-old fetuses were removed from pregnant transgenic mice, fixed in formalin, and embedded in paraffin. *In situ* hybridization was carried out with the same human gastrin exon II probe. In the 19-day-old fetus, no expression was detected in either the fetal pancreas or the fetal stomach. At three days postpartum, the expression of the rat gastrin transgene was still absent, but expression was detected at low levels in antral G cells. By ten days postpartum, expression in the mouse antrum was present at much higher levels. Thus, the rat gastrin promoter appeared to be correctly regulated developmentally in the gastric antrum, but not in the fetal pancreas.

The human gastrin promoter was then examined in transgenic mice, using a human gastrin minigene construct (Fig. 1). The human gastrin minigene contained 1.3 kb of human gastrin 5' flanking DNA plus the first (57 nt) non-coding exon, joined to the coding exons of the human gastrin gene. Three founder human gastrin (hGAS 1-3) transgenic lines were created, two of which (hGAS 1 and hGAS 2) expressed human gastrin mRNA at high levels. In contrast to the tissue-specific expression seen with the rat gastrin transgene, the human gastrin minigene was not expressed in the gastric antrum, but instead demonstrated aberrant expression in the adult liver. Northern blot analysis of RNA from both the hGAS 1 and hGAS 2 human gastrin minigene lines revealed that human gastrin mRNA was found in the liver, but not in the gastric antrum, corpus, duodenum, small intestine, colon, or pancreas. RNase protection studies revealed that transcription in both lines originated from the authentic gastrin promoter.

Since the Northern blot did not distinguish between hepatocyte and non-hepatocyte expression, *in situ* hybridization was performed on the human gastrin minigene lines at various stages of development [12]. *In situ* hybridization of formalin-fixed tissue from day 18 fetal mice revealed that the expression could be demonstrated at a high level in larger clusters of cells in the liver. At day 3 postpartum, the expression of the human gastrin transgene appeared to be repressed in the centrilobular region, which was even more prominent by day 10. By the adult stage, expression was apparent in only a small proportion (1-2 percent) of hepatocytes.

The finding that the human gastrin promoter demonstrated liver-specific expression was unexpected. Although this finding may be simply an aberrancy due to species difference between the human and rodent gastrin genes, it may also reflect the close relationship developmentally between the liver and the pancreas. Both the liver and the pancreas arise from an outpocketing of foregut endoderm. In addition, rats can exhibit "transdifferentiation" of pancreatic duct cells into hepatocytes under conditions of copper or methionine deficiency [15,16]. Therefore, there may exist a primitive endodermal stem cell that has the capacity to differentiate into either liver or pancreatic cell lineages, and repression of genes such as gastrin may be part of the normal differentiation process in the liver.

Finally, although the human gastrin minigene was not expressed in the adult pancreas, expression was plainly evident in the fetal pancreas. A high level of

	adult gastric antrum	fetal pancreatic islets	fetal & adult liver	small & large intestine
Rat Gastrin Transgene	++	-	-	+
Human Gastrin Transgene	-	++	++	-

FIG. 2. Messenger RNA expression patterns observed in transgenic lines bearing either the rat gastrin transgene or the human gastrin transgene. The expression pattern was determined by Northern blot and/or in situ hybridization assays in one or more lines.

expression was clearly demonstrated in proliferating islet-cell precursors at day 18 of fetal development. By day 3 postpartum, expression was present at low levels in the islet cells; by day 10 postpartum, expression was completely absent from the pancreas.

Thus, the human gastrin promoter, unlike the rat gastrin promoter, contains the islet-cell specific enhancer that both activates transcription of the gastrin gene in the pancreas, and represses transcription after birth. Previous transfection studies and gel mobility shift assays suggested that islet-cell expression of the human gastrin gene is controlled by a cis-regulatory domain between -108 and -76 bp upstream of the start site [4]. This region of the human gastrin gene contains some homology with the corresponding region of the rat gastrin gene; however, there are also significant differences. Further transgenic studies will be required to confirm that this region of the human gastrin promoter is responsible for regulating fetal islet expression. Such studies have been performed with the alpha feto-protein (AFP) gene, which is transiently expressed in the fetal liver but subsequently repressed after birth. Transgenic studies have confirmed that a cis-acting negative regulatory element (NRE) located 3.5 kb upstream is responsible for the postnatal extinction of the AFP transgene in liver cells [17]. A similar switch mechanism may be responsible for repressing gastrin in the neonatal pancreas.

Figure 2 summarizes the differences in the patterns of expression of the rat and human gastrin genes. The rat gastrin gene was expressed appropriately in the adult gastric antrum, but was not expressed in the fetal pancreas (within the limits of detection of our assay), and demonstrated inappropriate expression in the small intestine and colon. In contrast, the human gastrin transgene was expressed in the fetal pancreatic islets, but was not expressed in adult antrum, and demonstrated inappropriate expression in fetal and adult liver. The results suggest a complex pattern of expression that includes at least two different positive regulatory elements (islet and antral), and at least two negative regulatory elements (liver and intestinal). The absence of a correct pattern of transgene expression is, in most instances, attributed to the absence of necessary cis-acting regulatory elements due to insufficient amounts of 5' flanking DNA. Furthermore, some of the differences between the expression patterns of the rat and human transgenes may be due to different lengths of promoter used; however, recent studies suggest that very different mechanisms can be used to regulate gene expression in mouse and human species, or between two different mouse species [18]. Future studies will be directed at further

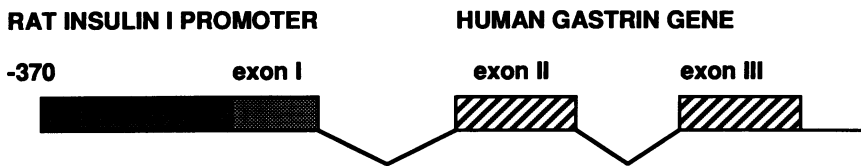


FIG. 3. Diagram of the insulin-gastrin transgene, which contained 370 bp of 5' flanking DNA and the first non-coding exon of the rat insulin 1 gene, joined to the coding portion of the human gastrin gene.

characterizing the cis-acting elements controlling these different patterns of tissue-specific expression.

EFFECT OF SUSTAINED PANCREATIC GASTRIN EXPRESSION IN TRANSGENIC MICE

The role of gastrin as an islet growth factor was then examined in transgenic mice expressing a chimeric insulin-gastrin (INS-GAS) transgene, in which the human gastrin gene was transcribed from the rat insulin I promoter. Since the insulin promoter continues to be highly transcribed after birth, this transgene will express high levels of gastrin in the pancreas after the endogenous gastrin is switched off. If gastrin regulates islet growth, then enhanced islet growth might result from a postnatal persistence of pancreatic gastrin.

The insulin-gastrin transgene (INS-GAS) comprised 370 bp of 5' flanking DNA and the first non-coding exon of the rat insulin I gene, ligated to the sequences (exons II and III) of the human gene encoding the pre-progastrin peptide precursor (Fig. 3). The INS-GAS fragment was isolated and microinjected into inbred one-cell mouse embryos. Three transgenic founder lines were generated. One of the three lines, INS-GAS 1, highly expressed human gastrin mRNA [13]. A Northern blot of total RNA isolated from the INS-GAS 1 line, probed with a human gastrin exon II riboprobe, demonstrated expression in pancreatic tissue, but in none of the other tissues examined, including stomach and small intestine. No gastrin mRNA was observed in pancreatic RNA from non-transgenic FVB mice. The gastrin transgene mRNA was efficiently translated into the bioactive gastrin peptide, since pancreatic extracts contained high levels of gastrin immunoreactivity, using a radioimmunoassay specific for bioactive C-terminal amidated gastrin. Immunohistochemistry showed gastrin was expressed only in the pancreatic islets; no expression was seen in the exocrine tissue.

Despite the active expression of gastrin in the postnatal pancreas, there was no obvious stimulation of islet-cell growth in INS-GAS 1 mice over that of controls. This fact was confirmed by point-counting morphometrics of pancreatic tissue from age-matched INS-GAS 1 and control mice, which showed no difference in islet-cell mass between INS-GAS transgenic and control mice (Fig. 4). Thus the sustained expression of gastrin in the postnatal pancreas alone is insufficient to stimulate islet-cell growth.

Islet cells develop from committed islet precursors, which proliferate and bud from the fetal pancreatic ducts [5]. Since these ductal islet-cell precursors disappear after birth, absence of these precursor cells in the postnatal pancreas may explain the failure of gastrin to stimulate islet growth in the INS-GAS transgenic mice. Consequently, an effect of transgenic gastrin expression may only be seen in mice which have persistence of islet precursors. For this reason, INS-GAS mice were mated with

MOUSE GENOTYPE

	WILD TYPE	INS-GAS	TGF-α	INS-GAS/ TGF-α
DUCT MASS	normal	normal	+++ increased	+ increased
ISLET MASS	normal	normal	normal	++ increased

FIG. 4. Effect of insulin-gastrin and metallothionein-TGF α transgene expression on ductular and islet mass, compared to wild-type mice. Islet and duct mass were determined by point-counting morphometrics of pancreatic sections from six or more mice. Duct mass was markedly (+++) increased in TGF α single transgenics, but only slightly (+) increased in INS-GAS/TGF α double transgenics. Islet mass was significantly (++) increased in the INS-GAS/TGF α double transgenics, but not changed in INS-GAS and TGF α single transgenics.

metallothionein-transforming growth factor α (MT-TGF α) transgenic mice which have pancreatic TGF α overexpression from a metallothionein promoter-human TGF α cDNA transgene [17–19]. Adult MT-TGF α transgenic mice have pancreatic changes resembling the proliferating ductules of the developing pancreas, which contain islet and exocrine precursor cells. TGF α causes numerous foci of metaplastic ductules in the MT-TGF α pancreas, which represent focally redirected differentiation of pancreatic epithelial cells. These metaplastic ducts have cells immunoreactive for amylase, indicating that acinar cell differentiation occurs within these metaplastic ductules [18].

To determine whether these metaplastic ductules also contain islet precursors, metaplastic ducts from MT-TGF α pancreas were examined immunohistochemically for insulin immunoreactivity. These studies showed that the metaplastic ductules contain insulin-expressing cells, indicating that differentiation along the islet lineage occurs within the metaplastic ducts [13]. Although TGF α -induced metaplastic ducts contain islet precursor cells, point-counting morphometrics indicated no increase in islet-cell mass of MT-TGF α mice, even though there was a greater than tenfold increase in pancreatic ductules (Fig. 4).

The effect of persistent pancreatic gastrin expression was examined in TGF α -induced metaplastic pancreas by crossing the homozygous MT-TGF α (MT-42) line with the heterozygous INS-GAS transgenic line. The offspring of this cross were either heterozygous MT-TGF α single transgenics (TGF α +) or double transgenics containing both INS-GAS and MT-TGF α transgenes (INS-GAS+/TGF α +). Age-matched wild-type mice with a similar genetic background were used as controls. All three groups of mice were placed on 50 mM ZnCl₂ (which induces the mouse metallothionein promoter) at three weeks of age.

At four months of age, the animals were sacrificed and the pancreas removed. The gross morphological appearance of the pancreas from the MT-TGF α and the INS-GAS/MT-TGF α mice were similar in being uniformly rigid and whitish in

appearance in both lines. There was, however, a striking difference in the pancreatic histology between the TGF α transgenic and the INS-GAS/TGF α double transgenics [13]. The pancreas from the MT-TGF α \times FVB mouse had the typical interstitial fibrosis and florid ductular metaplasia, similar to changes previously described [19–21] compared to the pancreas from the wild-type control. In contrast, the pancreas from the INS-GAS/MT-TGF α mice exhibited a histology that resembled that of the control pancreas more than that of the MT-TGF α mice. Thus, gastrin overexpression in the postnatal pancreas reduced the ductular metaplasia caused by TGF α overexpression. This result was confirmed by quantitating pancreatic ductular mass by point-counting morphometrics in the three groups of mice. The TGF α mice had a markedly increased ductular mass compared to controls (Fig. 4). Expression of the gastrin transgene in the INS-GAS/TGF α mice markedly reduced the TGF α -induced increase in ductal cell mass, although the ductal mass was still increased over control values.

Co-expression of gastrin and TGF α in the INS-GAS/TGF α pancreas also significantly increased the islet mass compared to controls, in contrast to the ineffectiveness of TGF α and gastrin expression alone (Fig. 4). The islet mass of the INS-GAS/MT-TGF α double transgenics was significantly (twofold) greater than the islet mass of the control mice, whereas the islet-cell mass of the MT-TGF α transgenics did not differ significantly from controls [13]. Since gastrin decreased the metaplastic duct mass, the increase in islet mass may result from gastrin inducing differentiation of insulin-positive cells in the TGF α -induced metaplastic ductules.

The differences in pancreatic morphology between the TGF α mice and the INS-GAS/MT-TGF α mice were not due to decreased levels of pancreatic TGF α expression in the INS-GAS/MT-TGF α mice. Northern blot analysis showed equivalent expression; furthermore, TGF α radioimmunoassay also demonstrated equivalent levels of TGF α immunoreactivity in pancreatic extracts from the MT-TGF α mice and INS-GAS/MT-TGF α double transgenic mice [13].

These synergistic effects of gastrin and TGF α transgene expression on islet development in the adult pancreas may elucidate part of the regulation of islet differentiation in the normal fetal pancreas. The pancreatic islets develop from endodermal stem cells that lie in the fetal pancreatic ducts, which also contain stem cells that differentiate into the exocrine pancreas. The development of the islets during the fetal period proceeds through a number of discrete stages that are regulated by peptide growth factors. Since TGF α is expressed in fetal pancreatic ducts [22], TGF α may stimulate proliferation of protodifferentiated islet precursors as in the postnatal TGF α transgenic pancreas. TGF α overexpression alone cannot, however, effect transition of the protodifferentiated islet precursors into fully differentiated islets. The increase in islet mass seen in the double (INS-GAS+/TGF α +) transgenic mice suggests that gastrin may have an analogous role during fetal development, when the endogenous gastrin gene is transiently expressed in the fetal pancreas, and may be one of the factors responsible for the transition of islet precursors into mature islet cells. The cellular mechanisms responsible for the synergistic interaction between TGF α and gastrin on islet formation are unknown. In addition, other peptide growth factors that are expressed in fetal life may also be required for normal islet development. These transgenic studies indicate, however, that islet growth can be stimulated by local expression of two growth factors, gastrin and TGF α .

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