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# Molecular structure and genetic mapping of the mouse gastrin gene

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**Abstract The mouse gastrin gene has three exons totalling 460 bp and a deduced preprogastrin of 101 amino acids. The sequence of murine gastrin-34 is 94% identical to rat gastrin-34 and 76% identical to human gastrin-34. At Arg[79], mouse progastrin has a unique cleavage site that might allow species-specific synthesis of gastrin-13. Northern analysis and RT-PCR demonstrated that gastrin gene transcripts are abundant in mouse stomach and duodenum and present at low levels in brain, ovary and pancreas, similar to the pattern described for other mammals. The gastrin gene was mapped to the distal region of mouse chromosome 11.**

*Key words:* Gastrin; cDNA; Gene; Chromosome mapping; Prohormone processing

## 1. Introduction

Gastrin is a peptide hormone that regulates acid secretion and mucosal growth in the stomach (for review see [1]). In adult vertebrates, the primary site of gastrin synthesis is the antral G-cells in the stomach. However, gastrin peptides have also been detected in the small and large intestine [2], vagal nerve [3], ovary [4], testis [5], hypothalamus and pituitary gland [6]. Moreover, during fetal life, gastrin is expressed in the pancreas [7], and colorectal tissues [8]. The biological significance of extra gastric gastrin is largely unknown, however, the trophic activity in the stomach mucosa suggests that gastrin might also be a growth factor in other regions of the intestinal tract and during gastrointestinal organogenesis.

One way to further elucidate the physiological and developmental significance of gastrin is to establish mouse models with altered gastrin expression. We report the cloning and chromosomal mapping of the mouse gastrin gene and cDNA and describe tissue distribution of gastrin gene transcripts.

## 2. Materials and methods

### 2.1. Isolation of the mouse gastrin gene and cDNA

A mouse gastrin probe was prepared by polymerase chain reaction (PCR) amplification of a 422 bp fragment from mouse genomic DNA using primers corresponding to positions 46–59 and 347–356 of the rat gastrin cDNA [9]. The fragment was gel purified and [$^{32}$P]dCTP-labelled by random oligonucleotide priming [10]. Using this probe, approx. $5.6\times10^6$ clones from a λ FIX II mouse 129/SvJ genomic library (Stratagene) were screened to isolate the gastrin gene [11]. With the same probe, $5\times10^4$ colonies from a mouse stomach cDNA library were screened. The cDNA library was prepared from mouse stomach RNA isolated as described [12]. After oligo(dT) primed cDNA synthesis using the Timesaver cDNA Synthesis System (Pharmacia), the library was inserted into λgt11 arms with an *Eco*RI/*Not*I adapter (Pharmacia) and in vitro packaged (Amersham). DNA from recombinant lambda clones was isolated using a liquid lysis method [13]. Nucleotide sequences were obtained on both strands, either manually using Sequenase 2.0 (Amersham/USB) or automatically using an ABI 373 sequencer. The sequence has been deposited in the EMBL data base with accessions numbers x94758, x94759 and x94760.

### 2.2. RNA analysis

Total RNA from stomach, duodenum, small intestine, colon, pancreas, ovary and oviduct, and brain was isolated from 3–10-week-old mice [12]. Poly(A)$^+$ RNA was isolated using oligo(dT) cellulose [14]. For Northern blot analysis, total RNA samples (20 μg) were electrophoresed in 1.0% agarose gels containing 2.2 M formaldehyde, transferred to Zeta-Probe nylon membrane (Bio Rad), and probed with a $^{32}$P-labelled mouse gastrin cDNA probe. Imaging was performed on a GS-250 Molecular Imager (Bio Rad). For reverse transcriptase (RT)-PCR analysis of gastrin gene expression 1 μg of total RNA was reverse transcribed for 15 min at 42°C in a mixture (20 μl) containing 5 units of AMV reverse trascriptase (Life Sciences), 20 units RNAsin (Promega), 50 pmol random hexamers, 1 mM dNTPs, 5 mM $MgCl_2$ and PCR buffer (Promega). PCR was performed on these samples using the exon 1 primer gas1 (5′-GCGCCACAACAGCCAACTATTCCCCAG-3′) and the exon 3 primer gas3′ (5′-CCAAAGTRCCATCCATCCGTAGGCCTCTTCT-3′). PCR reactions were analysed by electrophoresis in 1.5% agarose followed by Southern hybridisation with a mouse gastrin cDNA probe.

Primer extension reactions (12 μl) contained 0.5 pmol $^{32}$P-labelled 21-mer (5′-CAGTCGAGGCATCTTCTCCAC-3′) and 1 μg of mouse antrum poly(A)$^+$ RNA in 10 mM Tris-HCl (pH 6.9), 40 mM NaCl and 0.5 mM $MgCl_2$. Samples were heated to 95°C for 1 min, annealed at 56°C for 2 h, and extension was carried out for 30 min at 45°C after adding 8 μl of a mixture containing 125 mM Tris-HCl (pH 8.4), 25 mM $MgCl_2$, 5 mM dithiothreitol, 2.5 mM dNTPs and 1 unit of AMV reverse transcriptase (Life Sciences). Genomic DNA was sequenced (Amersham/USB) using a primer that has the same 3′ end and length as the oligonucleotide used for primer extension (5′-CTTCCCAAATTTCTCACCCAC-3′) and loaded in parallel with the primer extension product.

### 2.3. Genetic mapping

Primers GT5′ (5′-TCTTCTCATTTCCTTGGACATCTGTA-3′) and GT3′ (5′-GAATGGGCAGTCTTGGGAGGTTT-3′) surrounding the $(GT)_{23}$ repeat located 271 bp downstream of the gastrin gene were used to perform an allele specific typing of the (C57BL/6J × *Mus spretus*) $F_1$ × *Mus spretus* (BSS) backcross from The Jackson Laboratory [15]. To control for DNA loading in PCR reactions, a set of gastrin specific primers (gas5′ and gas3′) were used simultaneously with GT5′ and GT3′. Primer gas5′ was designed from sequences in exon 2 (5′-GCTGGCTCTAGCTACTACCTTCTCGGAACGTTC-3′) and gas3′ from sequences in exon 3 (as described above). PCR reactions were executed as described in [16]. Products were resolved on 2% agarose gels and stained with ethidium bromide. Statistical analysis of mapping data was calculated as previously described [16]. The symbol *Gast*, for the mouse gastrin gene, has been approved by the International Mouse Genome Nomenclature Committee.

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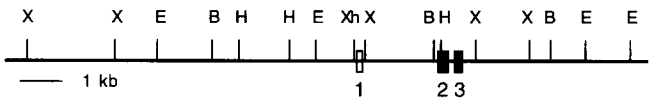
## 3. Results and discussion

### 3.1. Cloning and sequencing of the mouse gastrin gene

Two λ-clones were obtained after screening a mouse 129/SvJ genomic library with a mouse gastrin probe. The clones, λ1 and λ2, were 12.5 and 13 kb in length, respectively, and covered 15.6 kb of genomic DNA. The phage clones were characterised by restriction mapping and DNA sequencing to determine the molecular structure of the mouse gastrin gene (Fig. 1A). Exons were positioned by comparing the nucleotide sequence of the gastrin genomic DNA with the sequence of a gastrin cDNA clone isolated from a mouse stomach cDNA library. This comparison demonstrated that the murine gastrin gene consists of three exons spanning 2.6 kb of genomic DNA; exon 1 (52 bp) is non-translated, exon 2 (216 bp) contains the translational start site, and exon 3 (192 bp) contains the stop codon and poly(A)$^+$ site (Fig. 1B). The two introns are 1.8 kb and 111 bp in length. Southern blot analysis of 129/SvJ genomic DNA digested with *Bgl*II, *Eco*RI,

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-340 ttcattctct cccttccctc tcctctcagc cccgcccatg ggatgtagtc caatagaagc
-280 ctaggaagcc gatggcccaa ggcatacagt agaatgagct gctctggaga ccagggaatc
-220 tgtctcctgt ctgtctcctc cctgtttcag cccttccctt tccccatatt ctgaataaat
-160 aactaattga ttgacagctc tgtgacaggg tggggcagag tgacaggttg aacctacatt
-100 ccaggcccat ttctcttgct gtggggagtc tggcctcacc tggaaggagg gtttgggagg

-40  aacctcgagg gctttataag gcagacctgg agcagcgcac AGAGCAGAGC TGACCCAGCG

     CCACAACAGC CAACTATTCC CCAGCTCTGT GGgtgagaaa tttgggaagg gaaaagaggg

     aaagggaggg gagggaaggg acagcaagga tgagaattga caagcccccc gcctttagag
     gctccaggcc atggtgtctc cagagatggg gtcccttgct tggcccagag tggaaggaac

                                  ~ 1.8 kb

     cctttcctcc ctatctccct ttctctccct ctccctctct ctttttgtag cgaggcagat
     tagagctcat ctggtctagc cctttacctc tgggactatg gaatagcccc atcttgaagc
     ccagttctct tcccttctca gACAAG ATG CCT CGA CTG TGT GTG TAC ATG CTG
                                  Met Pro Arg Leu Cys Val Tyr Met Leu

     GTC TTA GTG CTG GCT CTA GCT ACC TTC TCG GAA GCT TCT TGG AAG CCC
     Val Leu Val Leu Ala Leu Ala Thr Phe Ser Glu ala Ser Trp Lys Pro

     CGC TCC CAG CTA CAG GAT GCA TCA TCT GGA CCA GGG ACC AAT GAG GAC
     Arg Ser Gln Leu Gln Asp Ala Ser Ser Gly Pro Gly Thr Asp Glu Asp

     CTG GAA CAG CGC CAG TTC AAC AAG CTG GGC TCA GCC TCT CAC CAT CGA
     Leu Glu Gln Arg Gln Phe Asn Lys Leu Gly Ser Ala Ser His His Arg

     AGG CAG CTG GGG CTC CAG GGT CCT CAA CAC TTC ATA GCA Ggtagtagct
     Arg Gln Leu Gly Leu Gln Gly Pro Gln His Phe Ile Ala A

     gctgagccag ccaggtttag ccatggttcc tccatactga ccccacagtt ctttgagact

     tggccttctt ctctccgttc ttacctcctg ttacctcctc agAC CTG TCC AAG AAG
                                                    sp Leu Asp Lys Lys

     CAG AGG CCA CGG ATG GAG GAA GAA GAA GAG GCC TAC GGA TGG ATG GAC
     Gln Arg Pro Arg Met Glu Glu Glu Glu Glu Ala Tyr Gly Trp Met Asp

     TTT GGC CGC CGC AGT GCT GAG GAA GAC CAG TAG GACTAGC AACACTCTTC
     Phe Gly Arg Arg Ser Ala Glu Glu Asp Gln End

     CAGAGCCCAG CCATCTCCAG CCACCCCTCC CCCAGCTCCG TCCTTACAAA ACATATTAAA

     AATAAGCTAG CTTCCAATGT atccctgagt catgtcatgt gcttgactgg agaggggttg

     aggcaggagg gagagctgag tacaccttag aggcagaccc aagagtagca ctaatcaagg
     atctacctgg gtgggcagtt aaacttactg ctctttcaaa ggattctggt tcaattccca
     gtacccatat tgggtggctc acagctgcct ataagtccag ttagagggaa tccaaccttc
     ttctcatttc cttggacatc tgtactcaca tgcatctaga cacataacat cgtgtgtgtg
     tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtatg tcaaaatgtg tcaatattgg
     aaggtttaaa cctcccaaga ctgcccattc catttacagt gaggccacga ctcca
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Fig. 1. (A) Schematic representation of the complete mouse gastrin gene. A composite restriction map of clones λ1 and λ2 is shown. Open box, noncoding exon; filled boxes, coding exons. B, *Bgl*I; E, *Eco*RI; H, *Hin*dIII; X, *Xba*I; Xh, *Xho*I. (B) Sequence of the mouse gastrin gene consisting of 3 exons (upper case) and two introns (lower case). The deduced amino acid sequence is indicated below the corresponding nucleotide sequence. The TATA box (underlined 5′ of the coding sequence), poly(A) site (underlined 3′ of the coding sequence), and $(GT)_{23}$ repeat (italics) are indicated. (We have previously reported nucleotides −300 to +9 in [34].)

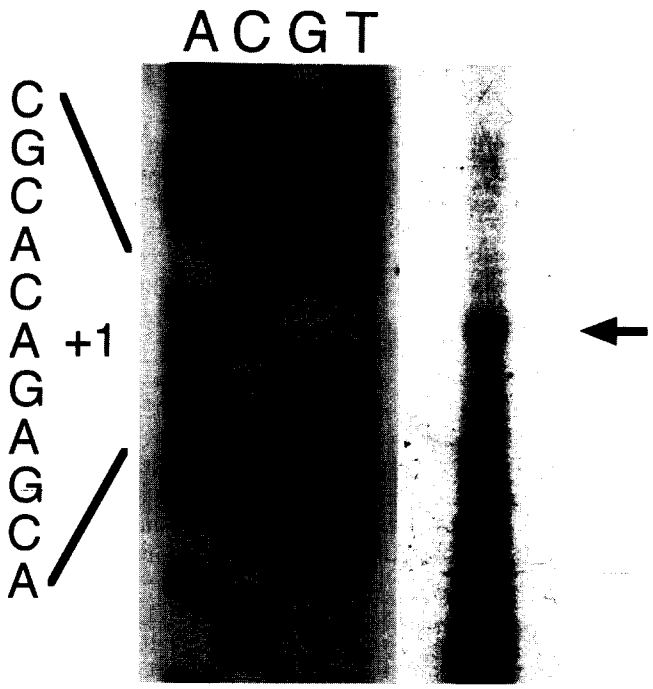


Fig. 2. Determination of the gastrin gene transcription start site. Poly(A)$^+$ from mouse stomach was annealed to a gastrin specific primer and primer extension was performed as described in section 2. Dideoxynucleotide sequencing products of a murine gastrin clone were electrophoresed on the same gel to localised the primer extension product (indicated by arrow) on the gastrin sequence. The first transcribed nucleotide is marked as +1.

*Hin*dIII and *Xba*I, all yielded a single band (data not shown) with sizes predicted from the restriction map of the λ1 and λ2 clones (Fig. 1), indicating that the gastrin gene is single copy in the mouse genome.

During the course of this study two other laboratories reported the partial characterisation of the murine gastrin gene [17,18]. However, the present study makes several important additions and corrections to the sequence [17], including a corrected sequence of the TATA element, the identification of the transcription start site, and a nucleotide difference in codon 76 changing the amino acid from Glu to Gln in this study. The basis for the nucleotide differences is unclear since the same mouse strain (129/SvJ) was analysed in both studies.

### 3.2. Identification of the transcription start site

A TATA-like sequence was identified upstream of the gastrin cDNA sequence (Fig. 1B, underlined), which corresponds to the position of the TATA element previously characterised in the human gene [19,20]. The transcriptional start site in the mouse was determined by primer extension analysis to be 26 bp downstream from the start of the TATA sequence (Fig. 2), in a position corresponding to the major start site for the human gastrin gene [19,20]. This indicates that the mouse gastrin cDNA clone isolated from the stomach library is truncated at the 5′ end by 16 bp.

When the proximal promoter sequences (−200 to +1) of the mouse are aligned with the promoter sequences of the rat [21] and human [19] gastrin genes (data not shown), several region of sequence identity are apparent, including an extensive region surrounding the TATA box (−48 to −20), and the following conserved consensus binding sites: an E-box (−64

CACCTG −59) [22,23], a β-interferon regulatory like element (−92 ATTTCTCT −85) [23,24], an SP1 site (−143 GGGTGGG −137) [25] (not present in the rat), and a homeodomain binding site (−159 ACTAATTG −152) [26] that has a single nucleotide difference in human (ACTAAATG). Notably, the EGF responsive element (GGGGCGGGGTGGGGGG) found in the human gastrin promoter [20] is absent from both the mouse and rat gastrin promoters.

### 3.3. Expression of the mouse gastrin gene

The pattern of gastrin gene expression in adult mice was studied by Northern blot and RT-PCR analysis. Northern analysis showed high levels of gastrin mRNA in the stomach and the duodenum, however, RT-PCR analysis also demonstrated gastrin mRNA in ovary, pancreas and brain (Fig. 3B). Thus, the sites and relative abundance of gastrin mRNA in mice are similar to those of other mammalian species, where stomach and the duodenum are the main sites of gastrin synthesis [1] and low amounts of gastrin are found in normal ovaries [4], pancreas [27], and certain regions of the brain [6,28,29]. Further studies are needed to identify the specific cell types expressing gastrin, and to determine to what extent the mRNA is translated to progastrin in the mouse.

### 3.4. Gastrin peptide sequence

The deduced mouse gastrin transcript consists of 460 nucleotides with an open reading frame encoding a 101 amino acid prepropeptide. Murine preprogastrin contains known processing sites to allow for the production of the bioactive α-carboxyamidated gastrins similar to the gastrins characterised in other mammals (Fig. 4) [30]. The N-terminus of the preprogastrin is hydrophobic in nature, putatively directing progastrin through the secretory pathway via the endoplasmatic reticulum. Dibasic endoproteolytic cleavage at $Arg^{94}$–$Arg^{95}$ followed by carboxypeptidase action would result in glycine-extended gastrins, which are the substrate for α-carboxyamidation. As is seen in other gastrins, differential cleavage of the N-terminal region of murine progastrin would result in numerous molecular forms (Fig. 4). Cleavage at $Arg^{57}$–$Arg^{58}$ would produce mouse gastrin-34 which has 94% and 76% amino acid identity to rat and human gastrin-34, respectively. The other major form of gastrin, gastrin-17 can be liberated by cleavage at $Lys^{74}$–$Lys^{75}$. Mouse progastrin contains other cleavage sites, of which the monobasic $Arg^{79}$ site is not observed in other species. Hence, synthesis of gastrin-13 may be a unique feature of murine gastrin (Fig. 4).

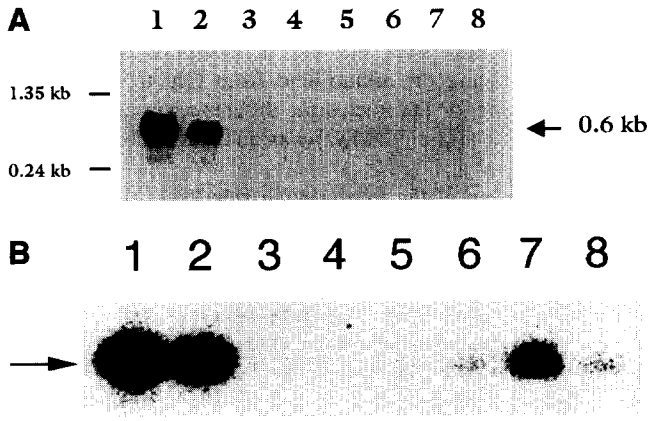


Fig. 3. Tissue distribution of gastrin transcripts in adult mouse. (A) Northern analysis of gastrin gene expression using 20 μg total RNA from stomach (1), duodenum (2), intestine (3), colon (4), liver (5), pancreas (6), ovary (7), and brain (8). The 0.6 kb gastrin transcript is indicated on the right (arrow) and the positions and sizes of RNA standards run on the same gel are indicated on the left. (B) RT-PCR analysis of gastrin gene expression was performed using exon 1 (gas1) and exon 3 (gas3′) primers. The 318 bp product was detected by Southern hybridisation with a gastrin cDNA probe. Lane designations as described in panel A.

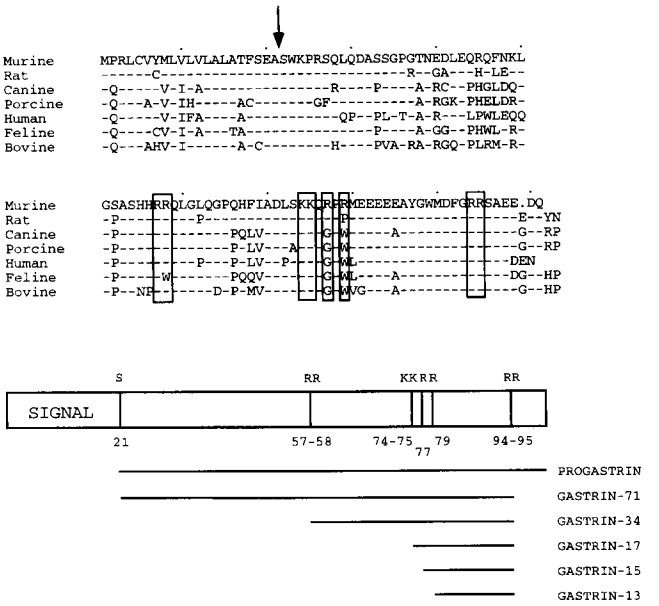


Fig. 4. Alignment of mammalian gastrin prepropeptides. The deduced amino acid sequence of mouse gastrin is aligned with that from rat [9], dog [35], pig [36], human [37], cat [38], and cow [39]. Dashes indicate identity with the mouse sequence and dots indicate spaces installed to maximise the similarity. The predicted cleavage site between the signal peptide and progastrin is marked by the arrow. Boxed amino acids indicate possible mono-/dibasic endoproteolytic cleavage sites. A diagram of the possible mono-/dibasic endoproteolytic cleavage sites in the deduced murine progastrin is shown below.

### 3.5. Genetic mapping

The chromosomal location of the mouse gastrin gene was determined by analysis of the BSS interspecific mouse backcross formed from the cross (C57BL/6J × *Mus spretus*) $F_1$ × *Mus spretus*. Individuals of this backcross were typed for inheritance of the parental C57BL/6J (B) or *M. spretus* (S) gastrin gene alleles by PCR amplification of a polymorphic dinucleotide $(GT)_{23}$ repeat located 271 bp downstream of exon 3 (Fig. 1B). Primers surrounding the GT repeat (GT5′ and GT3′) yielded the expected 152 bp PCR product from genomic DNA of mouse strain 129/SvJ (data not shown). When the parental strains for the backcross were tested, a 140 bp product was amplified from C57BL/6J and no product was amplified from *M. spretus* (Fig. 5A). Direct sequencing of a PCR fragment encompassing the repeat region in *M. spretus* demonstrated that the absence of an amplification product with the GT5′/GT3′ primer pair was due to nucleotide sequence changes that made the binding of the GT3′ primer impossible (data not shown).

The GT5′/GT3′ primer pair was combined with a control primer pair (gas5′/gas3′) to type the BSS backcross progeny for segregation of the gastrin B and S alleles. The control

primer pair amplified a 365 bp gastrin gene fragment from both C57BL/6J and *M. spretus* DNA, and thus was included to control for DNA loading in the reactions. When the four primers were combined to type the 94 individuals of the BSS backcross, three different fragments were observed (Fig. 5A). As predicted, the 365 bp fragment, corresponding to amplification from the control primer pair gas5′/gas3′, was observed in each sample. The 140 bp product amplified from primers

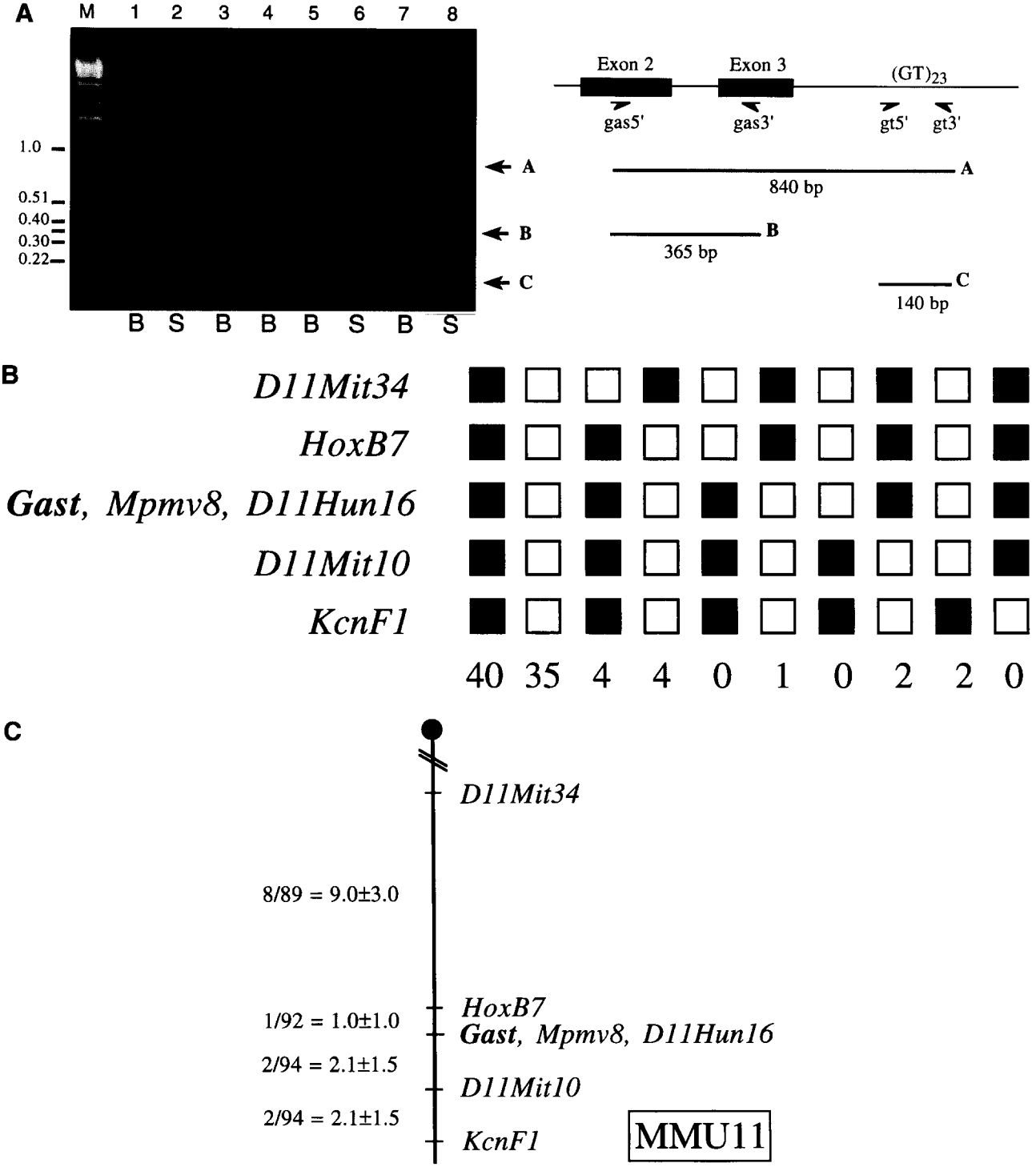


Fig. 5. Genetic localisation of the gastrin gene on mouse Chromosome 11. (A) Allele specific typing of the BSS backcross. Shown are eight samples subjected to PCR amplification with the gas5′, gas3′, GT5′ and GT3′ primers. The positions of the primers in the gastrin gene are diagrammed with the three possible amplification products (A–C). Allele typing is indicated below the lanes. Lanes: M, Gibco/BRL 1kb ladder; 1, C57BL/6J; 2, *Mus spretus*; 3–8, backcross individual DNAs 1–6. (B) Haplotype distribution of 88 individuals in the BSS backcross. Each column represents a chromosomal haplotype with the number of animals given below. Animals were scored at each locus as heterozygous for the S and B alleles (filled boxes) and homozygous for the S alleles (open boxes). Certain animals in the backcross for which there is ambiguous typing were not included. (C) Genetic map of mouse Chr 11 constructed from the haplotype distribution in part B. The map distances in cM were calculated from the recombination frequency observed in each interval.

GT5′/GT3′ was observed in samples containing the gastrin B allele. Another B specific product of 840 bp was also observed, which resulted from amplification from the gas5′ and GT3′ primers. The presence of this band verifies the allele specificity of the GT5′/GT3′ typing.

We established the unambiguous position of the gastrin gene on the basis of haplotype analysis of the 94 BSS backcross progeny to localised the mouse gastrin gene (*Gast* ) to Chr 11 (Fig. 5B). The intergenic distances (cM) were determined for *Gast* relative to other markers on Chr 11 (Fig. 5C). Mpmv8 and D11Hun16 segregated with *Gast* without recombination (0/94). This analysis placed the gastrin gene in the middle of a 35 cM region on distal mouse Chr 11 with synteny homology to human Chr 17q [31]. The human gastrin gene has been localised to human Chr 17q12-q21 by fluorescence in situ hybridisation [32] to within this extended region of synteny homology with mouse Chr 11. Thus, our localisation of the gastrin gene to the distal region of mouse Chr 11 is in agreement with the human mapping data. Although the gastrin and cholecystokinin (CCK) genes share an evolutionary origin, the mouse gastrin gene was not linked to the gene for CCK, which maps to mouse Chr 9 [33].

Based on the known physiological effects of gastrin in humans and mice, we predict that a gastrin deficient mouse could have stomach, intestinal or pancreatic abnormalities. A search of previously mapped mouse mutants on distal Chr 11 did not reveal one with a gastrointestinal defect that could be attributed to gastrin mutation. Thus, to access the phenotypic consequences of gastrin deficiency on mouse physiology and development, an induced mutation produced by gene targeting in embryonic stem cells would be required.

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