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27. Genomic DNAs were digested with Eco RI, separated by electrophoresis in 0.8% agarose, transferred to nylon membrane, and hybridized with the mouse genomic insert of plasmid pDP1171 as previously described (*2*).
28. Mouse (DBA/2J) chromosomes from cultured spleen lymphocytes were hybridized with ^{3}H-labeled pDP1055 at 42°C in 50% formamide, 2× standard saline citrate (SSC), 10× Denhardt's, 0.01% denatured salmon sperm DNA, and 10% dextran sulfate. Slides were washed three times for 2 min each at 39°C in 50% formamide, 2× SSC, exposed for 18 days, and stained for Q banding. The slides were examined by fluorescence microscopy to identify chromosomes and by transmitted light to localize autoradiographic grains.
29. We thank S. Swendeman for constructing pDP1055 and our colleagues, particularly E. Lander, for comments on the manuscript. Supported by grants from the National Institutes of Health, the Lucille P. Markey Charitable Trust, the March of Dimes, the American Cancer Society, and the Medical Research Council of Canada.

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Chromosome Mapping and Expression of a Putative Testis-Determining Gene in Mouse

CLAUDE M. NAGAMINE, KAIMIN CHAN, CHRISTINE A. KOZAK, YUN-FAT LAU*

Isolation and mapping of a mouse complementary DNA sequence (mouse Y-finger) encoding a multiple, potential zinc-binding, finger protein homologous to the candidate human testis-determining factor gene is reported. Four similar sequences were identified in Hind III–digested mouse genomic DNA. Two (7.2 and 2.0 kb) were mapped to the Y chromosome. Only the 2.0-kb fragment, however, was correlated with testis determination. Polymerase chain reaction analysis suggests both Y loci are transcribed in adult testes. A 3.6-kb fragment was mapped to the X chromosome between the T16H and T6R1 translocation breakpoints, and a fourth (6.0 kb) was mapped to chromosome 10. Hence, mYfin sequences have been duplicated several times in the mouse, although they are not duplicated in humans.

IN THE FETAL MOUSE, THE PAIRED GONADAL primordia are capable of developing into either ovaries or testes. If the fetus is of XY karyotype, the bipotential gonads differentiate into testes. The testes in turn secrete testosterone and Mullerian inhibiting substance, which induce development of the male accessory sex ducts and male secondary sexual characteristics. In the absence of a Y chromosome (for example, XX or XO karyotypes), the gonads develop into ovaries (*1*). The gene on the mouse Y chromosome initiating testis differentiation has been designated testis-determining Y (*Tdy*).

Recently Page *et al.* (*2*) isolated a human Y-chromosomal DNA fragment (pDP1007) that had characteristics making it a candidate for the testis-determining factor gene (*TDF*), the human equivalent of *Tdy*. A homologous sequence was also found on the X chromosome. The nucleotide sequence of pDP1007 encoded a protein containing multiple potential zinc-binding fingers. Zinc-finger proteins have been hypothesized to bind to and regulate DNA or RNA in a sequence-specific manner (*3*).

To isolate and characterize sequences in the mouse that are similar to pDP1007, a 36-base oligonucleotide (5′-CTC ATA CTC ACA GAA CTT GCA CTT GTG CAT CTT GTT-3′) deduced from amino acids 115 to 126 of the protein encoded by pDP1007 (*2*) was synthesized. It was used to probe a human Y chromosome–enriched library constructed in λ Charon 21A (*4*). A 1.3-kb Hind III DNA fragment (human Y-finger, hYfin) was isolated that is similar, if not identical, to pDP1007 on the basis of size and amino acid sequence. In addition, Southern blots of Eco RI–digested mouse genomic DNA revealed male-specific bands at 5.1 and 11.2 kb, similar to what was reported for pDP1007.

Using hYfin as a probe to screen a BALB/c adult testis cDNA library constructed in λ gt11 (*5*), we isolated a homologous 1.28-kb partial cDNA fragment (mouse Y-finger, mYfin). The Eco RI insert was subsequently isolated and subcloned into pUC18. On Southern blots of Eco RI–digested mouse genomic DNA, hYfin and mYfin probes detected the same 5.1- and 11.2-kb male-specific fragments and, in addition, 2.0- and 2.1-kb fragments in male and female DNAs.

In Hind III–digested genomic DNA from inbred strains, four DNA fragments hybridized to the mYfin probe in males and two DNA fragments in females (Fig. 1a). Since inbred male and female mice differ only in the presence of the Y chromosome, the fragments present only in males (2.0 and 7.2 kb) were mapped to the Y chromosome. When Southern blots of male mouse genomic DNA digested with Bam HI, Bgl II, Eco RI, Hind III, and Taq I were probed with mYfin, two Y-specific bands of different sizes but of equal hybridization intensities were observed. Our data suggest that either there are two copies of mYfin sequences on the Y chromosome or the cDNA spans an intron harboring these sites.

To determine whether mYfin sequences were closely linked on the Y chromosome, we analyzed the mouse sex-reversed mutation (*Sxr*). Male mice carrying the *Sxr* mutation (XYSxr) have a duplication of a segment of the Y comprising several thousand kilobases in length (*1*). This duplicated (*Sxr*) region, which is located on the telomere of the long arm of their variant Y chromosome, contains *Tdy*, the locus for the male-specific minor transplantation antigen (*H-Ya*) and banded krait minor satellite DNA repeat sequences (*Bkm*) (*1, 6*). Spermatozoa bearing a recombined X chromatid that possesses the *Sxr* region give rise to XXSxr males. When mYfin was used to probe Southern blots of genomic DNA from

C. M. Nagamine, K. Chan, Y.-F. Lau, Howard Hughes Medical Institute and Departments of Physiology and Medicine, University of California, San Francisco, CA 94143.
C. A. Kozak, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

*To whom correspondence should be addressed.

XXSxr, XY, and XYSxr siblings, both Hind III Y-specific bands were found in XXSxr males, indicating that both fragments are closely linked and are located in the *Sxr* region (Fig. 2a). As with other probes specific for the *Sxr* region (7), mYfin could also be used to determine the number of copies of the *Sxr* regions (1, 1, and 2 for XXSxr, XY, and XYSxr males, respectively) (Fig. 2a).

XXSxr′ mice possess a deletion in the *Sxr* region that includes the *H-Ya* locus but not *Tdy* (*8–10*). Only the 2.0-kb Hind III Y-fragment was present in XXSxr′ mice (Fig. 2b). The data confirm that mYfin sequences are duplicated in the *Sxr* region and demonstrate that the deletion in XXSxr′ mice encompasses one of the mYfin sequences. In keeping with current terminology, the two loci defined by the 2.0- and 7.2-kb sequences were designated zinc-finger-Y-1 (*Zfy-1*) and *Zfy-2*, respectively. Since XXSxr and XXSxr′ mice both have testes, only *Zfy-1* is linked to testis determination.

Two Hind III DNA fragments of 6.0 and 3.6 kb were recognized by mYfin in both male and female DNAs. Since the 3.6-kb Hind III DNA fragment consistently hybridized more intensely when two XX chromosomes were present [females versus males (Fig. 1, a and b), XXSxr or XX versus XYSxr or XY (Fig. 2a)], we hypothesized that it was an X-linked sequence and designated its locus as *Zfx*. We hybridized mYfin or hYfin probes to blots containing DNAs from a panel of 23 Chinese hamster–mouse somatic cell hybrids (*11*). The 3.6-kb Hind III fragment was correlated with the X chromosome (Table 1). Verification and more specific mapping was obtained with the Chinese hamster–mouse somatic cell hybrids: (i) VI-6, which harbors the entire mouse X chromosome (*12, 13*); (ii) B20c12, which contains a partial mouse X chromosome derived from a male carrying Searle's T(X;16)16H (T16H) translocation; and (iii) N15, which also has a partial mouse X chromosome but is derived from a male carrying the T(X;7)6R1 (T6R1) translocation (*14*). The presence of the 3.6-kb fragment in VI-6 and N15 but not in B20c12 maps the *Zfx* locus to the region of the X chromosome demarcated by the T16H and T6R1 translocation breakpoints (Fig. 1c). The T16H breakpoint is between the X probe DXPas8 (St14-1) and the mutation *tabby*; the T6R1 breakpoint is between the mutation *jimpy* (*PLP*) and the X probe DXPas1 (probe 45) (*14*). Gene loci known to occur between the T16H and T6R1 breakpoints include the X-inactivation locus (*Xce*), phosphoglycerate kinase–1 (*Pgk-1*), and α-galactosidase (*Ags*) (*14*). Comparative gene mapping of the human and mouse X chromosomes have identified at least four large, conserved segments (I to IV) (*15*). The *Zfx* locus is located in conserved segment III, which is homologous to the proximal long arm of the human X chromosome (*15*). Thus one would predict the X homologs of hYfin or pDP1007 would occur in this region of the human X chromosome. Page *et al.* (*2*), however, localized a pDP1007 homolog to the distal short arm of the human X chromosome (Xp21–Xp22.3). If confirmed by further studies, then pDP1007/hYfin sequences would represent an exception to the comparative gene mapping data for the human and mouse X chromosome.

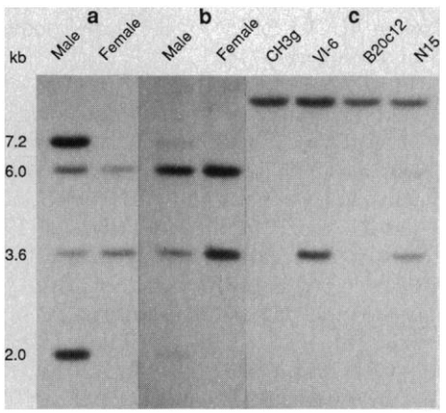

Fig. 1. (**a** and **b**) Southern blots of male and female mouse genomic DNA hybridized with the mYfin (a) and hYfin (b) probes showing similar patterns but different intensities of hybridizations. The filters were washed twice at low stringency only. Past experiments have shown that high-stringency washes do not alter the hybridization pattern of either probe. Exposure was for 1 (a) or 2 days (b). (**c**) Southern blot of DNA from the Chinese hamster cell line CH3g and the Chinese hamster–mouse somatic cell hybrids VI-6, B20c12, and N15. Cell hybrids are described in the text. The unlabeled high molecular weight band is a homologous Chinese hamster fragment. Hybridization was with mYfin probe, and exposure was for 4 days. Standard protocol: Fifteen micrograms of mouse spleen genomic DNA or 20 μg of DNA from tissue culture cells was digested with Hind III, separated by electrophoresis on 0.8% agarose gels, and transferred to Hybond-N membranes (Amersham) by capillary blotting (*25*). Transferred DNAs were fixed to the filters by ultraviolet irradiation. The blots were prehybridized for one to several hours at 65°C in a solution containing 0.25*M* NaH_2PO_4 (*p*H 7.2) and 7% SDS. The mYfin or hYfin probes were ^{32}P-labeled by nick-translation (*25*) or random priming (*26*) to a specific activity of 10^8 to 10^9 cpm per microgram of DNA and used at a concentration of 1.5×10^6 to 2×10^6 cpm/ml. Hybridization was performed at 65°C for 14 to 16 hours in a solution identical to the prehybridization solution but including 10% dextran sulfate. Filters were washed twice at low stringency (2× SSC, 0.1% SDS, 55°C, 30 min; 1× SSC = 0.15*M* NaCl and 0.015*M* sodium citrate) and once at high stringency (0.1× SSC, 0.1% SDS, 55°C, 15 min), unless noted otherwise. Autoradiography was with Kodak XAR5 film and one intensifying screen at −70°C.

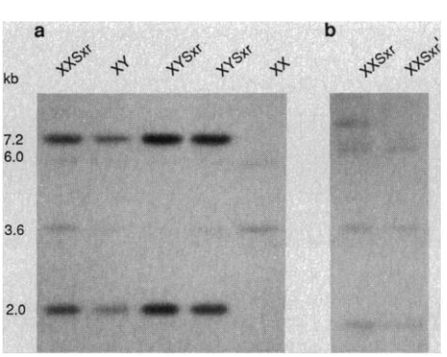

Fig. 2. (**a**) Southern blot of Hind III–digested DNA from XXSxr, Xy and XYSxr siblings, and a normal C3H/An female demonstrating linkage of the Y-specific fragments to the *Sxr* region. The darker Y-specific bands in the two XYSxr males suggest that two copies of the mYfin sequences are present. Hybridization was with mYfin probe, and exposure was for 4 days. (**b**) Absence of the 7.2-kb Y-specific band in Hind III–digested XXSxr′ DNA. Probing was with mYfin. After two low-stringency washes, the filter was washed once with 0.4× SSC and 0.1% SDS at 55°C for 15 min and autoradiographed for 1 day.

Table 1. Mapping of the 3.6- and 6.0-kb mYfin Hind III fragments to the X chromosome and chromosome 10, respectively, with Chinese hamster–mouse somatic cell hybrids. A discordancy of 0% indicates that the fragment was present in all hybrids containing and absent in all hybrids lacking a particular mouse chromosome. Percent discordancy calculated as total discordant observations (fragment either present when the cell line is lacking the particular mouse chromosome or absent when the cell line is possessing the particular mouse chromosome) divided by total number of cell lines tested (*n*).

Mouse chromosome	*n*	Percent discordancy	
		3.6 kb	6.0 kb
1	21	38.1	33.3
2	22	27.3	45.5
3	13	15.4	15.4
4	20	15.0	20.0
5	21	42.9	28.6
6	22	27.3	45.5
7	22	22.7	50.0
8	21	28.6	23.8
9	22	22.7	31.8
10	21	33.3	0.0
11	15/16*	46.7	18.8
12	15	20.0	60.0
13	20	30.0	35.0
14	19	26.3	36.8
15	15	40.0	73.3
16	16	43.8	25.0
17	20	20.0	50.0
18	17	41.2	29.4
19	19	31.6	31.6
X	20	0.0	35.0

*Fifteen cell lines were tested for the 3.6-kb fragment, 16 cell lines for the 6.0-kb fragment.

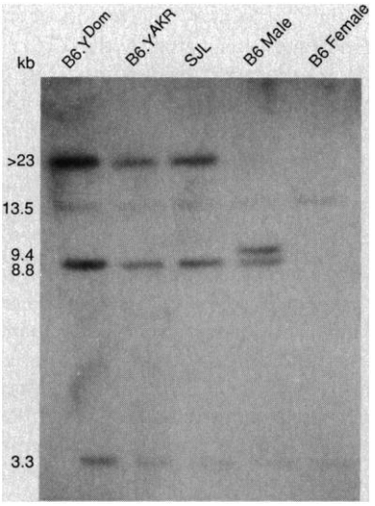

Fig. 3. Autoradiograph of a Southern blot of Bam HI–digested genomic DNAs from B6.Y^{Dom}, B6.Y^{AKR}, and SJL male mice and B6 male and female mice showing RFLP of the Y-specific bands between the Y chromosome of B6, which is derived from *M. m. musculus* ssp. (*17*) and the other three strains, which harbor different *M. m. domesticus*–derived Y chromosomes (*22*). The filter was probed with mYfin, and exposed for 3 days.

The 6.0-kb Hind III DNA fragment was absent from the somatic cell hybrids VI-6 and B20c12 (Fig. 1c), thereby excluding most of chromosome 16 (*12*) and the pseudoautosomal region of the sex chromosomes as possible sites, but was correlated with the presence of chromosome 10 (Table 1). Its locus was designated zinc-finger–autosomal (*Zfa*). The mapping of the *Zfa* locus to chromosome 10 precludes its being either of two autosomal genes known to be associated with testis determination in the mouse: testis-determining, autosomal-1 (*tda-1*), located on chromosome 2 or 4, or T-associated sex reversal (*Tas*), located on chromosome 17 (*1*). Whether the *Zfx* or *Zfa* loci represent functional genes is not known.

Although the hYfin and mYfin probes gave identical patterns of hybridization on Southern blots of mouse genomic DNA, hYfin consistently hybridized weakly to the Y-specific DNA fragments relative to the *Zfx* and *Zfa* homologs. Surprisingly, the reverse was true for mYfin; it hybridized strongly to the Y-specific fragments and weakly to the *Zfx* and *Zfa* homologs (Fig. 1, a and b). Based on these observations, we conclude that hYfin and mYfin sequences, although similar, have diverged during evolution.

The presence or absence of restriction fragment length polymorphisms (RFLPs) of mYfin sequences in laboratory mouse strains could provide insight as to its evolution. When Southern blots containing DNA of AKR, C57BL/6J, and DBA/2J strains digested with Bam HI, Bgl II, Eco RI, Hind III, Msp I, and Taq I were probed with mYfin, no RFLPs of the *Zfx* or *Zfa* homologs were observed, suggesting a relative stability of these two sequences (*16*). Preliminary analysis of 17 strains of mice [A, AKR, BALB/c, BFM/2, CBA, C3H/An, C3H/He, C57BL/6J, DBA/1, DBA/2, NCS, SJL, SWR, WLA, WMP, 129, *Mus musculus domesticus* (Tirano)] have revealed no RFLPs of the *Zfx* and *Zfa* loci except for *M. m. domesticus* (Tirano) and WMP strains, which had an Eco RI RFLP for the chromosome 10 homolog only (*16*).

In contrast to the relative absence of RFLP for the *Zfx* and *Zfa* homologs, RFLPs of the *Zfy*-specific bands were readily observed with the enzymes Bam HI, Msp I, and Taq I, but not with Bgl II, Eco RI, or Hind III (Fig. 3). Southern analysis of 19 strains of mice have demonstrated that the RFLPs correlated with whether their Y chromosomes had orginated from the *M. m. musculus* (A, BALB/c, CBA, C3H/An, C3H/He, C57BL/6J, DBA/1, DBA/2, 129) or *M. m. domesticus* [AKR, BFM/2, B6.Y^{Dom} B6.Y^{AKR}, NCS, SJL, SWR, WLA, WMP, *M. m. domesticus* (Tirano)] subspecies (*17*). The observed RFLP is of interest since when certain *domesticus* Y chromosomes were backcrossed into the C57BL/6J (B6) strain, the introduced *domesticus* Y chromosome did not induce complete testicular differentiation, resulting in XY hermaphrodities and XY females (*18–22*). The B6 strain has a *musculus*-derived Y chromosome and shows no evidence of abnormal testicular differentiation. It has been postulated that XY sex reversal is due in part to incompatible *Tdy* alleles on the *domesticus* and *musculus* Y chromosomes (*19*). If additional research confirms that *Zfy-1* is *Tdy* and that *Tdy* alleles exist, then the observed RFLP may illustrate the alleles.

Since mYfin was isolated from a cDNA library constructed from polyadenylated RNA of adult mouse testes, contained a stretch of adenosine residues at the 3′ end of the corresponding mRNA, and hybridized strongly to the *Zfy*-specific versus *Zfx* and *Zfa* homologs in Southern blots, we surmised it was derived from a testicular transcript originating from either the *Zfy-1* or *Zfy-2* locus. To address the questions of origin and expression of mYfin, we amplified a 600-bp fragment of mYfin, first, in genomic DNA using the polymerase chain reaction technique (PCR), and, second, in adult testes cDNA using the reverse transcriptase-PCR technique (RT-PCR) (*23*). PCR amplification of normal male genomic DNA revealed four male-specific fragments of approximate sizes of 1030, 780, and 618 bp (A, B, C) in addition to the 600-bp cognate fragment (D) (Fig. 4, lane 2). No female fragments were observed, demonstrating that the amplified sequences were Y-linked (Fig. 4, lane 3). Although all four fragments were amplified with XXSxr DNA (Fig 4, lane 4), only C was amplified with XXSxr′ DNA (Fig. 4, lane 5). In keeping with the Southern blot data, the locus represented by the fragment amplified with XXSxr′ DNA (C) corresponds to *Zfy-1*; the locus represented by the fragment not amplified in XXSxr′ DNA (D) corresponds to *Zfy-2*. Furthermore, the data suggest that the mYfin sequence was derived from the *Zfy-2* locus (D). The origin of fragments A and B is unknown.

RT-PCR amplification of adult testes RNA resulted in all four male-specific fragments (A to D) being amplified, suggesting that both *Zfy-1* and *Zfy-2* loci are transcribed in adult mouse testes (Fig 4, lanes 6 and 8). The signal from the *Zfy-1* locus (C) appeared less than that from the *Zfy-2* locus (D). This is in contrast to the equal amplification of these two (C and D) fragments with normal male or XXSxr DNA (Fig. 4, lanes 2 and 4).

The detection of transcripts from both *Zfy-1* and *Zfy-2* loci in adult testes suggests that both loci are functional. Only *Zfy-1* is linked to testis determination and can be considered as the candidate gene for *Tdy*. Significantly, the *Zfy-2* locus is linked to *H-*

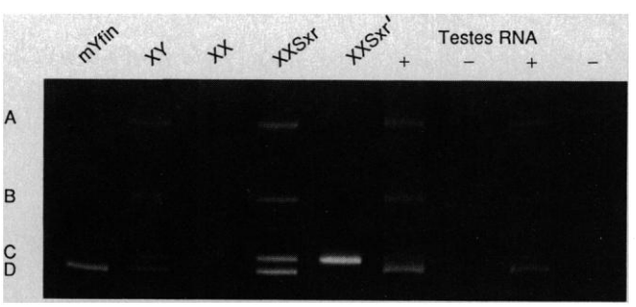

Fig. 4. PCR analysis of mYfin and genomic DNA and RT-PCR analysis of testes RNA. Lanes 1 to 5: lane 1, pUC18 containing the mYfin insert; lanes 2 and 3, B6 male and female DNA, respectively; lanes 4 and 5, XXSxr and XXSxr′ DNA, respectively. Lanes 6 to 9: RT-PCR of two different adult testes RNA preparations that have (+) and have not (−) first undergone reverse transcription (*23*). Volumes of amplified mixture loaded on the gel: 0.5 μl (lane 1), 5 μl (lanes 2 to 5), and 10 μl (lanes 6 to 9). Amplification of the mYfin sequence (lane 1) delineates the cognate 600-bp fragment spanning the specific primers. A to D = Y-specific fragments.

Ya, the gene controlling expression of the H-Y antigen as defined by transplantation and cytotoxic T cell assays (*10*) and a closely linked spermatogenesis gene (*24*). It has been suggested that the genes controlling H-Y expression and spermatogenesis are the same (*24*). It is tempting to speculate that the expressed *Zfy-2* gene is *Hya* or the spermatogenesis gene. The exact functions of *Zfy-1* and *Zfy-2*, however, cannot be elucidated without further molecular characterization and functional assessments, such as transgenic mouse construction or gene inactivation by targeted gene transfer.

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Pavlovian Conditioning of Rat Mucosal Mast Cells to Secrete Rat Mast Cell Protease II

GLENDA MACQUEEN,* JEAN MARSHALL, MARY PERDUE, SHEPARD SIEGEL, JOHN BIENENSTOCK

Antigen (egg albumin) injections, which stimulate mucosal mast cells to secrete mediators, were paired with an audiovisual cue. After reexposure to the audiovisual cue, a mediator (rat mast cell protease II) was measured with a sensitive and specific assay. Animals reexposed to only the audiovisual cue released a quantity of protease not significantly different from animals reexposed to both the cue and the antigen; these groups released significantly more protease than animals that had received the cue and antigen in a noncontingent manner. The results support a role for the central nervous system as a functional effector of mast cell function in the allergic state.

SEVERAL STUDIES SUGGEST THAT ANAphylaxis can occur in the absence of any antigenic stimulus or physical irritant (*1, 2*) and may represent a conditional response (*2*). That is, neutral stimuli, such as sensory events (conditional stimulus; CS) may become associated with antigens such that subsequent exposure to these stimuli will elicit anaphylactic responses. In Pavlovian conditioning terms, the antigen is an unconditional stimulus (US) that elicits an unconditional response, anaphylaxis.

That the immune system is influenced by Pavlovian conditioning (*3*) has provided evidence for nervous-immune system interactions. Cues paired with both immunosuppressive (*3*) and immunoenhancing agents (*4*) induce conditional immunomodulation. Conditional alterations of immune function also occur in animals trained with antigens as the US (*5*). Conditional secretion of histamine in the guinea pig was previously reported, although the cellular source of the conditional histamine release was not identified (*5*). This suggested that mast cells, which are targeted in allergic disease (*6*), might be influenced by the nervous system. Evidence to support this hypothesis was lacking, however, as histamine may be released from a variety of cells at many sites within the body (*5*). We measured a protease specific to mucosal mast cell activity and demonstrated that mucosal mast cell activity is subject to central nervous system modulation.

Mast cells have high-affinity binding re-

G. MacQueen and S. Siegel, Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

J. Marshall, M. Perdue, J. Bienenstock, Molecular Virology and Immunology Programme, Department of Pathology, and Intestinal Diseases Research Unit, McMaster University Medical Center, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

*To whom correspondence should be addressed.