vascular beds. In additional studies we examined the potentencies and half-lives of two other nitrosothiols, *S*-nitrosoglutathione and *S*-nitrosomercaptoethanol. Both were found to be potent vasodilators with 50% effective doses of approximately 10 nM or less. The half-lives of both compounds, however, were substantially longer than that of EDRF released from bovine aortic endothelial cells, exceeding 2 min ($n = 3$ for both).

The incorporation of nitric oxide into a nitrosothiol compound such as *S*-nitrosocysteine may have several important biological implications. When nitric oxide is incorporated into *S*-nitrosocysteine, its absolute stability and the potency is substantially enhanced. The release of such a compound enables a finer instantaneous control of vascular tone than the release of a less potent compound. The incorporation of nitric oxide into *S*-nitrosocysteine may be important in the control of EDRF secretion. It is conceivable that transmembrane transport relies on the association of nitric oxide with a carrier molecule such as an amino acid or other thiol compounds. Uptake of a nitrosothiol into the vascular smooth muscle may also depend on a transport mechanism. Alternatively, the nitrosothiol may degrade at the smooth muscle cell membrane to yield nitric oxide. This process may more efficiently deliver nitric oxide to the cytoplasm of the vascular smooth muscle.

Several pathological processes are associated with abnormalities of endothelium-dependent vascular relaxation. These include acute hypertension[10], diabetes[11], ischaemia with subsequent reperfusion[12], and atherosclerosis[13]. Many of these processes are associated with the generation of oxygen free radicals within endothelial cells which may oxidize free sulphydryl groups to the disulphide form. This may result in either an inability of the endothelium to synthesize nitrosothiols, resulting in the release of either nitrite (a compound with essentially no vasodilator activity) or nitric oxide (a compound substantially less potent than EDRF). In either instance, endothelium-dependent vascular relaxation would be impaired by the oxidation of sulphydryl groups within endothelial cells. □

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Similarity of the product of the *Drosophila* neurogenic gene *big brain* to transmembrane channel proteins

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CELLS in the neurogenic region of *Drosophila* embryos are initially bipotential; they can become either neuroblasts or epidermoblasts[1,2]. Cell–cell interaction seems to play an important part in this developmental decision[3], which involves the function of a group of genes (the neurogenic genes). Loss-of-function mutations in any of the neurogenic genes result in nervous system hyperplasia and epidermal hypoplasia[4,5]. Of the six known zygotic neurogenic genes, *big brain* (*bib*) is unique in several aspects[6–9]. Most notably, all the other known neurogenic genes seem to fit into a cascade defined by genetic interactions, whereas *bib* does not show any detectable interaction with them[9]. To understand how *bib* functions, we have now cloned the *bib* genomic and complementary DNAs. The predicted *bib* product shows significant sequence similarity to a family of transmembrane proteins[10–13], some of which form channels permeable to small molecules[14,15]. Together with genetic studies, our results indicate that the *bib* product may mediate intercellular communication in a pathway separate from the one involving the products of the other neurogenic genes.

The *bib* locus has been mapped genetically to 30F on the left arm of the second chromosome[5]. By screening embryonic lethal mutations caused by the insertion of a P-element-derived vector[16], we found two neurogenic mutant lines, *l(2)neo66* and *l(2)neo91*, that did not complement *bib* and had the P-element insertion at 30F. To test whether the insertion is the cause of the *bib* phenotype, we have done a reversion experiment in which the P-element in *l(2)neo91* was excised from its insertion site after exposure to transposase activity carried on a different chromosome. The neurogenic phenotype and lethality reverted with the removal of the P-element, demonstrating that the insertion at 30F in this case was indeed responsible for the phenotype.

Genomic DNA surrounding the P-element insertion sites in *l(2)neo66* and *l(2)neo91* has been cloned by plasmid rescue[17] (Fig. 1*a*). When northern blots of embryonic RNA were hybridized with these genomic DNA fragments, we found several transcription units in the region between position −18 and −3 kilobases (kb), and one transcription unit between position 0 and +12.5 kb which gives rise to two messenger RNAs of 3.4 and 3.1 kb (Fig. 1*d*). The latter transcription unit has been identified as the *bib* gene because: (1) it overlaps with genomic alterations in different *bib* alleles (a deletion in $bib^{1119\text{-}5}$, an inversion breakpoint in bib^{C7a}, and a small deletion in *l(2)neo121*) as revealed by Southern blot analyses (Fig. 1*b*); (2) the P-element in *l(2)neo66* is inserted into the transcribed region of this transcription unit (at base pair (bp) 147 of a cDNA, c13, see below), whereas the insertion site in *l(2)neo91* is only 19 bp upstream of that cDNA; (3) introduction of a genomic DNA fragment including this transcription unit (PWB in Fig. 1*c*) by P-element mediated transformation[18,19] rescued the neurogenic phenotype and lethality of *bib* mutants.

We have examined the distribution of *bib* mRNA by *in situ* hybridization to whole-mount embryos (Fig. 2). Expression of *bib* begins soon after the cell membrane starts to form around the nuclei in the syncytial blastoderm and is detectable in all the somatic cells that are forming in an early stage-5 embryo (staging is according to ref. 2).The *bib* mRNA soon disappears in a ventral region; the width of this region is about 10 cells at the stage of blastoderm formation when roughly one fifth of the cell membrane is formed (Fig. 2*a*) and increases to about 17 cells when cellularization is complete (late stage 5). This region of about 17 cells includes all presumptive mesodermal cells and two rows of ectodermal cells. During gastrulation at stages 6 and 7, the mesoderm invaginates, leaving one row of ectodermal

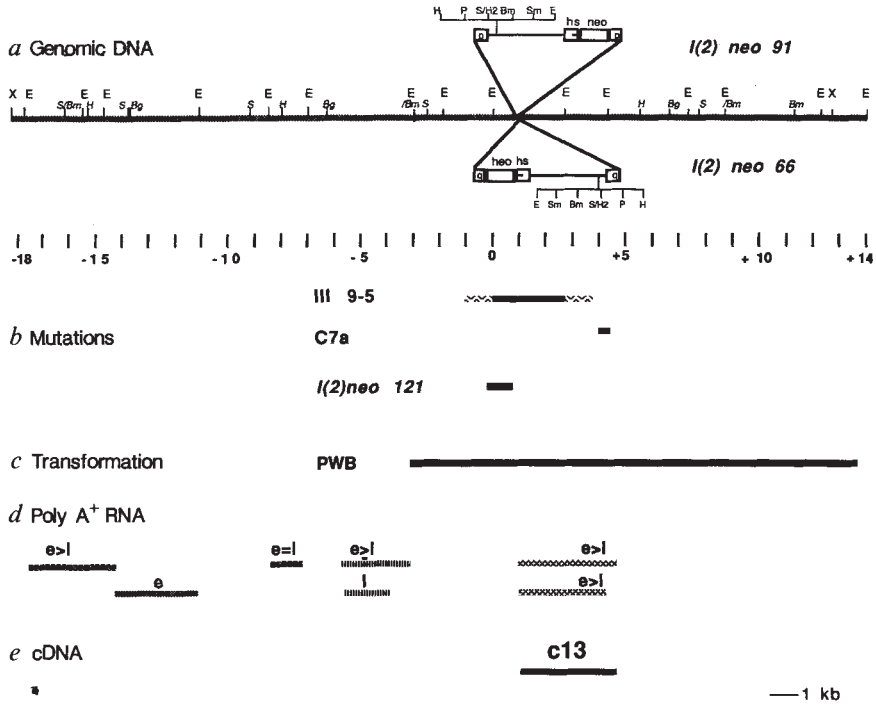

FIG. 1 Molecular map of *bib*. *a*, Restriction map of the *bib* genomic locus, as well as location of P-elements. Bg: *Bgl*I, Bm: *Bam*HI, E: *Eco*RI, H: *Hin*dIII, S: *Sal*I, X: *Xho*I. The *Eco*RI fragment at position 0–2.6 kb is polymorphic so that the second chromosome balancer, CyO (Curly derivative of Oster[40]), has a 2.9 kb instead of a 2.6 kb fragment. The insertions of a P-element-derived vector pUChsneo in *l*(2)*neo66* and *l*(2)*neo91* are in opposite orientations. *b*, Location of X-ray induced mutations of *bib*: *bib*$^{III9\text{-}5}$ has a deletion that includes the fragment indicated by the solid line and the ends could not be mapped in the cloned region; *bib*^{C7a} is an inversion allele with one of its breakpoints indicated by the solid line; *l*(2)*neo121* has a deletion in the region indicated by the solid line. *c*, PWB is a 15-kb DNA used in P-element mediated germ-line transformation. *d*, Poly(A)$^+$ RNA panel shows the results of transcriptional analysis. The size of each transcript is indicated by the length of the line and the transcripts are positioned roughly under the genomic fragments that hybridize to them; e > I indicates that a transcript is more abundant in the early embryonic RNA (1–14 h) than in late embryonic RNA (8–21 h). Probes from position 1–2.6 kb and 2.6–3.2 kb hybridize to the same two messages of 3.4 and 3.1 kb. Position 8.7–12.3 kb contains repetitive sequences. *e*, cDNA panel shows c13, one of the longest of 17 cDNAs isolated with probes made from genomic fragments (1–3.2 kb); c13 hybridizes to both messages on northern blots and to genomic DNA fragments, at positions 1–2.6, 2.6–3.2 and 8.7–12.3 kb. The sequence of c13 contains *opa* repeats.

METHODS. Poly(A)$^+$ RNA used in northern blots was described previously[35]. Embryonic cDNA libraries were from N. Brown[36]. Insertion sites of pUChsneo in *l*(2)*neo66* and *l*(2)*neo91* have been determined by sequencing genomic fragments rescued from these lines. In addition to the deletion shown above, *l*(2)*neo121* has a P-element insertion at 49D on the right arm of the second chromosome. This insertion is not responsible for the *bib* phenotype because a recombination event which separated the two arms of the second chromosome in *l*(2)*neo121* revealed that the lethality segregated with the left arm rather than with the pUChsneo insertion on the right arm. Moreover, deficiency of 49D does not give rise to any neurogenic phenotype (unpublished observation).

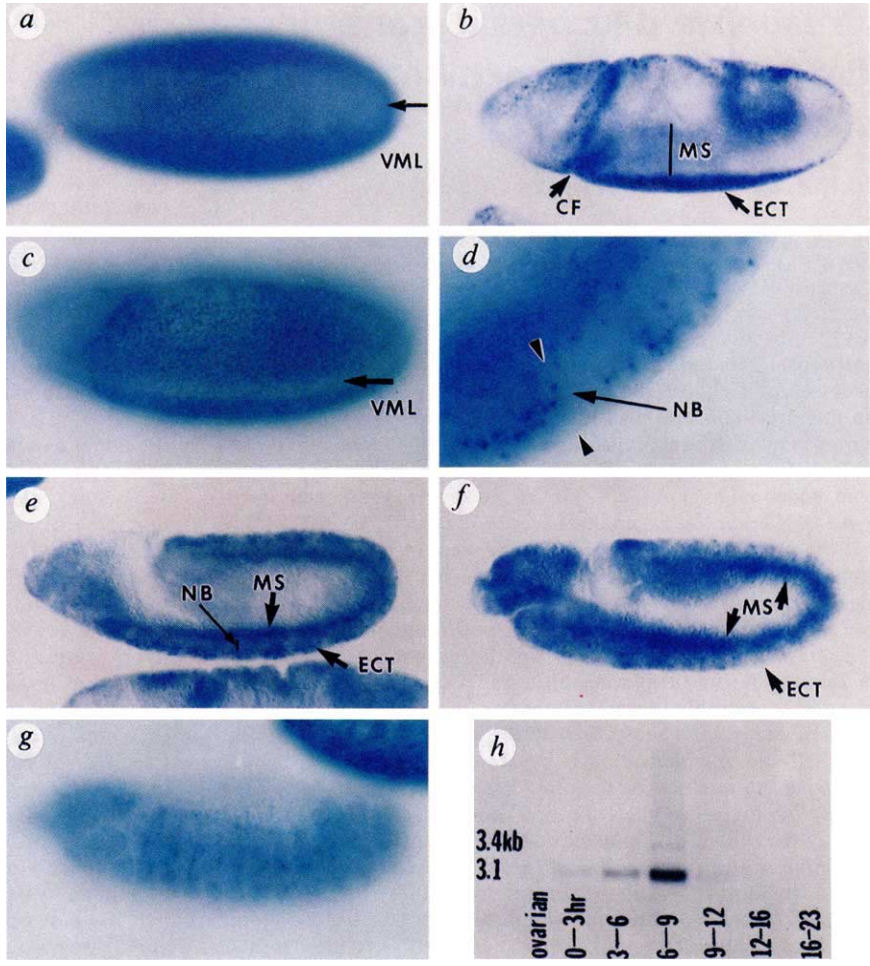

FIG. 2 Embryonic expression of *bib*. Orientation of the embryos is anterior to the left (in *a*, *b*, *c*, *e*, *f*, *g*), and dorsal to the top (in *b*, *c*, *e*, *f*, *g*). Staging is according to ref. 2. No *bib* expression is seen before the cell membrane starts to form. *a*, Ventral view of a stage-5 embryo showing absence of expression in the presumptive mesoderm. VML indicates the ventral midline. *b*, Side view of a stage-7 embryo showing *bib* expression only in the ectoderm and not in the mesoderm during gastrulation. *c*, After ventral furrow formation, no *bib* mRNA is detectable in two rows of ectodermal cells, one row on each side of the ventral midline in an early stage-8 embryo; *d*, *bib* expression in a segregating neuroblast in a stage-9 embryo. Arrowheads point to the neuroblast which has its nucleus moved inward, but has its cell membrane still in contact with its ectodermal neighbours; *e*, *bib* expression in both the epidermal and the mesodermal layers but not in the neuroblast (NB) layer in a stage-10 embryo after germ-band elongation. *f*, In a stage-11 embryo, *bib* expression is still in the mesoderm, but not in the ectoderm. *g*, After germ-band shortening in a late stage-12 embryo, there are *bib* transcripts in a few cells per segment but the identity of these cells cannot be resolved in these embryos. *h*, Developmental northern analysis: no *bib* message is found in ovarian RNA. Numbers in the other lanes denote the age of the embryos from which the RNA was prepared.

METHODS. Whole-mount *in situ* hybridization[37] was carried out with a digoxigenin-labelled probe (template: genomic DNA at coordinate 0.9 to 8.7 kb). The staining is absent in *bib*$^{III9\text{-}5}$ mutant embryos.

cells without eletectable *bib* expression on either side of the ventral furrow (Fig. 2*c*).

During germ-band elongation (at stage 8), *bib* expression is maintained in the ectoderm (Fig. 2*b*). As a presumptive neuroblast delaminates (at late stage 8 and stage 9), *bib* mRNA is detectable when the nucleus has moved inward but the cell membrane is still adjacent to its original neighbours in the ectoderm (Fig. 2*d*); *bib* expression disappears from the neuroblasts after they have completely segregated from the epidermal layer. Toward the end of germ-band extension (from stage 9 to early stage 11), the mesoderm starts to express *bib*, so that there is *bib* mRNA in the epidermis and mesoderm, but not in the neuroblasts that are sandwiched between them (Fig. 2*e*). Just before germ-band shortening (late stage 11), *bib* expression begins to disappear first from the epidermis (Fig. 2*f*) and then from the mesoderm. After completion of germ-band shortening (from stage 12 onwards), *bib* mRNA is barely detectable in the embryo (Fig. 2*g*).

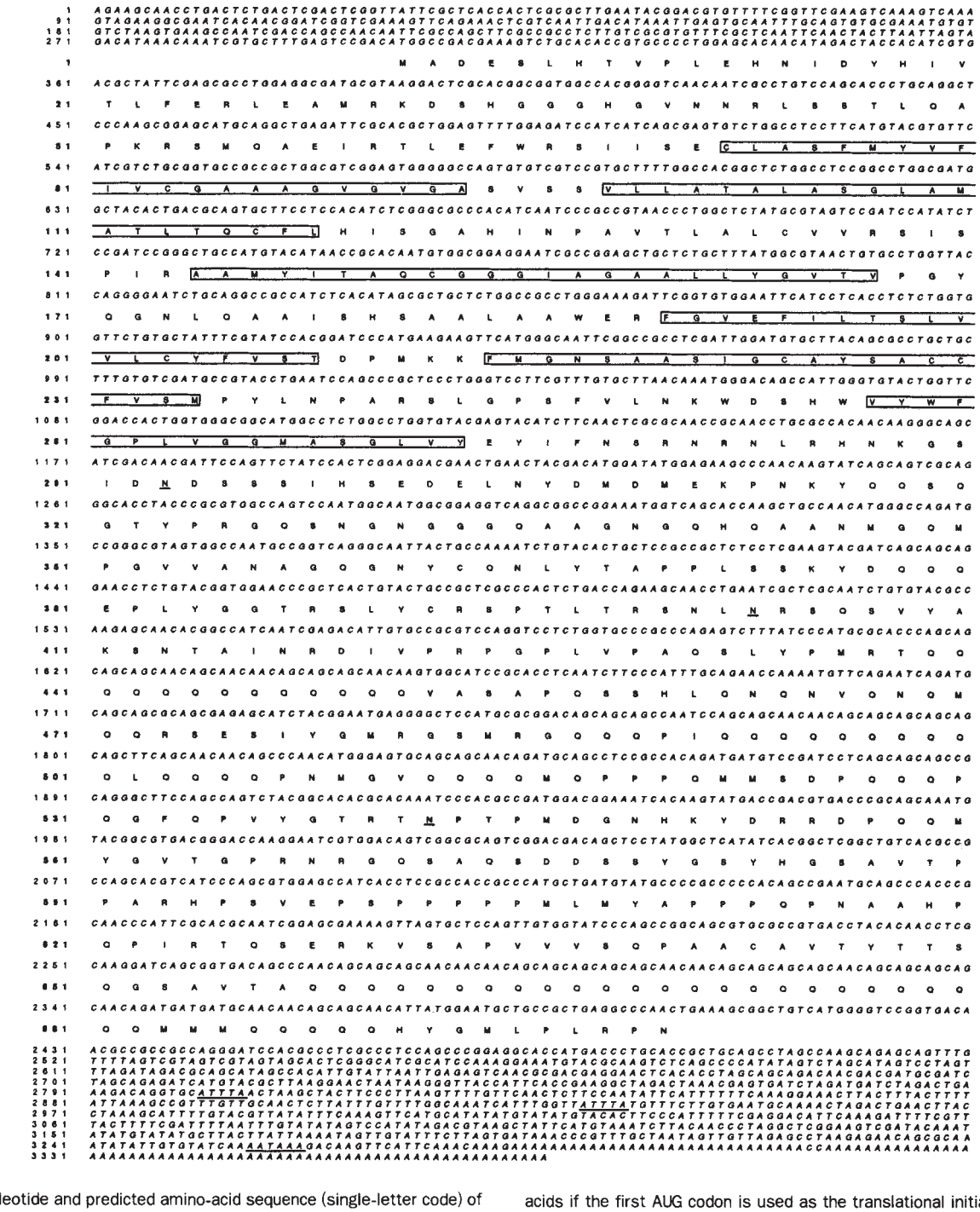

FIG. 3 Nucleotide and predicted amino-acid sequence (single-letter code) of *bib*, as determined from the sequence of c13. Both strands of the cDNA have been sequenced by the dideoxy chain-termination method with Sequenase (USB). The 3′ untranslated region of *bib* mRNA has several copies of the AUUUA motif, a signal for RNA instability[38]. The cDNA has an open reading frame of 2,134 base pairs and predicts a protein of 700 amino acids if the first AUG codon is used as the translational initiation site. The first three AUG codons conform to the consensus sequence for *Drosophila* translation initiation sites[39], and all three precede the region of sequence similarity shown in Fig. 4*b*. A polyadenylation signal is underlined, as are sites conforming to *N*-linked glycosylation consensus. Putative transmembrane domains are boxed.

The result of developmental northern blot analysis correlates with the temporal expression pattern detected by *in situ* hybridization (Fig. 2*h*). Furthermore, no *bib* message is found in the ovary, consistent with the observation that *bib* is the only known neurogenic gene that has no detectable maternal contribution[6].

We have analysed a possibly full-length cDNA, c13, which corresponds to the identified *bib* gene. The predicted 700 amino-acid *bib* product has some notable structural features (Fig. 3). Its N-terminal half is highly hydrophobic (Fig. 4*a*), whereas the C-terminal portion is generally hydrophilic and glutamine-rich. There are six hydrophobic stretches of more than 21 amino-acid residues (boxed in Fig. 3) that could potentially span the membrane[20]. As there is no apparent signal peptide, both the N- and the C-termini may be intracellular.

The *bib* protein has significant sequence homology to the major intrinisic protein (MIP) of bovine lens fibre cell membrane[10]; 40% of the 224 amino acids (residues 56–279) in the N-terminal half are identical to those in MIP. This region also has sequence similarity to a soybean membrane protein, nodulin (nod) 26 (with 27% amino-acid identity)[11,12], and an *Escherichia coli* protein, GlpF (with 23% amino-acid identity)[13] (Fig. 4*b*). The *bib* protein is about twice as large as the other proteins; the C-terminal half of *bib* shows no significant homology to any known proteins.

MIP is the most abundant membrane protein in mammalian lens fibre cells[21]. It is localized on the cell membrane[22–25]. As the lens is avascular, cell–cell coupling is essential to ensure extensive metabolite exchange. There are abundant lens junctional structures between the fibre cells, some of which resemble gap junctions. It has been postulated that MIP is involved in forming gap junctions between the lens fibre cells[22,23,26], but there is conflicting evidence. Unlike connexins, MIP has not been shown to form gap junctions, and ultrastructurally, MIP is localized not only to gap junctions but also to other types of junctions[24,25]. Injection of MIP mRNA into *Xenopus* oocytes also failed to cause gap junction formation[27]. However, MIP has been reconstituted into the lipid bilayer and shown to form non-selective channels with large conductance[14].

Nod 26 is a transmembrane protein made by soybeans. In legume-*Rhizobium* symbiosis, after the bacteria infect the plant cells and are endocytosed into the cytoplasm, a series of events leads to the formation of nitrogen-fixation nodules. Rhizobia within the soybean cytoplasm are individually surrounded by the plant-derived peribacteroid membrane. There are about 20 proteins (the nodulins, including nod 26) made by soybean specifically for the peribacteroid membrane, which are believed to have important roles in morphogenesis and function of nitrogen-fixation nodules[28]. Although the function of nod 26 is unknown, the peribacteroid membrane must be involved in regulating the exchange of nutrients and fixed nitrogen compounds between plant and bacteria.

GlpF is the glycerol facilitator of the *E. coli* inner membrane[13]. It is a passive channel permeable to neutral molecules smaller than 0.4 nm in diameter, including glycerol and glycine[15]. Although the *E. coli* outer membrane has several pore-type transporters, glpF is the only channel that allows bidirectional transport of molecules through the inner cytoplasmic membrane.

The sequence similarity between *bib*, glpF and MIP implies that the *bib* product may allow passage of small molecules across the membrane, which could mean that *bib* directly mediates

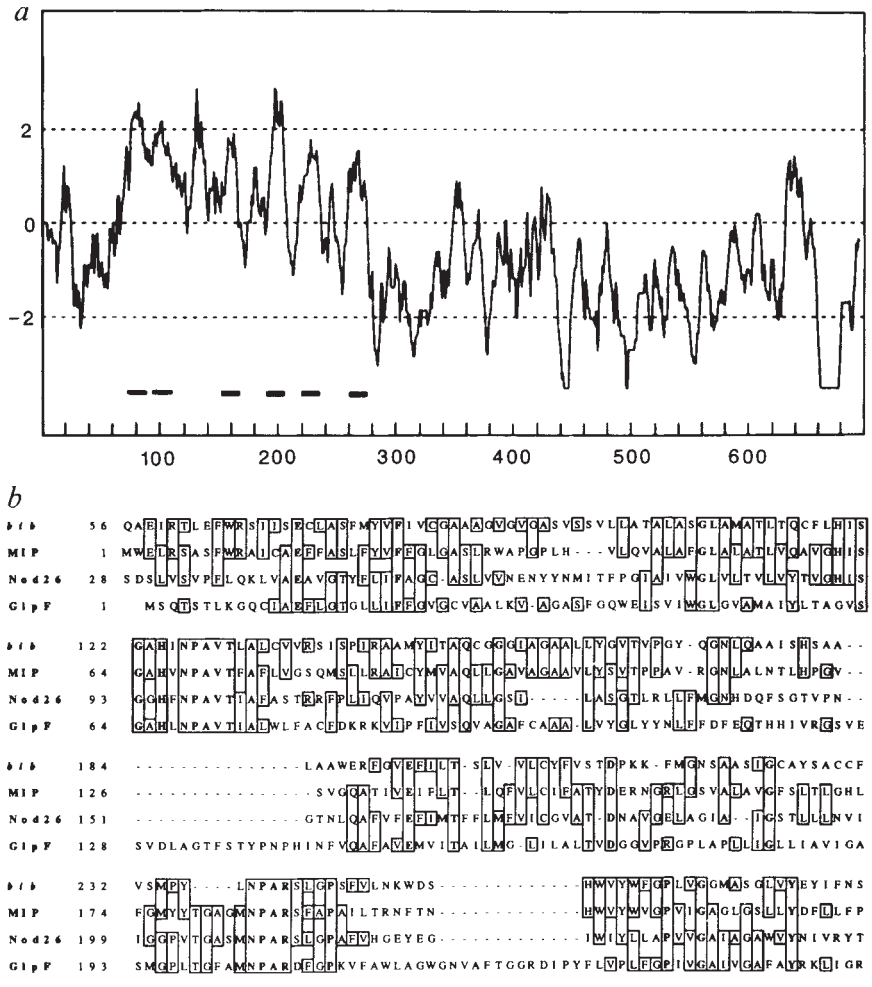

FIG. 4 Characterization of *bib* protein. *a*, Hydropathy plot of the predicted *bib* protein. The Kyte and Doolittle program was run over 9-amino-acid stretches (the plot was similar when run with a window of 19 residues). The third and eighth hydrophobic peaks are not predicted by other programs as transmembrane domains, perhaps because there are several β-turns predicted in each region. The other six hydrophobic peaks indicated by the bars are boxed in Fig. 3 as the putative transmembrane domains. *b*, Sequence alignment of *bib*, MIP, nod 26 and glpF. The predicted 700-residue *bib* protein is longer than MIP (263 residues), nod 26 (271 residues) or glpF. The carboxy-half of *bib* contains many polyglutamine stretches and proline-rich regions and does not have significant sequence similarity with other proteins.

cell-cell interaction. Previous studies have suggested that, except for *bib*, the other known neurogenic genes belong to one pathway[9]. The products of two neurogenic genes, *Notch*[29,30] and *Delta*[31], are transmembrane proteins with epidermal growth factor-like repeats on their extracellular domains, that could directly mediate the intercellular interaction, whereas other genes in this pathway are either upstream (for example *neuralized*, or *mastermind*), or downstream (such as the *Enhancer of split* complex[32,33]) of *Notch* and *Delta*. The *bib* product, however, may mediate intercellular communication in a manner independent of this pathway, as *bib* is the only neurogenic gene that shows no detectable genetic interaction with the other known neurogenic genes[9].

Potentially, *bib* can mediate interaction either between neuroblasts and their surrounding ectodermal cells (the presumptive epidermoblasts), or among the epidermoblasts. In the former case, the *bib* mutant phenotype could result from the disruption of lateral inhibition exerted by the neuroblasts on their neighbouring cells. But if *bib* mediates interaction among epidermoblasts, such interaction could be essential for the presumptive epidermoblasts to stay in the epidermogenesis pathway rather than to take the default pathway of neurogenesis.

Results of a transplantation experiment[34] suggest that *bib* may act on the signalling side of cell-cell interaction. On the basis of the similarity between the *bib* protein and a number of channel proteins, we hypothesize that *bib* functions by allowing the release of certain molecule(s) and thereby sending a signal for an ectodermal cell to become an epidermoblast instead of a neuroblast. □

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Abnormal sexual development in transgenic mice chronically expressing Müllerian inhibiting substance

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MÜLLERIAN inhibiting substance (MIS), also known as anti-Müllerian hormone, is a glycoprotein[1–4] normally secreted by the Sertoli cells of the fetal and adult testis[5,6] and by granulosa cells of the postnatal ovary[7,8]. The production of MIS in the male fetus brings about the regression of the Müllerian ducts, the anlagen of the uterus, oviducts, and upper vagina[9–11]. In addition, purified MIS induces the formation of seminiferous cord-like structures in fetal rat ovaries cultured *in vitro*, suggesting that MIS may influence testicular differentiation[12]. We have produced transgenic mice chronically expressing human MIS under the control of the mouse metallothionein-1 promoter to investigate its role during sexual development. In females, chronic expression led to the inhibition of Müllerian duct differentiation, resulting in a blind vagina and no uterus or oviducts. At birth the ovaries had fewer germ cells than normal; during the next two weeks germ cells were lost and the somatic cells became organized into structures resembling seminiferous tubules. Apparently, these structures degenerate as they are undetectable in adult females. The majority of transgenic males developed normally. But in two lines with the highest levels of MIS expression, some males showed feminization of the external genitalia, impairment of Wolffian duct development, and undescended testes. These results suggest that MIS has several distinct roles in mammalian sexual development.

We generated nine founder transgenic mice (two females and seven males) carrying a metallothionein-1 (MT)-MIS fusion gene (Fig. 1) by pronuclear injection of fertilized mouse eggs[13]. The MT promoter can direct expression of heterologous genes to a variety of fetal and adult tissues in transgenic mice[14]. Seven of the founders, including both females, had circulating levels

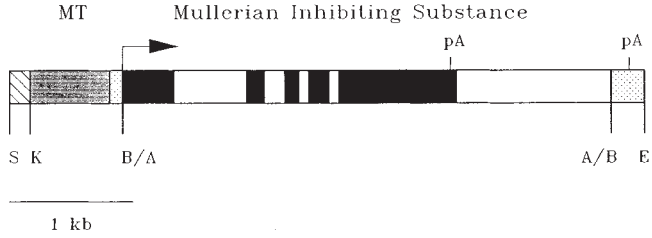

FIG. 1 Map of MT-MIS gene construct used to generate transgenic mice. The construct contains 650 base pairs (bp) of the mouse metallothionein-1 promoter (MT) (heavy shading) fused to the human MIS structural gene (exons, solid boxes; introns and 3′ flanking region, open boxes). Mouse protamine-1 5′ and 3′ untranslated sequences are included (light shading) and 94 bp of simian virus 40 from *Kpn*I to *Sph*I (diagonal shading). S, *Sph*1; K, *Kpn*1; B, *Bam*H1; A, *Afl*II; E, *Eco*R1; *pA, polyadenylation signal.* A 4,164-bp *Afl*II fragment containing the MIS structural gene was isolated from pBG311.hmis (ref. 3) and inserted into the *Bam*H1 site of the MTPr.SVD expression vector. A 5-kbp *Sph*I-*Eco*RI fragment was isolated from vector sequences and microinjected into fertilized mouse eggs as described[13].