molecules. The demonstration of HLA-G expression in cytotrophoblasts identifies a molecule and a gene that could be involved in placental-maternal interaction and can be studied quantitatively and qualitatively in connection with certain types of unexplained infertility and spontaneous abortion.

Note added in proof: In agreement with observations described above, a survey of human tissues detected HLA-G mRNA only in first trimester placental cells and, subsequently, only in extra-embryonic membranes upon completion of gestation (*33*).

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20 October 1989; accepted 8 February 1990

A Promoter That Drives Transgene Expression in Cerebellar Purkinje and Retinal Bipolar Neurons

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A genomic clone encoding the Purkinje cell–specific L7 protein has been isolated and utilized to drive the expression of β-galactosidase in mice. Three independent transgenic lines, germ line transformed with an L7–β-galactosidase fusion gene, exhibit β-galactosidase expression in both cerebellar Purkinje cells and retinal bipolar neurons. This distribution is the same as that previously determined for the L7 protein by immunohistochemistry. The transgenic murine lines can be used to obtain populations of marked Purkinje and bipolar neurons. Similar L7 promoter constructs can be used to express other foreign genes specifically in these two classes of neurons.

MUCH OF THE PRESENT UNDERstanding of mammalian neural development has relied on the availability of markers that identify specific nerve cell populations. Although such markers are useful for analyzing cell lineage and fate in complex nervous systems, they can also serve as probes with which to elucidate the cellular and transcriptional mechanisms that orchestrate neuroembryogenesis. For example, by examining the expression of a panel of marker genes, it may be possible to define specific genetic regulatory elements and transcription factors that confer cell type specificity. Such regulatory sequences could then be used to drive the expression of exogenous genes that encode proteins that might be involved in, or interfere with, the normal maturation of a particular set of neurons.

The properties of two neural structures, the cerebellum and the retina, make them particularly suitable for an investigation of neurodevelopment. Such properties include their late and well-characterized developmental progression (*1*), their relatively simple and ordered structure (*2*), and the availability of viable murine mutants that affect their development (*3*). This has led us, and others, to identify and characterize molecules that mark specific sets of neurons and glia within these areas (*4*). One of the most restricted markers identified in these studies is L7, which is expressed only in cerebellar

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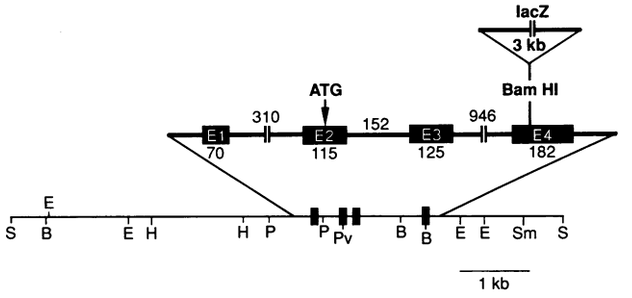

Fig. 1. L7 gene structure and restriction map. The filled boxes represent exons, and the numbers signify lengths in base pairs. The start of translation occurs in exon 2 (E2) as indicated by ATG, and the lacZ fragment was inserted into the Bam HI restriction site of the fourth exon (*8*). The filled boxes indicate exon positions, and the letters indicate restriction sites. S, Sal I; B, Bam HI; E, Eco RI; H, Hind III; P, Pst I; Pv, Pvu I; and Sm, Sma I.

Purkinje cells (*5*) and retinal rod bipolar neurons (*6*).

Here we report the faithful expression of an L7–β-galactosidase (L7-βGal) fusion gene in cerebellar Purkinje neurons and retinal bipolar neurons of the mouse. This result indicates that a similar strategy could be used to express other genes of interest in these cells. Furthermore, by using the founder mice, we have established homozygous transgenic lines that represent unambiguous marker strains for the further analysis of Purkinje and bipolar cell development and function.

An 8-kb mouse genomic DNA fragment was isolated by hybridization to an L7 cDNA probe (*5*) and partially sequenced. The exon-intron structure and restriction map of this clone are shown in Fig. 1. The transcribed region of the L7 gene comprises 1.9 kb and is interrupted by three introns. The genomic clone also includes 4 kb upstream of the start of transcription, and 2 kb downstream of the polyadenylation signal. To make the construct for injection into mouse embryos, a 3-kb βGal fragment (*7*) was inserted, in frame, into the Bam HI site of the fourth exon (*8*). The resultant fusion gene is predicted to produce a βGal protein with 80 amino acids of L7 fused at the amino terminal end. The entire 8-kb L7 genomic fragment was used so as not to inadvertently remove any essential control elements and also to leave the L7 gene structure as little changed as possible. Since we had also sequenced a rat L7 genomic clone and had found extensive sequence identity to mouse in the 5′ flanking region and in the first two introns (*9*), important regulatory elements might reside well downstream from the promoter.

The purified 11-kb L7-βGal fusion gene was injected into the paternal pronucleus of fertilized mouse eggs by standard procedures (*10*). Three independent founder animals (SZ-1, JM-2, and JM-13) were identified by Southern blot analysis of tail DNA by the use of the 3-kb βGal insert as probe. All three founders were male and were mated with normal (nontransgenic CD-1) females to produce F_1 offspring. The germ line of JM-13 was completely transformed, whereas those of SZ-1 and JM-2 were approximately 10% transformed. All three transgenic lines in the heterozygous state showed normal viability, without evidence of any abnormal behavioral patterns.

To assay for the expression of the fusion gene, frozen sections of brain were prepared from F_1 animals of each of the three identified transgenic lines and processed for βGal activity with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (*11*). High levels of βGal expression were detected in cerebellum and retina of all three transgenic lines (Figs. 2 and 3).

Within the cerebellum, only the Purkinje neurons expressed the transgene. The βGal product was found throughout the soma, dendritic tree, and axon (Fig. 2). Histochemically reactive tendril-like fibers were also observed in the region of the deep cerebellar nuclei and the lateral vestibular nuclei. These fibers represent the axonal processes of cerebellar Purkinje cells that project to these locations. All of the Purkinje cells express the transgene, although there are differences in the level of βGal reaction product from cell to cell (Fig. 2). However, this patchy distribution is not evident when two distinct L7 polyclonal antisera are used to perform immunocytochemistry (*5, 12*). This quantitative discrepancy could be attributed to cell-specific variations in βGal stability or variations in levels of expression of the transgene (*13*).

Within the retina, the only cell type that expresses βGal was the bipolar neuron. Like

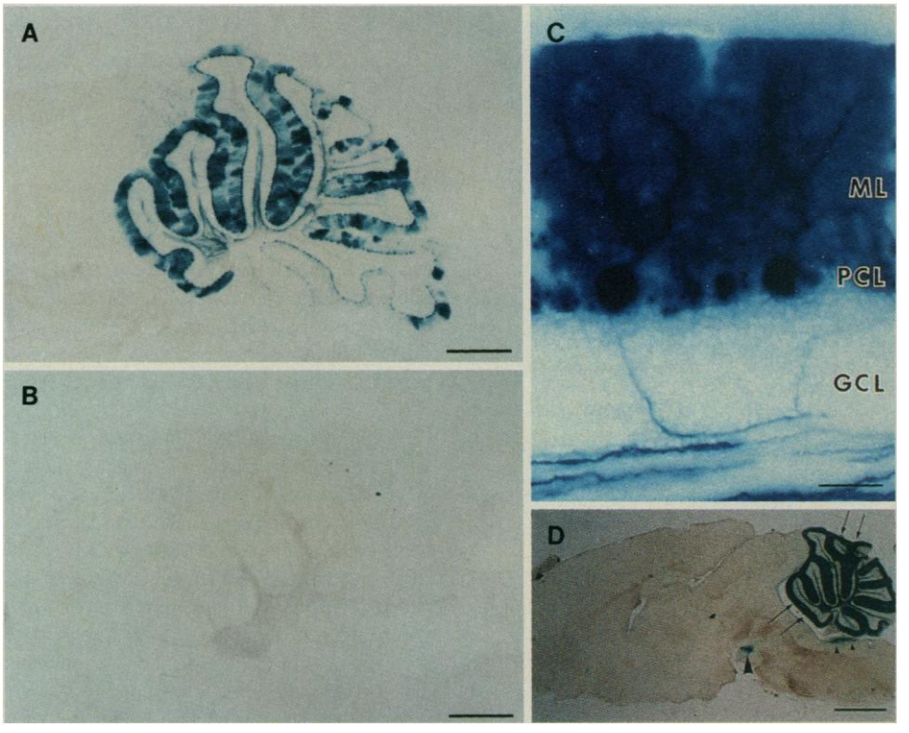

Fig. 2. Expression of βGal in the brain of transgenic mice. (**A**) Photomicrograph demonstrating X-Gal staining in cerebellar cortex in a 15-μm-thick section from heterozygous F_1 transgenic offspring. (**B**) Control section through the cerebellum of a nontransgenic F_1 littermate of animal shown in (A), also processed with the substrate X-Gal. Scale bar for (A) and (B), 550 μm. (**C**) Two Purkinje cells are shown in the cerebellar cortex after X-Gal staining. Blue reaction product is seen throughout the soma, the dendritic tree in the outermost molecular layer (ML), and the axon traversing the granular cell layer (GCL). PCL, Purkinje cell layer. Scale bar, 35 μm. (**D**) Low-power photomicrograph showing X-Gal staining in a 25-μm-thick midline brain section from an F_1 transgenic mouse. Three areas contain cells or fibers that express the L7-βGal transgene: (i) the cerebellar Purkinje cell somata and dendritic trees situated in the outermost molecular layer (arrows), (ii) the lateral vestibular nucleus (small arrowheads), and (iii) the interpeduncular nucleus (large arrowhead). Scale bar, 1000 μm. Methods are described in (*11*).

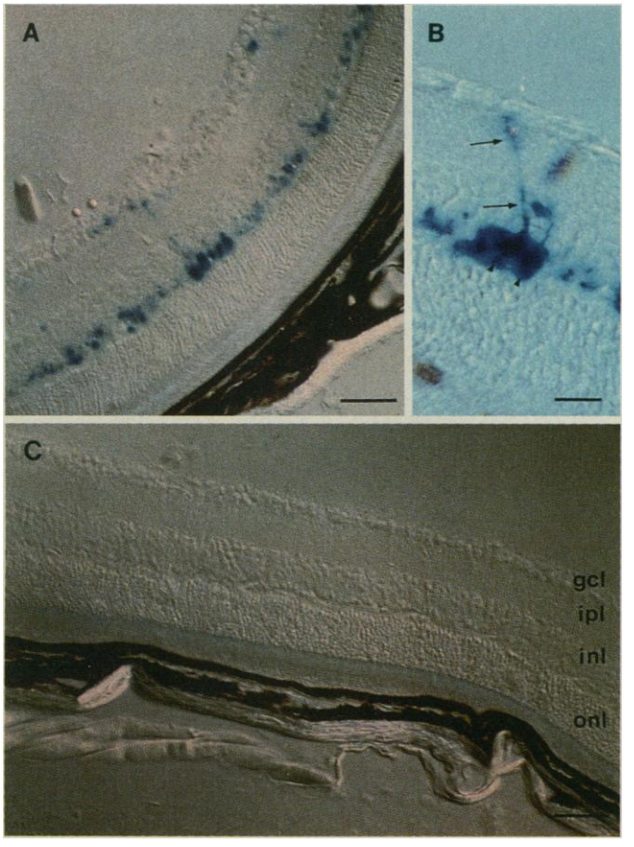

Fig. 3. Expression of βGal in the transgenic retina. (**A**) The X-Gal staining reveals that a subset of bipolar cells, situated in the inner nuclear layer, expresses the transgene. A single bipolar cell axon can be seen extending to the ganglion cell layer. Nomarski optics. Scale bar, 75 μm. (**B**) X-Gal reaction product is present in the axon (arrows), soma, and dendrites (arrowheads) of the bipolar neurons. Scale bar, 80 μm. (**C**) Control retina. No specific X-Gal staining is seen in any layer of the retina. Abbreviations: gcl, ganglion cell layer; ipl, inner plexiform layer; inl, inner nuclear layer; and onl, outer nuclear layer. Nomarski optics. Scale bar, 75 μm.

the cerebellar Purkinje cell, the entire bipolar cell, including the soma, dendrite, and axon, contained the reaction product (Fig. 3). The fibers of these retinal cells projected through the inner plexiform layer to the ganglion cell layer. The distribution of the histochemically reactive cells in the retina suggests that not all of the bipolar cells express βGal, much like the results obtained with immunohistochemical staining with antibodies to L7 protein (*14*). At this time, however, it is not known if a particular subset of bipolar cells (for example, rod versus cone, long versus short cells) expresses the transgene.

One region outside of the cerebellum and retina that expressed the L7-βGal transgene in at least two of the three transgenic lines is the interpeduncular nucleus (IPN) (Fig. 2D). This site has not been previously identified as a site of L7 production by standard immunocytochemical procedures (*14*), and it is unclear whether this IPN staining is due to a greater sensitivity of the βGal assay or aberrant expression from the L7 promoter. It should be emphasized that the level of expression of βGal in the IPN is much lower than in cerebellar Purkinje cells and retinal bipolar neurons. Since all three lines appear identical in their pattern of transgene expression, correct transcriptional control of the L7 gene, as constituted in this construction, must be relatively insensitive to the site of genomic integration.

Strain-specific markers have been useful in mouse chimera analysis as a means of identifying mutant versus wild-type cell contributions (*15, 16*). The L7-βGal transgenic strain will be useful as a source of unambiguously marked Purkinje cells. The high sensitivity of the βGal assay would allow easy detection of these cells and thus would bypass many of the problems associated with other markers (*17*). Furthermore, this strain might be used as a source of Purkinje and bipolar cells for transplantation and cell culture studies (*18*).

By progressively deleting the L7 promoter, the DNA elements required for accurate cell- and stage-specific expression of the L7 gene can be identified. This will provide us with the means for characterizing Purkinje cell trans-activating factors. Additionally, the L7 promoter can be used to direct expression of any foreign protein specifically to Purkinje cells and bipolar neurons. With appropriate constructs one could produce novel degeneration mutants or interfere with the terminal phase of differentiation of these neurons.

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8. The 8-kb L7 genomic clone was isolated from a mouse EMBL-3 library prepared by partial Mbo I digestion of liver genomic DNA (Clontech) by hybridization to an L7 cDNA (*5*). About 2.5 kb, spanning the entire coding region, was subcloned and sequenced. In addition, the entire 8-kb genomic DNA fragment was restriction mapped. The fragment was inserted into the Bam HI site of EMBL-3, but was excised with Sal I. Consequently, the L7 fragment has about 100 bp of λ DNA on each end. The L7-βGal construct was prepared as follows: The 8-kb L7 fragment was subcloned into pGEM-3 (Promega) and this plasmid was partially restricted with Bam HI. The linearized form was purified by electroelution from a 0.8% agarose gel [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)]. A 3-kb Bam HI fragment from pMC1871 (*7*) containing the βGal sequence was ligated randomly into the partially restricted L7 plasmid, and the correct construct was identified by hybridization to a synthetic oligonucleotide, the sequence of which spanned the overlap between the βGal and the L7 gene. Both the 5′ and 3′ junctions between βGal and L7 were subcloned and sequenced to confirm the construct.
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11. Mice were injected intraperitoneally with an overdose of sodium pentobarbitol. After the animals were deeply anesthetized, they were perfused transcardially with 0.9% normal saline followed by 2% paraformaldehyde in 0.1*M* Pipes (1,4-piperazinediethanesulfonic acid) (*p*H 6.9) containing $MgCl_2$ (2 m*M*) and EGTA (2 m*M*). Brains and eyes were removed and then fixed in fresh perfusion solution for 1 hour, after which the tissue was transferred overnight to a 30% sucrose in 1× phosphate-buffered saline solution (PBS) (*p*H 7.3) containing 1 m*M* $MgCl_2$. Frozen sections were cut at 10 to 30 μm at −22°C and mounted onto 0.5% gelatin–coated slides and reacted with X-Gal (1 mg/ml) (Boehringer Mannheim) in dimethyl sulfoxide (40 mg/ml). This was added to a solution containing 5 m*M* potassium ferricyanide, 5 m*M* potassium ferrocyanide, 2 m*M* $MgCl_2$, 0.02% (w/v) NP-40, and 0.01% (w/v) sodium deoxycholate. After staining, slides were rinsed in 1× PBS and cover slips were applied with glycerol-gelatin (Sigma).
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19. We thank T. Curran and C. Stewart for critical reading of the manuscript and our long-time collaborator, E. Mugnaini, for valuable advice. Partially supported by National Research Service Award 1 F32 NS 08680-01 (to R.J.S.).

21 November 1989; accepted 6 February 1990

Unusual Topogenic Sequence Directs Prion Protein Biogenesis

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Biosynthetic studies of the prion protein (PrP) have shown that two forms of different topology can be generated from the same pool of nascent chains in cell-free translation systems supplemented with microsomal membranes. A transmembrane form is the predominant product generated in wheat germ (WG) extracts, whereas a completely translocated (secretory) form is the major product synthesized in rabbit reticulocyte lysates (RRL). An unusual topogenic sequence within PrP is now shown to direct this system-dependent difference. The actions of this topogenic sequence were independent of on-going translation and could be conferred to heterologous proteins by the engineering of a discrete set of codons. System-dependent topology conferred by addition of RRL to WG translation products suggests that this sequence interacts with one or more cytosolic factors.

SECRETORY AND TRANSMEMBRANE proteins acquire their orientation with respect to the membrane of the endoplasmic reticulum (ER) in a manner that is usually both predictable and absolute (*1*). This reflects the action of discrete regions within such nascent proteins, termed signal and stop-transfer sequences, that initiate and terminate translocation across the ER (*2*). Studies in eukaryotic cell-free systems have revealed that at least some topogenic sequence actions are mediated by receptor proteins (*3–6*). The subcellular components that function in such systems appear conserved. Thus, mRNA from any tissue or species can be translated in cytosolic extracts of either WG or RRL (*7, 8*). When these translation systems are supplemented with microsomal membranes derived from the ER, the newly synthesized protein achieves a topology identical to that observed in the ER in vivo (*9*). Topology can be assayed by means of proteases to distinguish domains protected by the lipid bilayer (*10, 11*).

An exception to this conservation of components occurs during biogenesis of the prion protein (PrP). PrP, a brain glycoprotein, exists in two isoforms: (i) PrP^C, which is found in normal brain and is expressed at specific times during development (*12, 13*), and (ii) PrP^{Sc}, which is a component of the infectious agent causing scrapie (a degenerative neurologic disease of animals that is related to several diseases of humans) (*14*). The primary amino acid sequences of PrP^C and PrP^{Sc} are identical (*14, 15*). Both isoforms contain a phosphatidylinositol glycolipid anchor at their COOH-terminals that is cleavable by a phosphatidylinositol-specific phospholipase C (PIPLC) (*16*). However, only PrP^C is released from cells when treated with PIPLC (*17*). These findings are consistent with a difference between the two isoforms in either subcellular localization or transmembrane orientation.

Likewise, PrP synthesized in vitro displays two system-dependent topologies. When a cloned cDNA encoding PrP is expressed by transcription-linked translation in WG, a transmembrane form predominates (*18*). When the same transcript is translated in RRL, the predominant product is a completely translocated (that is, secretory) protein (*19*). Some feature or process occurring in the cytosolic fraction seems to recognize information within nascent PrP to direct transmembrane or secretory forms.

The characteristic transmembrane form of PrP observed in WG spans the bilayer twice, such that both the NH_2- and COOH-terminal domains are within the lumen of the ER (Fig. 1A) (*18*). The first membrane-spanning domain synthesized, TM1, is approximately 90 amino acid residues from the NH_2-terminal–cleaved signal sequence. Therefore it seemed plausible that the difference in PrP topology observed in WG versus RRL was a reflection of a difference in the ability of these systems to stop translocation of the chain at TM1. One explanation for this difference might be that the rate of chain elongation (and hence the rate of chain translocation) is unequal in WG versus RRL, thereby influencing the extent of stop transfer at TM1.

Thus, the first step toward analysis of the molecular basis for the alternate fates in PrP biogenesis was to uncouple translocation of the chain from protein synthesis. Normally, translocation occurs only while peptide chains are in the process of being synthesized (co-translationally). By truncation of a cDNA within the coding region, it becomes possible to generate mRNA lacking a termination codon. In this case, the initial engaged ribosome will be unable to release the nascent peptide chain. These nascent chains remain translocation-competent even in the absence of further chain elongation (*20*).

PrP cDNA was truncated at a Hinc II site 74 codons 5′ to the termination codon. Transcription-linked translation of this DNA (PrP/HcII) in either WG (Fig. 2) or RRL (Fig. 3) in the absence of microsomal membranes resulted in a nascent chain–ribosome complex in which PrP had been synthesized from the NH_2-terminal through TM1. The translation product was presented to the membrane either co-translationally (with membranes present during its synthesis) or posttranslationally (with membranes added after completion of synthesis and in the presence of translation inhibitors). As expected for translocation-competent chains, cleavage of the signal sequence occurred in the presence of membranes either co- or posttranslationally in both WG and RRL (Figs. 2 and 3). Unlike native PrP (*18, 19*), these truncated products were not glycosylated, since the recipient asparagine residues for N-linked carbohydrates were lost after truncation. Glycosylation has no influence on generation of either form of PrP, as shown by use of a tripeptide inhibitor of N-linked glycosylation in both WG and RRL (*21*).

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