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Swaying Is a Mutant Allele of the Proto-Oncogene *Wnt*-1

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Summary

Mice homozygous for the recessive mutation *swaying* (*sw*) are characterized by ataxia and hypertonia, attributed to the malformation of anterior regions of the cerebellum. We show that *sw* is a deletion of a single base pair from the proto-oncogene *Wnt*-1. The deletion is predicted to cause premature termination of translation, eliminating the carboxy-terminal half of the *Wnt*-1 protein. Histological examination shows that *sw* is phenotypically identical to a previously described *wnt*-1 mutation introduced into mice by gene targeting. Although both mutations in *Wnt*-1 disrupt primarily the development of the anterior cerebellum, they also exhibit a variability in expressivity such that rostrally adjacent structures in the midbrain and caudally adjacent structures in the posterior cerebellum can also be affected.

Introduction

Processes as seemingly different as insect segmentation and vertebrate neural development require the activity of genes from the *Wnt* family (Nusse et al., 1991). This family of genes is found in genera throughout the animal kingdom, including Caenorhabditis (Kamb et al., 1989), Drosophila (Cabrera et al., 1987; Rijsewijk et al., 1987), Xenopus (Noordermeer et al., 1989), and Homo (Van't Veer et al., 1984). Members of the *Wnt* family are related by sequence similarity and share a signal sequence motif, potential glycosylation sites, and a highly conserved array of 21 cysteine residues concentrated toward the carboxyl half of the protein. In Drosophila, the *Wnt* gene family is represented by the segment polarity gene *wingless* (*wg*), which is required for pattern formation within each embryonic segmental unit as well as for the normal development of wing and leg imaginal disks (Morata and Lawrence, 1977; Baker, 1987; Martinez-Arias et al., 1988). The *wg* gene acts in a non–cell autonomous fashion, presumably via a secreted protein that stimulates the growth and/or differentiation of adjacent cells (Morata and Lawrence, 1977; Van den Heuvel et al., 1989). In mice there are at least ten expressed members of this family (McMahon and McMahon, 1989; Gavin et al., 1990; Roelink et al., 1990; Roelink and Nusse, 1991). The protein coded by the murine *Wnt*-1 gene is secreted by cultured cells (Brown et al., 1987; Papkoff et al., 1987; Papkoff, 1989; Bradley and Brown, 1990; Papkoff and Schryver, 1990) and, by analogy with its Drosophila counterpart, is suspected to act inductively upon neighboring cells (Burgess, 1988; Nusse, 1988).

Wnt-1 was originally identified in mice as the proto-oncogene *int*-1 (Nusse and Varmus 1982). Integration of proviral DNA from mouse mammary tumor virus in the vicinity of this gene is associated with mammary carcinogenesis, presumably the result of ectopic expression of *Wnt*-1 in mammary tissue. Normally, the *Wnt*-1 gene is not expressed in mammary tissue, but in adult testes (Jakobovits et al., 1986; Shackleford and Varmus, 1987) and in the midgestation embryonic neural tube (Shackleford and Varmus, 1987; Wilkinson et al., 1987). Analysis of mice homozygous for targeted mutations in *Wnt*-1 demonstrated a role for *Wnt*-1 in neurogenesis (McMahon and Bradley, 1990; Thomas and Capecchi, 1990). Although *Wnt*-1 mRNA is detectable at numerous sites along the entire length of the neural tube, the effect of the *wnt*-1$^-$ mutation is restricted to a small subset of these sites and is most strongly manifest as an underdevelopment of the midbrain and cerebellum.

Normal development of the vertebrate cerebellum involves a series of proliferation, differentiation, and cell migration steps that begins in midgestation embryogenesis and proceeds until well past the birth of the organism (Jacobsen, 1978). A number of the morphological changes and cell lineage relationships involved in cerebellar genesis have been described (Altman and Bayer, 1985a, 1985b, 1985c; Martinez and Alvarado-Mallart, 1989; Hallonet et al., 1990), and to date, mutational analysis has identified approximately 20 murine genes involved either in cerebellar development or in the maintenance of adult cerebellar integrity (Sidman, 1983; Mullen, 1984; Lyon and Searle, 1989). One of these mutations is the autosomal recessive allele *swaying* (*sw*) (Lane, 1967). The *sw* phenotype is very similar to that described for an adult mouse homozygous for the targeted mutation in *Wnt*-1 (Thomas and Capecchi, 1990). Mutants of both types are characterized by ataxia and lack the anterior half of their cerebellum. Moreover, the *Wnt*-1 gene has been localized to mouse chromosome 15, approximately 45 cM from the centromere, a site quite close to *sw* (Lane, 1970; Nusse et al., 1984; Meruelo et al., 1987). In this report, we show that the *sw* mutation is due to a frameshift mutation in the *Wnt*-1 structural gene and that mice homozygous for *sw* are indistinguishable in phenotype from mice homozygous for the targeted *wnt*-1$^-$ allele or from the compound heterozygote.

Results

The *Wnt*-1^{neo} and *sw* Mutations Are Phenotypically Similar

The targeted *Wnt*-1 allele *wnt*-1^{neo} was created by inserting

a 1 kb bacterial *neo*^{r} gene into the second protein-coding exon of the murine *Wnt*-1 gene (McMahon and Bradley, 1990; Thomas and Capecchi, 1990). The allele used in this study contains a tandem duplication of the mutant *wnt*-1 sequence, the result of the conversion of the original *Wnt*-1 locus by a dimer of the gene targeting vector. The insertion site of the *neo*^{r} gene lies between codons 54 and 55 of the 370 amino acid *Wnt*-1 protein and is likely to result in a null mutation. Biological activity of *Wnt*-1 is thought to require most, if not all, of the amino acid residues of the native protein. Not only are the highly conserved functional motifs, such as the signal sequence, glycosylation sites, and cysteine residues, dispersed throughout the length of the protein, but no mouse mammary tumor virus proviral insertion events (identifiable by ectopic expression of the wild-type *Wnt*-1 protein) have ever been found within the protein-coding domains of this gene. Despite the assessment of *wnt*-1^{neo} as a loss-of-function mutation, the phenotype caused by this allele varies in expressivity (Thomas and Capecchi, 1990).

This variability is manifested in two ways: in lifespan, ranging from prenatal death to survival to adulthood, and in the quantity of cerebellar and midbrain tissue at a given stage of development. At one extreme, *wnt*-1^{neo} homozygotes lack all evidence of cerebellar primordium, and large portions of the midbrain appear defective (McMahon and Bradley, 1990; Thomas and Capecchi, 1990). At the other extreme, the *wnt*-1^{neo}/*wnt*-1^{neo} mice that survive to weaning age exhibit extensive cerebellar development. This is illustrated in Figure 1 by a comparison of sagittal sections of a cerebellum from a 4-week-old *wnt*-1^{+}/*wnt*-1^{neo} mouse (Figure 1a) with those from two *wnt*-1^{neo}/*wnt*-1^{neo} animals (Figures 1b and 1c). Although neither *wnt*-1^{-} cerebellum shows much folial development anterior to the primary fissure, posterior foliation is quite extensive. Both brains also show additional abnormalities—the presence of detached layers of granule and Purkinje cells (see arrowheads, Figures 1b and 1c) and a lack of midline cerebellar fusion (not visible in sagittal section).

The *wnt*-1^{-} cerebella depicted in Figure 1 are remarkably similar in structure to those described for *sw* mice. *sw* was originally identified as a recessive mutation that causes ataxic behavior (Lane, 1967). Analyses of the brains from *sw* mice show that anterior folia and vermis of the cerebellum are absent, while posterior structures appear normal. In addition, the *sw* cerebellum is not joined at the midline, the posterior cerebellar vermis is fused with the white matter of the midbrain, and islands of cerebellar cellular layers are found in the midbrain (Sidman, 1968).

Sw Is a Mutant Allele of *Wnt*-1

To test the hypothesis that *sw* is due to a defect in the *Wnt*-1 gene, a heterozygous, +/*sw* male was mated with five females heterozygous for the targeted *wnt*-1 allele *wnt*-1^{neo}. Of 34 newborn mice from these intercross litters, 7 died shortly after birth. All seven showed a brain morphology (Figure 2c) virtually identical to that found in *wnt*-1^{neo}/*wnt*-1^{neo} homozygotes (Figure 2b): a reduction in the size of the tectum and the virtual absence of a defined cerebellum compared with the heterozygous control (Figure 2a). The

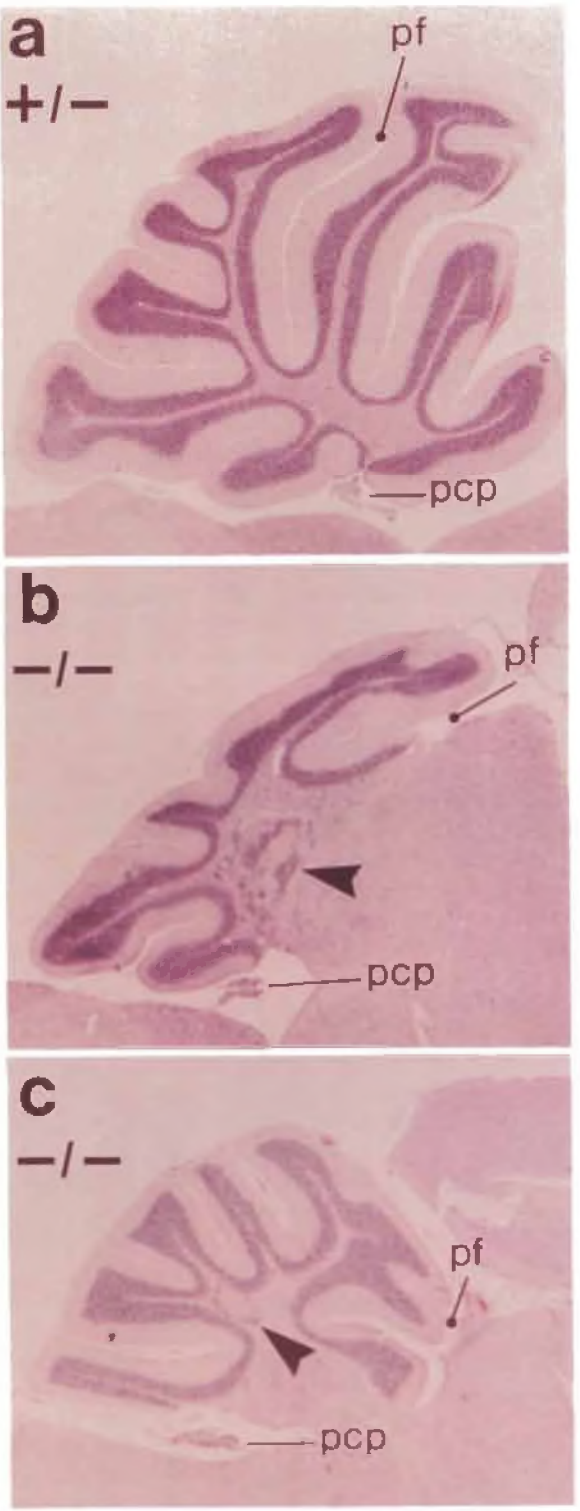

Figure 1. Cerebella from *wnt*-1^{-} Mice

Brains were prepared and sectioned as described (Thomas and Capecchi, 1990). (a) Normal cerebellum from a *wnt*-1^{+}/*wnt*-1^{neo} mouse. (b and c) Cerebella from *wnt*-1^{neo}/*wnt*-1^{neo} mice. +, *wnt*-1^{+}; −, *wnt*-1^{neo}; pcp, posterior choroid plexus; pf, primary fissure; arrowheads indicate detached granular and Purkinje layers. The position of the primary fissure was assigned following examination of serial sections. All three examples are from 4-week-old females. The field is 2.5 × 4 mm.

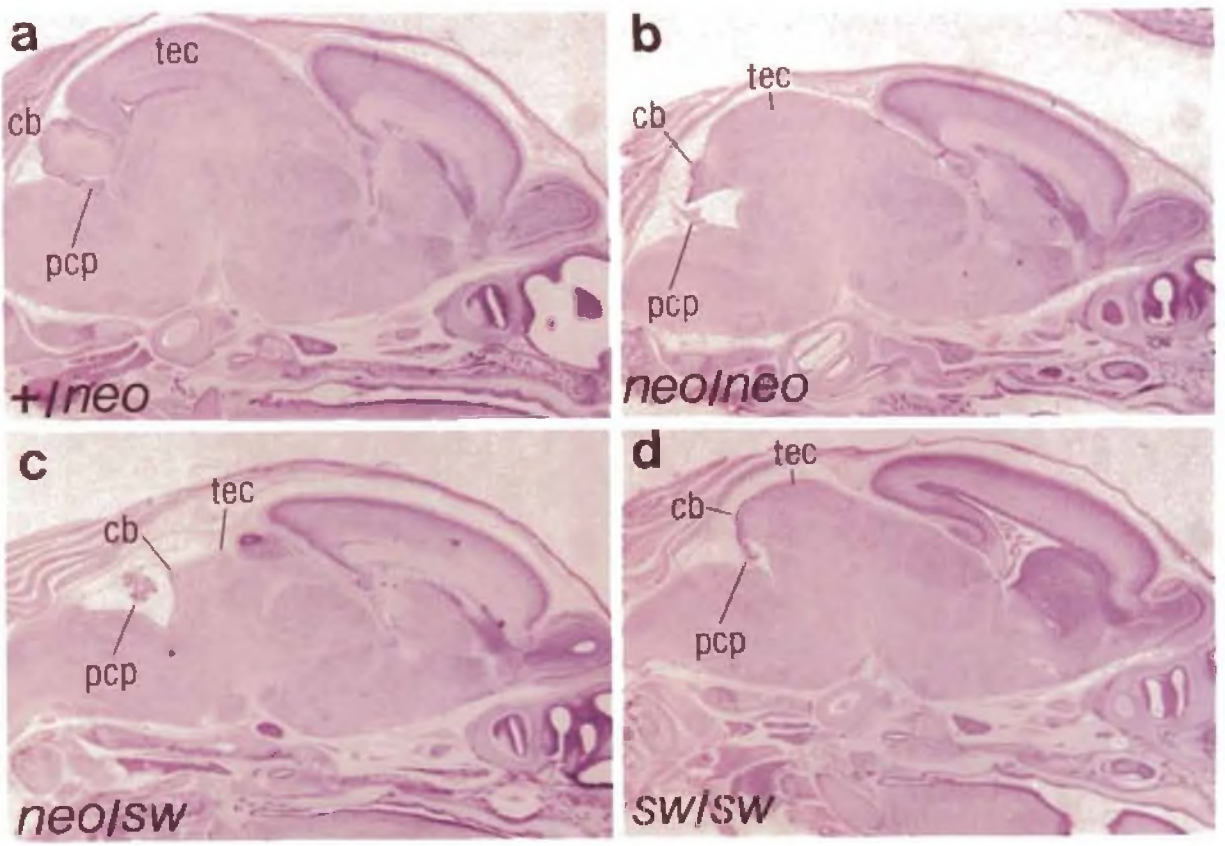

Figure 2. Midsagittal Sections of Newborns

Heads were removed from newborn individuals, fixed in Bouin's reagent, embedded in paraffin, sectioned (8 μm), and stained with hematoxylin and eosin. Genotypes were determined by Southern transfer analysis of DNA purified from tails. +, *wnt*-1^{+}; neo, *wnt*-1^{neo}; sw, *wnt*-1^{sw}; cb, cerebellum; tec, tectum; pcp, posterior choroid plexus. The field is 4 × 7 mm.

phenotypic similarity between the intercross progeny and the *wnt*-1^{neo}/*wnt*-1^{neo} homozygotes and the frequency of its inheritance (21%) provided strong evidence that the *sw* mutation affects the *Wnt*-1 locus.

To determine the molecular basis of the *sw* mutation, DNA surrounding and including both *wnt*-1 alleles from one of the compound heterozygous mice was analyzed at the molecular level. Genomic DNA was purified from a phenotypic *wnt*-1^{-} individual, cloned into a λ phage vector, and screened with a *Wnt*-1-specific probe. Two classes of phage clones were identified by this protocol. One class contained a 13.5 kb BglII fragment derived from the *wnt*-1^{neo} allele; the other contained a 12.5 kb BglII fragment, presumably originating from the +/*sw* parent.

The *sw* mutation was identified by sequencing all four exons, all intron–exon boundaries, and the 5′ untranslated regions of the *Wnt*-1 DNA in the 12.5 kb BglII fragment (Figure 3a) and comparing this sequence with that from the wild-type gene (van Ooyen and Nusse, 1984; Fung et al., 1985; Nusse et al., 1990). A single deviation from the published sequence of *Wnt*-1 was found: the deletion of 1 guanosine residue from a run of 4 consecutive guanosines in the coding strand beginning at genomic nucleotide 1888. The deletion was reaffirmed by sequencing the appropriate regions of the *sw* and the wild-type alleles in parallel (Figure 3b). Henceforth we will refer to this mutation in *Wnt*-1 as *wnt*-1^{sw}.

The *wnt*-1^{sw} mutation is predicted to generate two restriction endonuclease polymorphisms that permit easy detection of this allele. Deletion of the guanosine residue simultaneously removes a BsaJI site (CCN_2GG) and creates a BslI site (CCN_7GG). As a result of the latter change, a 254 bp BslI fragment found in *Wnt*-1^{+} DNA should be replaced by two BslI fragments of 178 bp and 86 bp in *wnt*-1^{sw} DNA. When DNA from the progeny of a *wnt*-1^{+}/*wnt*-1^{sw} × *wnt*-1^{+}/*wnt*-1^{sw} cross is genotyped by BslI digestion and Southern transfer analysis, all three predicted genotypes are detectable (Figure 3c).

The predicted consequence of the deletion found in *wnt*-1^{sw} is depicted in Figure 3d. The new reading frame of the *Wnt*-1 gene, beginning at codon 189, will terminate at a TGA stop codon ten codons downstream from the deletion. As a result, the *wnt*-1^{sw} protein will contain only the amino-terminal 188 of the 370 wild-type *Wnt*-1 amino acids. Among the deleted amino acids are 16 cysteine residues predicted to play critical roles in the biological activity of the native protein (van Ooyen and Nusse, 1984; Fung et al., 1985; McMahon and Moon, 1989). Such an

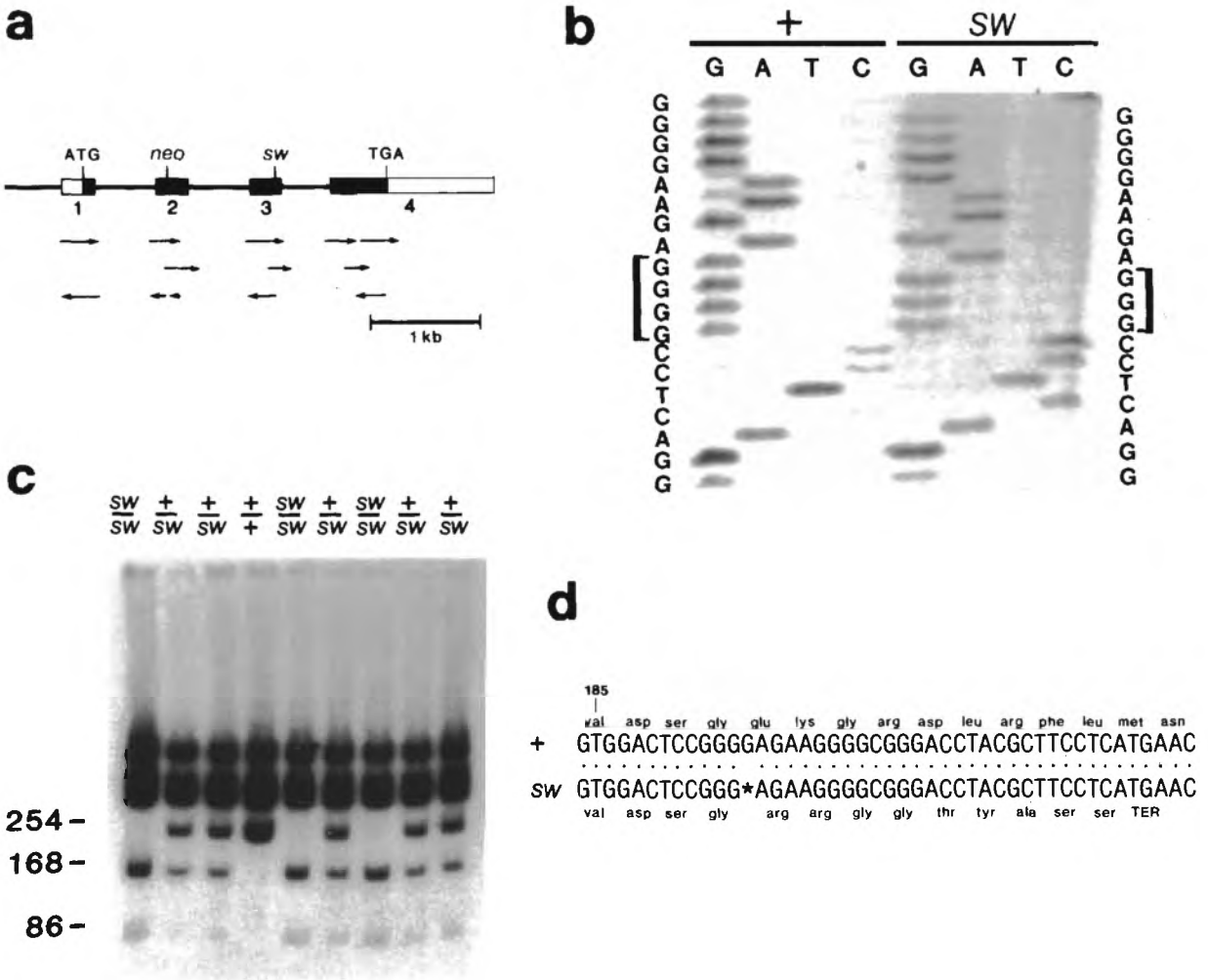

Figure 3. Molecular Analysis of *wnt*-1^{sw}

(a) Sequencing strategy for the *Wnt*-1 locus. Thin lines represent introns; closed boxes represent protein-coding exons; open boxes represent untranslated regions; exons are numbered; ATG and TGA signify the start and stop of translation respectively; neo indicates the site of the *neo* insertion in *wnt*-1^{neo}; *sw* indicates the position of the *sw* mutation. Arrows beneath the *wnt*-1 map show the direction and extent of the DNA sequence analysis of the *wnt*-1^{sw} allele.

(b) Sequence of *wnt*-1^{+} and *wnt*-1^{sw}. DNA from the *wnt*-1 locus was sequenced as described in Experimental Procedures. Shown is a polyacrylamide gel analysis of DNA corresponding to genomic nucleotides 1881–1899 from the coding strand. +, DNA from *wnt*-1^{+}; *sw*, DNA from *wnt*-1^{sw}.

(c) Restriction endonuclease identification of *sw*. DNA was extracted from tails of newborn mice produced from a *wnt*-1^{+}/*wnt*-1^{sw} × *wnt*-1^{+}/*wnt*-1^{sw} cross. Following digestion with BsII, DNA was electrophoresed through polyacrylamide, transferred to a membrane, and probed for *Wnt*-1 sequences. Size of DNA hybridizing to the probe is indicated in base pairs to the left. Above each lane are the deduced genotypes. +, *wnt*-1^{+}; *sw*, *wnt*-1^{sw}.

(d) Consequence of the *sw* mutation. DNA and predicted amino acid sequences are from the *wnt*-1^{+} and *wnt*-1^{sw} alleles, extending from codons 185–199. Asterisk shows the position of the G deletion in the *sw* allele. TER: termination codon, TGA.

extreme truncation makes it likely that *wnt*-1^{sw} represents a severe, if not complete, loss-of-function mutation of *Wnt*-1.

Both *wnt*-1 Mutations Show Variability in Expressivity

The assessment of *sw* as a null allele of *Wnt*-1 is somewhat surprising if one compares the published phenotype of *sw* mice with that of the *wnt*-1^{neo}/*wnt*-1^{neo} homozygotes. The former is described as a condition of adult mice plagued with ataxia (Lane, 1967; Sidman, 1968), whereas the latter generally results in neonatal death (McMahon and Bradley, 1990; Thomas and Capecchi, 1990). One interpretation of these phenotypes is that *sw* represents a less severe mutation than does *wnt*-1^{neo}. Alternatively, the described *sw* phenotype may represent one phenotypic extreme of the variable expressivity described for *wnt*-1^{neo} homozygotes (Thomas and Capecchi, 1990).

To compare directly the phenotypic effects of the two mutant alleles, inter- and intra-allelic crosses were performed between mice heterozygous for either *wnt*-1^{neo} or *wnt*-1^{sw}. Some litters were observed undisturbed to determine the effect of the mutations on viability, while the progeny from others were examined histologically. From 15

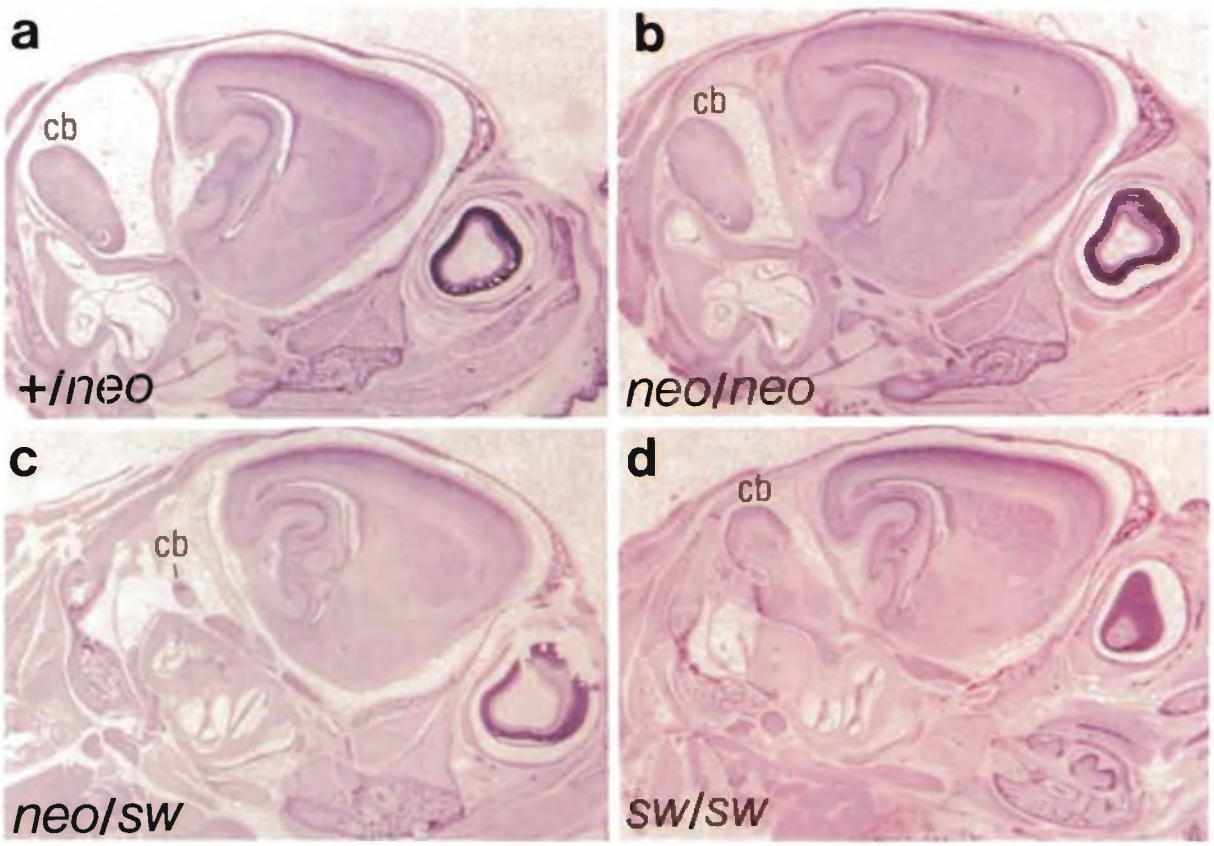

Figure 4. Parasagittal Sections of Newborns

Sections (8 μm) are from the same brains prepared for Figure 2, but from between 0.9 and 1.0 mm from the midline. cb, cerebellum. Field is 3.5 × 5 mm.

undisturbed litters, five from each of the three possible crosses, all but one mutant, a *wnt*-1^{neo}/*wnt*-1^{neo} female, which survived until weaning, were stillborn or dead within 2 days of birth. Thus, with respect to survivability, there appears to be no difference between the two mutant *wnt*-1 alleles.

The effect of the mutant alleles on brain development was determined by histological comparisons of neonatal *wnt*-1^{-} progeny of the three crosses. Based on morphological criteria, the brains from 13 *wnt*-1^{-} individuals fell roughly into two classes. In one class (8 of 13 mice), significant amounts of cerebellar tissue were detected, and the presence of midbrain structures such as the colliculi was quite obvious. In the other class, the cerebellum was nearly undetectable, and the midbrain was markedly reduced in size. No correlation could be made, however, between these two phenotypic classes and the three *wnt*-1^{-} genotypes.

Examples of the first class are shown in Figures 2 and 4 as mid- and parasagittal brain sections from individuals homozygous for *wnt*-1^{neo} (Figure 2b; Figure 4b) and *wnt*-1^{sw} (Figure 2d; Figure 4d). The two mutant brains, although different in genotype, are quite similar morphologically. At the midline (Figure 2), the cerebellum and the tectum (tec) are reduced in size compared with a heterozygous *wnt*-1^{+}/*wnt*-1^{neo} control (Figure 2a). As sectioning proceeds laterally, however, additional cerebellar tissue is revealed, and at the positions shown in Figure 4, the *wnt*-1^{-} cerebella are indistinguishable from the control.

The presence of distinct cerebellar tissue in the lateral regions was not as evident in 5 of the 13 *wnt*-1^{-} brains examined. An example of this second class, from a *wnt*-1^{neo}/*wnt*-1^{sw} mouse, is shown in sagittal section in Figure 2c and Figure 4c. At the midline (Figure 2c), both cerebellum and tectum are noticeably smaller than the other mutant brains (Figures 2b and 2d), and even in lateral sections, only minor amounts of deeply staining cells (presumably representing the cerebellar granular layers) are detected (Figure 4c). Both classes of *wnt*-1^{-} brain morphologies are found in mice homozygous for both mutant alleles as well as in the compound heterozygote, suggesting strongly that the two alleles are equivalent.

Discussion

We have shown that *sw* is a mutant allele of the *Wnt*-1 locus. The mutation is a deletion of a guanosine residue from the coding strand in the third exon of the *Wnt*-1 gene. This deletion shifts the normal *Wnt*-1 reading frame, resulting in premature translation termination, deleting the

carboxy-terminal 50% of the *Wnt*-1 protein. Such a deletion would be predicted to alter severely, if not abolish, activity of the protein. Comparison of mice homozygous for the *wnt*-1^{sw} allele with mice homozygous for the presumed null allele *wnt*-1^{neo} suggests that this is the case. When examined at the same developmental stage, mice homozygous for either allele (or compound heterozygous mice) are indistinguishable morphologically. In addition, mice of all three mutant genotypes show the same characteristic variations in expressivity.

It has been suggested that this variability in expressivity, originally associated with only one *wnt*-1^{neo} allele (Thomas and Capecchi, 1990), could be the result of instability of the targeted insertion mutation. The *wnt*-1^{neo} allele used in this study does contain a direct repeat of two mutant *wnt*-1 genes, which could presumably recombine with each other. However, because both members of this duplication contain the *neo*^{r} insertion, an intrachromosomal recombination event would serve only to reduce the number of mutant sequences (from two to one) and not to eliminate the mutagenic insertion. The data presented above demonstrate that the *wnt*-1^{sw} allele also varies in expressivity. This suggests that the variation is not due to allele-specific affects and that the previously published nonequivalent phenotypes of the *wnt*-1^{neo} and *wnt*-1^{sw} homozygotes are due to differences in either genetic backgrounds or husbandry protocols used in the original studies. It should be noted that both *wnt*-1$^-$ alleles originated and have been maintained as intercrosses between different inbred strains of mice. Should other loci influence the phenotype of *wnt*-1$^-$ homozygotes, individual variations in expressivity of *wnt*-1 mutations might represent the random assortment of different alleles of these other genes.

The distinction between anterior and posterior cerebellum seen in adult *wnt*-1$^-$ mice is reminiscent of at least three other mouse mutations. *Meander tail* (*mea* [Ross et al., 1990]) and *rostral cerebellar malformation* (*rcm* [Lane et al., 1990]) are recessive alleles characterized by a disorganization of cellular patterning, restricted to the anterior lobes of the cerebellum. Similarly, mice carrying the *leaner* allele (*tg*^{la}) of the *tottering* locus exhibit a degeneration of cortical neurons in the anterior cerebellar lobe (Sidman et al., 1965; Herrup and Wilczynski, 1982) and show abnormal anterior versus posterior regulation of tyrosine hydroxylase expression (Hess and Wilson, 1991). Although the morphology and histology of all these mutants are somewhat different, together they suggest a discrete compartmentalization of the cerebellum not obvious from the examination of wild-type mice.

There is also nongenetic precedent in defining an anterior/posterior cerebellar delineation. In the embryonic rat brain, for example, Purkinje cells fated for anterior regions are distinguished spatially and temporally (with respect to migration) from those destined for posterior regions (Altman and Bayer, 1985c). Transplantation experiments of neural tube tissue between chick and quail have suggested that a similar anterior/posterior division occurs in the avian cerebellum as a consequence of a developmental program (Martinez and Alvarado-Mallart, 1989; Hallonet et al., 1990): cells in anterior regions can be traced to a mesencephalic origin, whereas those in the posterior originate from the metencephalon. If in fact there are two parallel pathways of cerebellar development, it is tempting to speculate that *wnt*-1 mutations exhibit a more profound effect on one, the anterior.

An attractive hypothesis to explain this effect is that in the absence of *wnt*-1, embryonic cellular proliferation in the midbrain region is retarded. Thus, the ultimate quantity of cells required for appropriate development is reduced in what eventually will become anterior cerebellum. It should be noted that in avians, the mesencephalic contribution to the cerebellum is distributed rostromedially (Hallonet et al., 1990). An absence of mesencephalic-derived cells would thus be expected to affect the midline as well as the anterior cerebellum. However, differentiation, per se, may not be absolutely dependent upon *Wnt*-1 functions, since normal cerebellar cell types are found in the *wnt*-1$^-$ background (Sidman, 1968; Thomas and Capecchi, 1990).

Although *wnt*-1 mutations exert the strongest effects upon anterior cerebellum, they also influence, in variable fashion, adjacent structures. Histological comparisons of newborn, *wnt*-1$^-$ mice reveal a gradient of expressivity extending into other cerebellar regions and the midbrain. If the mutant alleles do represent loss-of-function mutations, the individual variation in phenotype is thus a result of other components, environmental or genetic, which influence brain development. Because the *wnt*-1$^-$ mutants do not yet exist in isogenic backgrounds, such variations in phenotypes are not totally unexpected. It is possible, for example, that other members of the *Wnt* gene family can complement *wnt*-1$^-$ mutations. Genetic or epigenetic factors that modulate the expression of other *Wnt* genes could thus potentially affect the expressivity of the *wnt*-1$^-$ mutations. Whatever the mechanism, the complementation of the *wnt*-1$^-$ phenotype occurs only in cells destined for posterior cerebellum and the tectum. In the rostromedial cerebellum, *Wnt*-1 is epistatic to these other postulated components, as that structure is never seen in a *wnt*-1$^-$ background. This region of *Wnt*-1 epistasis may in fact correspond to a ring of cells at the midbrain–hindbrain junction known to express high levels of *Wnt*-1 mRNA at embryonic day 10.5 (Wilkinson et al., 1987).

In conclusion, we have identified *sw* as a single base pair deletion in the *Wnt*-1 gene. This mutation results in a phenotype indistinguishable from that caused by the other characterized *wnt*-1$^-$ allele *wnt*-1^{neo}. Analyses of mice mutant for *wnt*-1 have shown this gene to be essential for the development of the anterior lobe of the cerebellum. This same analysis also implies a role for *Wnt*-1 in the normal development of the posterior cerebellum and the midbrain, although these functions can apparently be complemented by as yet undescribed factors.

Experimental Procedures

Mice Strains

Mice heterozygous for the *wnt*-1^{neo} insertion were generated as described (Thomas and Capecchi, 1990). Although the original mutation was created in a strain 129/Sv/Ev mouse, mice in this study were

the product of two backcrosses into a C57BL/6J inbred line. Mice heterozygous for *sw* were obtained from the Jackson Laboratory on a C57BL/6J × C3HeB/FeJ hybrid background.

Histology

Collection and processing of embryonic and adult tissues have been described previously (Thomas and Capecchi, 1990).

Heads from newborn mice were fixed in Bouin's reagent (Sigma) for a minimum of 3 days prior to infiltration and embedding. Samples destined for sagittal sectioning were then halved along the midline with a razor blade. Infiltration of the samples began with an overnight wash (two changes) in 5% sucrose, 0.1 M sodium phosphate (pH 7.4), followed by sequential washes of 50%, 70%, 95%, and 100% ethanol, followed by ethanol/xylene (1/1 [v/v]), xylene, xylene/paraffin (1/1 [v/v]), and finally infiltration with two changes of paraffin (Paraplast, Oxford Labware). Sections (8 μm) were prepared using a Leica 1512 microtome and were mounted on acid-washed glass slides. Hematoxylin and eosin staining was performed using standard protocols. Photography was through a Wild MPS Photoautomat mounted on a Wild 78 dissecting microscope, using Kodak Ektar 125 film.

Genotype Determination

DNA was extracted from tails of adult and newborn mice and yolk sacs of embryos using the protocol of Hogan et al., 1986. The *wnt*-1^{neo} allele was distinguished from *wnt*-1^{+} by Southern transfer analysis as described previously (Thomas and Capecchi, 1990). Identification of *wnt*-1^{sw} was performed as follows: 10 μg of tail-derived DNA was digested with 20 U of BsII (New England Biolabs) at 55°C for 12 hr in a 40 μl reaction mix containing buffer supplied by the manufacturer, supplemented with 4 mM spermidine. Following ethanol precipitation, the digested DNA was resuspended in 15 μl of a loading buffer consisting of 6% sucrose, 1 mM EDTA (pH 8.0). Samples were electrophoresed through 6% polyacrylamide and electrotransferred to an Immobilon-N membrane (Millipore). Prehybridization and hybridization of the filter were under conditions suggested by the manufacturer. The probe used to detect the *Wnt*-1 sequences was a random-primed (Pharmacia), ^{32}P-labeled DNA fragment consisting of 1.3 kb of *Wnt*-1 genomic DNA extending from an EcoRI site in intron 2 to an AccI site in exon 4 (van Ooyen and Nusse, 1984). Filters were washed under supplier-recommended conditions, with two final washes at 68°C in 0.3 × SSC, 0.1% SDS, and 0.1% sodium pyrophosphate.

Cloning and Sequencing of *wnt*-1^{sw}

The source of the *wnt*-1^{sw} DNA was a phenotypic *wnt*-1^{-} newborn offspring from a cross between *wnt*-1^{neo} and *sw* heterzygotes. DNA was extracted from the liver under conditions used for tail DNA extraction. Purified liver DNA (170 μg) was digested to completion with BglII (Gibco-BRL) and centrifuged through a 10%–40% sucrose gradient buffered in 1 M NaCl, 20 mM Tris (pH 8.0), 1 mM EDTA. Samples containing DNA from 10–15 kb in length were pooled and dialyzed into Tris–EDTA buffer. Following ethanol precipitation, 250 ng of BglII-digested DNA was cloned into BamHI-cut λ-Dash II (Stratagene). Recombinant phage (10^6) were screened by filter hybridization, probing with a 2 kb ClaI–BglII fragment containing the 3′ end of *Wnt*-1 genomic DNA (van Ooyen and Nusse, 1984). *Wnt*-1-containing DNA was removed from the phage by digestion with SalI, subcloned into pUC9, and mapped by restriction endonuclease digestion. Individual restriction fragments were subcloned further prior to sequencing.

Sequencing of *wnt*-1^{sw} was performed on double-stranded plasmids using Sequenase (U. S. Biochemicals) and [^{35}S]dATP. Sequencing reactions used either specific oligonucleotide primers (synthesized on an ABI model 380B DNA synthesizer) or universal primers. Verification of the *wnt*-1^{sw} sequence was performed using the primer, 5′-GCTGCAGTGACAACATC-3′, corresponding to coding strand nucleotides 1835–1852 from the wild-type *Wnt*-1 genomic sequence.

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References

Altman, J., and Bayer, S. A. (1985a). Embryonic development of the rat cerebellum. I. Delineation of the cerebellar primordium and early cell movements. J. Comp. Neurol. *231*, 1–26.

Altman, J., and Bayer, S. A. (1985b). Embryonic development of the rat cerebellum. II. Translocation and regional distribution of the deep neurons. J. Comp. Neurol. *231*, 27–41.

Altman, J., and Bayer, S. A. (1985c). Embryonic development of the rat cerebellum. III. Regional differences in the time of origin, migration, and settling of Purkinje cells. J. Comp. Neurol. *231*, 42–65.

Baker, N. E. (1987). Molecular cloning of sequences from *wingless*, a segment polarity gene in *Drosophila*: the spatial distribution of a transcript in embryos. EMBO J. *6*, 1765–1773.

Bradley, R. S., and Brown, A. M. C. (1990). The proto-oncogene *int-1* encodes a secreted protein associated with the extracellular matrix. EMBO J. *9*, 1569–1575.

Brown, A. M. C., Papkoff, J., Fung, Y. K. T., Shackleford, G. M., and Varmus, H. E. (1987). Identification of protein products encoded by the proto-oncogene *int-1*. Mol. Cell. Biol. *7*, 3971–3977.

Burgess, A. W. (1988). Int-1 and Int-2: oncogenic proteins, mitogens and morphogens? Bioessays *8*, 40–42.

Cabrera, C. V., Alonso, M. C., Johnston, P., Phillips, R. G., and Lawrence, P. A. (1987). Phenocopies induced with antisense RNA identify the *wingless* gene. Cell *50*, 659–663.

Capecchi, M. (1989). Altering the genome by homologous recombination. Science *244*, 1288–1292.

Fung, Y.-K. T., Shackleford, G. M., Brown, A. M. C., Sanders, G. S., and Varmus, H. E. (1985). Nucleotide sequence and expression in vitro of cDNA derived from mRNA of *int-1*, a provirally activated mouse mammary oncogene. Mol. Cell. Biol. *5*, 3337–3344.

Gavin, B. J., McMahon, J. A., and McMahon, A. P. (1990). Expression of multiple novel *Wnt-1/int-1*-related genes during fetal and adult mouse development. Genes Dev. *4*, 2319–2332.

Hallonet, M. E. R., Teillet, M.-A., and Le Douarin, N. M. (1990). A new approach to the development of the cerebellum provided by the quail-chick marker system. Development *108*, 19–31.

Herrup, K., and Wilczynski, S. L. (1982). Cerebellar cell degeneration in the leaner mutant mouse. Neuroscience *7*, 2185–2196.

Hess, E. J., and Wilson, M. C. (1991). Tottering and leaner mutations perturb transient developmental expression of tyrosine hydroxylase in embryologically distinct Purkinje cells. Neuron *6*, 123–132.

Hogan, B., Costantini, F., and Lacy, E. (1986). Manipulating the Mouse Embryo (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Jacobsen, M. (1978). Developmental Neurobiology (London: Plenum Publishing Corp.).

Jakobovits, A., Shackleford, G. M., Varmus, H. E., and Martin, G. R. (1986). Two proto-oncogenes implicated in mammary carcinogenesis, *int-1* and *Int-2*, are independently regulated during mouse development. Proc. Natl. Acad. Sci. USA *83*, 7806–7810.

Kamb, A., Weir, M., Rudy, B., Varmus, H., and Kenyon, C. (1989). Identification of genes from pattern formation, tyrosine kinase, and potassium channel families by DNA amplification. Proc. Natl. Acad. Sci. USA *86*, 4372–4376.

Lane, P. W. (1967). Mouse News Lett. *36*, 40.

Lane, P. W. (1970). Mouse News Lett. *42*, 30.

Lane, P. W., Bronson, R., and Spencer, C. (1990). Mouse Genome *86*, 237.

Lyon, M. F., and Searle, A. G. (1989). Genetic Variants and Strains of the Laboratory Mouse (Oxford: Oxford University Press).

Martinez, S., and Alvarado-Mallart, R.-M. (1989). Rostral cerebellum originates from the caudal portion of the so-called 'mesencephalic' vesicle: a study using chick/quail chimeras. Eur. J. Neurosci. *1*, 549–560.

Martinez Arias, A., Baker, N. E., and Ingham, P. W. (1988). Role of

segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. Development *103*, 157–170.

McMahon, A. P., and Bradley, A. (1990). The *Wnt*-1 (*int*-1) proto-oncogene is required for development of a large region of the mouse brain. Cell *62*, 1073–1085.

McMahon, J. A., and McMahon, A. P. (1989). Nucleotide sequence, chromosomal localization and developmental expression of the mouse *int-1*-related gene. Development *107*, 643–650.

McMahon, A. P., and Moon, R. T. (1989). Ectopic expression of the proto-oncogene *int*-1 in Xenopus embryos leads to duplication of the embryonic axis. Cell *58*, 1075–1084.

Meruelo, D., Rossomando, A., Scandalis, S., D'Eustachio, P., Fournier, R. E. K., Roop, D. R., Saxe, D., Blatt, C., and Nesbitt, M. N. (1987). Assignment of the *Ly-6 - Ril-1 - Sis - H-30 - Pol-5/Xmmv-72 - Ins-3 - Krt-1 - Int-1 - Gdc-1* region to murine chromosome 15. Immunogenetics *25*, 361–372.

Morata, G., and Lawrence, P. A. (1977). The development of *wingless*, a homeotic mutation of *Drosophila*. Dev. Biol. *56*, 227–240.

Mullen, R. J. (1984). Ontogeny and genetics of the mammalian nervous system. In Chimeras in Developmental Biology (London: Academic Press), pp. 353–368.

Noordermeer, J., Meijlink, F., Verrijzer, P., Rijsewijk, F., and Destree, O. (1989). Isolation of the *Xenopus* homolog of *int-1/wingless* and expression during neurula stages of early development. Nucl. Acids Res. *17*, 11–18.

Nusse, R. (1988). The *int* genes in mammary tumorigenesis and in normal development. Trends Genet. *4*, 291–295.

Nusse, R., and Varmus, H. E. (1982). Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell *31*, 99–109.

Nusse, R., van Ooyen, A., Cox, D., Fung, Y. K. T., and Varmus, H. E. (1984). Mode of proviral activation of a putative mammary oncogene (*int-1*) on mouse chromosome 15. Nature *307*, 131–136.

Nusse, R., Theunissen, H., Wagenaar, E., Rijsewijk, F., Gennissen, A., Otte, A., Schuuring, E., and van Ooyen, A. (1990). The *Wnt-1* (*int-1*) oncogene promoter and its mechanism of activation by insertion of proviral DNA of the mouse mammary tumor virus. Mol. Cell. Biol. *10*, 4170–4179.

Nusse, R., Brown, A., Papkoff, J., Scambler, P., Shackleford, G., McMahon, A., Moon, R., and Varmus, H. (1991). A new nomenclature for *int*-1 and related genes: the *Wnt* gene family. Cell *64*, 231.

Papkoff, J. (1989). Inducible overexpression and secretion of *int-1* protein. Mol. Cell. Biol. *9*, 3377–3384.

Papkoff, J., and Schryver, B. (1990). Secreted *int-1* protein is associated with the cell surface. Mol. Cell. Biol. *10*, 2723–2730.

Papkoff, J., Brown, A. M. C., and Varmus, H. E. (1987). The *int-1* proto-oncogene products are glycoproteins that appear to enter the secretory pathway. Mol. Cell. Biol. *7*, 3978–3984.

Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D., and Nusse, R. (1987). The Drosophila homolog of the mouse mammary oncogene *int*-1 is identical to the segment polarity gene *wingless*. Cell *50*, 649–657.

Roelink, H., and Nusse, R. (1991). Expression of two members of the *Wnt* family during mouse development—restricted temporal and spatial patterns in the developing neural tube. Genes Dev. *5*, 381–388.

Roelink, H., Wagenaar, E., Lopes de Silva, S., and Nusse, R. (1990). *Wnt-3*, a gene activated by proviral insertion in mouse mammary tumors, is homologous to *int-1/Wnt-1* and is normally expressed in mouse embryos and adult brain. Proc. Natl. Acad. Sci. USA *87*, 4519–4523.

Ross, M. E., Fletcher, C., Mason, C. A., Hatten, M. E., and Heintz, N. (1990). Meander tail reveals a discrete developmental unit in the mouse cerebellum. Proc. Natl. Acad. Sci. USA *87*, 4189–4192.

Shackleford, G. M., and Varmus, H. E. (1987). Expression of the proto-oncogene *int*-1 is restricted to postmeiotic male germ cells and the neural tube of mid-gestational embryos. Cell *50*, 89–95.

Sidman, R. L. (1968). Development of interneuronal connections in brains of mutant mice. In Physiological and Biochemical Aspects of Nervous Integration, F. D. Carlson, ed. (Englewood Cliffs, New Jersey: Prentice-Hall, Inc.), pp. 163–193.

Sidman, R. L. (1983). Experimental neurogenetics. In Genetics of Neurological and Psychiatric Disorders, S. S. Kety, L. P. Rowland, R. L. Sidman, and S. W. Matthysse, eds. (New York: Raven Press), pp. 19–46.

Sidman, R. L., Green, M. C., and Appel, S. (1965). Catalogue of Neurological Mutants of Mice (Cambridge, Massachusetts: Harvard University Press).

Thomas, K. R., and Capecchi, M. R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell *51*, 503–512.

Thomas, K. R., and Capecchi, M. R. (1990). Targeted disruption of the murine *int-1* proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. Nature *346*, 847–850.

van den Heuvel, M., Nusse, R., Johnston, P., and Lawrence, P. A. (1989). Distribution of the *wingless* gene product in Drosophila embryos: a protein involved in cell–cell communication. Cell *59*, 739–749.

van Ooyen, A., and Nusse, R. (1984). Structure and nucleotide sequence of the putative mammary oncogene *int*-1; proviral insertions leave the protein-encoding domain intact. Cell *39*, 233–240.

Van't Veer, L. J., Geurts van Kessel, A., van Heerikhuizen, H., van Ooyen, A., and Nusse, R. (1984). Molecular cloning and chromosomal assignment of the human homolog of *int-1*, a mouse gene implicated in mammary tumorigenesis. Mol. Cell. Biol. *4*, 2532–2534.

Wilkinson, D. G., Bailes, J. A., and McMahon, A. P. (1987). Expression of the proto-oncogene *int*-1 is restricted to specific neural cells in the developing mouse embryo. Cell *50*, 79–88.