Winged helix transcription factor Foxb1 is essential for access of mammillothalamic axons to the thalamus

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Accepted 16 December 1999; published on WWW 8 February 2000

SUMMARY

Our aim was to study the mechanisms of brain histogenesis. As a model, we have used the role of winged helix transcription factor gene Foxb1 in the emergence of a very specific morphological trait of the diencephalon, the mammillary axonal complex. Foxb1 is expressed in a large hypothalamic neuronal group (the mammillary body), which gives origin to a major axonal bundle with branches to thalamus, tectum and tegmentum. We have generated mice carrying a targeted mutation of Foxb1 plus the tau-lacZ reporter. In these mutants, a subpopulation of dorsal thalamic ventricular cells (“thalamic palisade”) show abnormal persistence of Foxb1 transcriptional activity; the thalamic branch of the mammillary axonal complex is not able to grow past these cells and enter the thalamus. The other two branches of the mammillary axonal complex (to tectum and tegmentum) are unaffected by the mutation. Most of the neurons that originate the mammillothalamic axons suffer apoptosis after navigational failure. Analysis of chimeric brains with wild-type and Foxb1 mutant cells suggests that correct expression of Foxb1 in the thalamic palisade is sufficient to rescue the normal phenotype. Our results indicate that Foxb1 is essential for diencephalic histogenesis and that it exerts its effects by controlling access to the target by one particular axonal branch.

Key words: Axonal navigation, Diencephalon, Fkh5, Forebrain, Hypothalamus, Mf3, Regionalization, Thalamic palisade, TWH

INTRODUCTION

We were interested in the developmental mechanisms by which genetic information is translated into region-specific traits in the mouse brain. The role of winged helix transcription factor gene Foxb1 (also known as Fkh5, Kaestner et al., 1996; Mf3, Sasaki and Hogan, 1993; HFH-e5.1, Ang et al., 1993; TWH, Dou et al., 1997) in the establishment of the mammillary axonal complex offered a good model for the study of these processes. We have previously reported (Wehr et al., 1997) that mice homozygous for a targeted mutation of Foxb1 show specific deletion of a diencephalic neuronal group, the medial mammillary nucleus. A large number of transcription factors of the winged helix family (Weigel and Jackle, 1990; Lai et al., 1993) are involved in brain development (BF-1, Tao and Lai, 1992; Xuan et al., 1995; BF-2, Hatini et al., 1994; Fkh4, Fkh5, Kaestner et al., 1996; Hfh2, Labosky and Kaestner, 1998; Mf2, Wu et al., 1998). Foxb1 is known to participate also in spinal cord differentiation (Dou et al., 1997) as well as in the control of lactation (Labosky et al., 1997).

The mammillary body is a large neuronal group in the caudal/ventral hypothalamus formed by three nuclei – the lateral and medial mammillary nuclei, and the dorsal premammillary nucleus (Fig. 1; reviewed by Canteras and Swanson, 1992; Risold and Swanson, 1995). All of them express Foxb1 during development (Wehr et al., 1997). The mammillary axonal complex is formed by the axons from these nuclei, which share a branching pattern – every neuron gives off one axonal stem that bifurcates into two branches. One of the branches is directed dorsally to the thalamus and another caudally to the midbrain (Cajal, 1911; Canteras and Swanson, 1992; Guillery, 1955; Hayakawa and Zyo, 1989; Kölliker, 1896). This peculiar pattern of connections bridging forebrain and midbrain, also shown by other nuclei in this boundary region, is considered a major feature of diencephalic circuitry with important behavioral implications (Canteras and Swanson, 1992; Risold et al., 1997). In particular, the mammillothalamic tract connects the two major subdivisions of the diencephalon (hypothalamus and thalamus) and closes the circuit originally described by Papez (1937). This circuit is probably involved in spatial memory (reviewed in Sziklas and Petrides, 1998).

In this study, we show that correct expression of Foxb1 in a particular subset of thalamic ventricular cells (which we have termed “thalamic palisade”) makes possible the entrance of mammillothalamic axons in the thalamus, and that the navigation of the other two mammillary axonal branches (mammillotectal and mammillotegmental) is independent from Foxb1 control.

MATERIALS AND METHODS

Histological analysis

All histological material was fixed in formaldehyde
Paraformaldehyde, Merck, Darmstadt) 4% in PBS. Some brains were processed for embedding in paraffin wax using standard techniques and sectioned at 10 μm, while others were sectioned in a vibrating microtome at 100 μm. In situ hybridization was performed according to Simmons et al. (1989).

The Foxb1 plasmid used for the preparation of riboprobe was provided by K. H. Kaestner (University of Pennsylvania). The TUNEL staining for apoptosis detection (Gorczyca et al., 1993) was performed with ApopTag (Oncor, Gaithersburg, MD) following the manufacturer’s instructions. Detection of β-galactosidase by X-gal reaction was performed as described (Koenen et al., 1982; MacGregor et al., 1987; Voss et al., 1998a).

For immunocytochemistry, we used 2H3 antibody (made by J. Dodd and T. Jessell, maintained by Developmental Hybridoma Bank, Iowa University), anti-β-III-tubulin (BabCo, Richmond, CA) and anti-β-galactosidase (ICN Pharmaceutical, Costa Mesa, CA). Immunostaining was performed using standard techniques and developed with the ABC kit (VectorLabs, Burlingame, CA) or fluorescent second antibodies (Molecular Probes, Eugene, OR).

Generation of mutants
We added cDNA for tau-β-galactosidase fusion protein (courtesy of P. Mombaerts; Mombaerts et al., 1996) to our Foxb1-targeting construct (Wehr et al., 1997). Mutants were generated following current protocols (Hogan et al., 1994). The vector was electroporated into R1 ES cells, and transfectants were selected with G418 and gancyclovir. Chimeric mice were generated by morula aggregation and germline transmission was assessed by scoring the F1 generation for agouti coat colour, and by Southern analysis. Germline chimeras were mated to 129/SvPas mice. All experiments were performed on a pure 129/SvPas genetic background.

Generation of chimeric pups
For chimeric analysis of the mutant phenotype, 8-cell-stage embryos were recovered from crosses between superovulated Foxb1 homozygous females carrying Foxb1 mutant alleles without lacZ marker gene insertions, and Foxb1 heterozygous males carrying one Foxb1 mutant allele with lacZ insertion (Fig. 7A). The 8-cell-stage embryos were aggregated with 8-cell-stage wild-type embryos and, after 1 day of culture in vitro, were transferred to recipient females, as previously described for ES cell aggregation (Nagy and Rossant, 1993; Voss et al., 1998a,b). Chimeric pups were recovered at postnatal day 0. The brains were analyzed histologically. Cells contributed by homozygous embryos were distinguished from cells contributed by wild-type or heterozygous embryos by β-galactosidase immunohistochemistry (Fig. 7). The genetic background of all the mice used was 129Sv. The results presented here were obtained from 8 chimeric animals.

RESULTS

The Foxb1 mutant fails to develop a mammillothalamic axonal tract
We had previously reported that, in Foxb1 homozygous mutants, the medial mammillary nucleus (MM) develops in...
appearance normally, then disappears perinatally (Wehr et al., 1997). We hypothesized that deficiency in Foxb1 could lead to failure of the MM to make proper connections, and this could be the cause of the death and disappearance of this nucleus. To test this hypothesis, we first analyzed the development of the mammillary region of wild-type and mutant mice on cresyl violet-stained sagittal sections of embryonic brains of different ages (Fig. 2). The profile of the MM is easily distinguishable on sagittal sections of the forebrain from early in development. The principal mammillary tract emerges from the dorsal surface around embryonic day 14 (e14) and starts to course rostrally directed, that reaches its target (the anterior subdivision of the dorsal thalamus) within 24 hours and is well established by e19/postnatal day 0 (e19/P0; Fig. 2C). In mutants, the mammillothalamic tract could not be seen on parasagittal sections of an e18.5 heterozygous brain. The inset shows the region represented. The dotted line outlines the ventral side of the brain. The arrow in J,K shows a lateral migration of Foxb1-expressing cells in the developing hypothalamus. Abbreviations: HY, hypothalamus; LM, lateral mammillary nucleus; MB, midbrain; MBO, mammillary body; MMm, medial mammillary nucleus, medial subdivision; MMn, central subdivision; MMp, posterior subdivision; mr, mammillary recess; SP, spinal cord.

**Foxb1-driven tau-lacZ expression in heterozygotes is a reliable indicator of Foxb1 transcriptional activity**

To analyze the development of the mammillary axonal complex in Foxb1 mutants, we replaced parts of exons 1 and 2 of the Foxb1 locus (Fig. 3A) with DNA sequences coding for a tau-β-galactosidase fusion protein (Mombaerts et al., 1996). We electroporated this construct into ES cells and generated mice carrying this modified Foxb1 locus. The modified locus lacks the sequences that code for the start of translation and for functional Foxb1 protein, since the mRNA fails to develop or maintain a mammillothalamic tract.

Since the tau protein is a normal constituent of the axonal cytoskeleton, the tau-β-galactosidase fusion protein was localized to the axons (Mombaerts et al., 1996) of those neurons that show Foxb1 transcriptional activity. We have
previously performed a detailed study of the pattern of expression of Foxb1 in developing and adult wild-type mouse brains (Alvarez-Bolado et al., 1999 and G. A.-B. et al., unpublished data). In addition, we knew that the mammillary region of heterozygotes does not show an abnormal phenotype (Wehr et al., 1997). On the basis of these studies, we have compared (1) expression of Foxb1 in wild type to expression of Foxb1 in heterozygous brains (compare Fig. 3B to G), and (2) expression of Foxb1 to expression of lacZ in heterozygous brains. In both cases, we have focused particularly on the diencephalic region. We have found (1) expression of tau-lacZ in our mutants is a reliable indicator of Foxb1 transcriptional activity, since it matches expression of Foxb1 in wild-type embryos (compare Fig. 3B to E) and adults (Fig. 3F,G), and (2) expression of tau-lacZ in heterozygous embryos also matches the pattern of Foxb1 expression (compare Fig. 3H to K). In addition, Dou et al. (1997) have reported expression of lacZ matching the wild-type expression of Foxb1 (TWH) in mice targeted with a construct very similar to ours and with the same 5′ insertion point. Therefore, we considered the cells that expressed lacZ as reflecting Foxb1 transcriptional activity.

Abnormal pattern of Foxb1 transcriptional activity around the mutant thalamus

β-galactosidase activity in heterozygous and homozygous developing brains cut into two (Fig. 4) revealed the development of the mammillary axonal complex. By e18.5, the two most caudal branches of the mammillary axonal complex, as well as the tip of the mammillothalamic outgrowth (mtt-og, Fig. 4A-D) were present in heterozygous and homozygous brains. The ventral border of dorsal thalamus showed β-galactosidase activity in both (asterisk). At e19.5, the mtt-og contacted the ventral border of dorsal thalamus (Fig. 4E-H). By this stage, the border showed very low Foxb1 transcriptional activation in the heterozygote (Fig. 4E,F). In the homozygote, a region of β-galactosidase-expressing tissue separated the thalamus from the hypothalamus (Fig. 4G,H). By P1, the heterozygote mtt-og has become a full-grown mammillothalamic bundle (mtt, Fig. 4I,J), and β-galactosidase activity is absent from the region under the dorsal thalamus. The homozygote showed an arrested or deflected mtt-og, which does not enter the dorsal thalamus (Fig. 4K,L), together with persistence of β-galactosidase activity in the region under the dorsal thalamus (see also Fig. 8F). Accordingly, two consequences of the Foxb1 mutation seemed to be abnormally persistent Foxb1 transcriptional activation in the ventral side of the dorsal thalamus, together with failure of the mtt-og to become a full-grown mtt.

The mammillothalamic outgrowth does not enter the target area

β-galactosidase detection on histological sections revealed that, while by P0 the heterozygous mammillothalamic outgrowth had already become a full grown axonal bundle reaching the anterior thalamic complex (Fig. 5A), in the homozygous diencephalon (Fig. 5B), the mammillothalamic tract had obviously failed to enter the thalamus through the region showing Foxb1 transcriptional activity (K,L; asterisk). Asterisk, Foxb1 transcriptional activity area in the dorsal thalamus; DOR, dorsal thalamus; MBO, mammillary body; mtc, mammillotectal tract; mtg, mammillotegmental tract; mtt, mammillothalamic tract; mtt-og, mammillothalamic outgrowth; P, pons; pm, principal mammillary tract.
target region. β-galactosidase activity was intense along the Foxb1-expressing thalamic boundary in homozygotes (asterisk in Fig. 5B,D,F), but only a remnant of β-galactosidase activity could be detected in heterozygotes (asterisk in Fig. 5A,C).

These observations suggested that, in homozygotes, the mammillothalamic tract was either not attracted by its intended target (abnormal lack of attraction), or was deflected upon contacting a certain dorsal thalamic boundary region (abnormal presence of repulsion) where Foxb1 transcriptional activation was abnormally high and persistent.

A subpopulation of Foxb1-expressing ventricular cells surrounds the ventral side of the dorsal thalamus

Foxb1 is normally expressed in the wild-type early thalamic primordium (Wehr et al., 1997) by cells in the ventricular layer (not shown). The level of expression slowly decreases during development. By e18.5, Foxb1 expression was still detectable by in situ hybridization in the ventral border of the dorsal thalamus (Fig. 6A,B). In heterozygotes, both Foxb1 and lacZ are expressed in this region (arrow, Fig. 6C,D).

In homozygotes, immunodetection of β-galactosidase revealed that this area is formed by a group of cells with long prolongations extending away laterally from the ventricular zone. In transverse sections, the long cellular processes (Fig. 6E,F) separated the dorsal thalamus from underlying structures. Comparison of sections taken in different planes (compare Fig. 6E to H) shows that these cells are arranged forming a flat, horizontal structure, oriented rostrocaudally in the middle of the diencephalon. This structure has the appearance of a “barrier” or “palisade”.

Because of their morphology, we hypothesized that these cells were not neuronal. We tested this hypothesis by treating sections of e18.5 homozygous brains with antibodies against one of several neuron-specific markers and against β-galactosidase. In Fig. 6I-K, we show the results obtained with antibody against β-III-tubulin (neuron-specific; Easter et al., 1993; Lee et al., 1990; Moody et al., 1989). The processes of the cells expressing β-galactosidase (Fig. 6J) are clearly distinct from the surrounding neurons (green in Fig. 6J). Some processes seem to form bundles (arrows in Fig. 6J). Small, elongated, β-galactosidase-expressing cell bodies are apparent (arrowhead in Fig. 6J), quite different from the large, round, β-III-tubulin-expressing neurons. Some β-galactosidase-expressing somata are also evident in the diencephalic neuroepithelium (Fig. 6K), forming a clearly separated population from the surrounding β-III-tubulin-expressing neurons. Similar information was obtained with anti-neurofilament monoclonal antibodies 2H3 (anti-NF-M; Dodd et al., 1988; Guthrie and Pini, 1995) and NR4 (anti-NF-L), which label neuron-specific molecules known to be expressed already at that time in development (Cochard and Paulin, 1984; Julien et al., 1986; not shown).

These results suggested that the dorsal thalamic boundary region through which (in Foxb1 homozygous mutants) mammillothalamic axons cannot proceed is formed by a palisade of non-neuronal cells, some of whose bodies are in the ventricular layer, and some probably migrating away from it. The word palisade is used here in reference to the appearance of the flat and compact structure formed by these non-neuronal cells on horizontal sections (Fig. 6G,H).

Fig. 5. The mutant mammillothalamic tract failed to enter the target region. (A,B) Sagittal paraffin sections through heterozygous (A) and homozygous (B) newborn mouse brains reacted for the detection of β-galactosidase. In B, a smaller bundle of principal mammillary axons can be seen forming a second bifurcation towards the thalamus (arrowhead). (C,D) Sagittal vibrating microtome sections through the brains of heterozygous (C) and homozygous (D) newborn mouse brains treated with anti-β-galactosidase antibody. The framed portions are shown enlarged in E,F. (E,F) Enlarged parts of C and D showing the mammillary bifurcation and the choice point of the mammillotectal axons (arrowhead). Asterisk, Foxb1 transcriptional activity area in the dorsal thalamus; MBO, mammillary body; mtt, mammillothalamic tract; mtc, mammillotectal tract; mtg, mammillotegmental tract.

pass through the ventral border of the thalamus. Use of an antibody against β-galactosidase (Fig. 5C-F) allowed a more detailed analysis. In heterozygous brains, the mammillothalamic tract followed a straight path to the target (Fig. 5C,E). In homozygous brains (Fig. 5D,F), the mammillothalamic tract formed correctly as an outgrowth of the principal mammillary tract. This outgrowth was oriented in the correct direction (rostral and dorsal, towards the dorsal thalamus). In the vicinity of the ventral boundary of the dorsal thalamus, however, it abnormally changed direction rostrally, without entering the...
The axonal defect of Foxb1 mutants is not cell autonomous

We wanted to delimitate the respective roles of thalamic palisade and mammillothalamic axons in the generation of the mutant phenotype. Accordingly, we generated chimeric mice composed of wild-type and Foxb1−/− cells (Fig. 7A). We knew that lacZ expression is massive in the (non-chimeric) homozygous thalamic palisade (Figs 4G, K, 5B, D, 6H; see also Fig. 8F); we showed before that lacZ expression is a reliable indicator of Foxb1 expression (compare Fig. 3H to K, and 6C to D). According to these data, we consider that it is at least very likely that, in our chimeras, a thalamus devoid of lacZ expression is a wild-type thalamus. Some chimeras presented homozygous, lacZ-expressing cells in the mammillary body, together with a thalamus free of lacZ expression (Fig. 7B, C). This allowed the behavior of mutant (lacZ-expressing) axons
to be followed in the presence of a wild-type (non-lacZ-expressing) thalamus. In these cases, the labeled axons were able to enter a non-labeled thalamus (Fig. 7B,C).

This result suggests that the failure of mammillary axons to reach the thalamus is not cell autonomous, that is, it depends on the thalamic palisade and not on the axons themselves (summarized in Fig. 7D).

The medial mammillary nucleus of Foxb1 mutants disappears perinatally by apoptosis

The size and shape of the MM were normal in homozygotes on e19/P0, as assessed by means of cresyl violet staining (not shown). During the next 3 days, the nucleus decreased rapidly in size, and it showed numerous round, dense bodies (pyknotic figures) that suggested cell death (not shown). By P4, at least most of the MM, as assessed on cresyl violet-stained sections (Wehr et al., 1997) has disappeared. By using the TUNEL method on sagittal and on transverse sections of wild-type and homozygous fetal brains of the appropriate ages, we confirmed the presence of massive apoptosis in the homozygous MM in coincidence with the time of its disappearance (Fig. 8A-D). The lateral mammillary nucleus (LM), as well as the dorsal premammillary (PMd), express Foxb1 during development and in the adult. They contribute axons to the mammillothalamic bundle (see Fig. 1). Consequently, these nuclei in homozygotes are expected to be affected. Examination of adult heterozygous and homozygous brains sagittally cut and reacted for β-galactosidase detection (Fig. 8E,F) shows that at least some cells are left in the mammillary region even in homozygotes, and that these cells generate labeled axons that course in the appropriate direction (Fig. 8F).

DISCUSSION

We wanted to study the role of genes in brain histogenesis, i.e., how genetic information is translated into the visible distinct characteristics of each brain region. As a model, we have used the development of the diencephalon in Foxb1 mutants. We knew that deficiency in transcription factor Foxb1 has major consequences for diencephalic organization in the mouse (Wehr et al., 1997). Here we have found that Foxb1 was essential for the emergence of a regionally specific morphological trait with important behavioral roles, the mammillothalamic tract. This role was exerted through a novel group of ventricular cells, the thalamic palisade, which showed permissive or attractive abilities for mammillothalamic axons only through correct expression of Foxb1. Additionally, our results indicated that the product of this gene was required specifically for the navigation of one particular branch of the mammillary axonal complex. Finally, massive apoptosis in the MM followed navigational failure.

A specific subpopulation of ventricular cells bounds the dorsal thalamus

An intriguing finding of our study is the subpopulation of ventricular cells that showed Foxb1 transcriptional activity. We have called them “thalamic palisade”; radial glial cells in the chiasmatic region, which have an important role in retinofugal axon guidance, have been described as forming a palisade (Marcus et al., 1995; Marcus and Mason, 1995). However, here we use the term “palisade” as a concise, descriptive word, without functional connotations. The thalamic palisade is formed by the radially oriented processes of non-neuronal cells anchored to the ventricular layer of the developing diencephalon; some of these cells are monopolar, with cell bodies situated in the neuroepithelium, while most have a bipolar appearance, with one proximal process (in contact with the neuroepithelium) and a distal one.

The pathfinding process can be divided into a series of steps, each one involving growth cone decisions that are influenced by the different molecules that are found at every step (Holt and Harris, 1998). The decisions are taken at “choice points” (Cook et al., 1998; Raper et al., 1983; Tessier-Lavigne and
The trajectories of the axons coming from the mammillary body can also be described in this way. First, the primary axon (the principal mammillary tract, see Fig. 1) chooses a caudal direction; at the same time, the branching point is probably selected in advance by the primary growth cone on passing through the future site of the bifurcation (Szebenyi et al., 1998). Then, the newly formed branches have to decide if they should take the thalamic direction or the tectal direction. Finally, the axons going towards the thalamus have to gain entrance in the target region.

Several choice points are currently being characterized, like the midline/floor plate (reviewed in Flanagan and Van Vactor, 1998; Tessier-Lavigne and Goodman, 1996), the intersegmental (Keynes et al., 1991; Wills et al., 1999) and the tract of the postoptic commissure (Anderson and Key, 1996). For all of these, secreted and membrane-bound proteins have been found that would be directly involved in different phenomena of attraction and repulsion of axons (for instance, netrins, semaphorins, ephrins and their corresponding receptors, reviewed in Puschel et al., 1995; Cook et al., 1998).

Our analysis of chimeric brains suggests that the failure of Foxb1 homozygous axons to enter the thalamus is not cell autonomous, that is, that the defect lies probably in the mutant thalamic palisade and not in the mutant axons. Therefore, the thalamic palisade can be described as a choice point for the axons coming from the mammillary body.

**Foxb1 has a role in the thalamic palisade choice point**

The present results indicate that, of the different types of decision needed at the mammillary choice point (branching, defasciculating, turning, entering a target region), Foxb1 specifically controls one step (gaining entrance in the target region) of only one axonal tract (the mammillothalamic). A current model (Tessier-Lavigne and Goodman, 1996) represents axon guidance as the product of four forces (long- and short-range attraction and repulsion), which cooperate in order to “hem” axons along a particular pathway. At present, we do not know if the control of the thalamic choice point by Foxb1 is exerted through attraction (the mutant phenotype would be due to abnormal lack of attraction towards the target) or repulsion (the mutant phenotype would be due to abnormal presence or persistence of repulsion). In recent years, repulsion of navigating axons by membrane-bound molecules has been revealed as a frequent and important mechanism in axonal pathfinding (reviewed in Cook et al., 1998; Keynes and Cook, 1992; Orioli and Klein, 1997; Walter et al., 1990). The semaphorins constitute a family of molecules with axonal repulsive properties. A number of semaphorins are abundantly expressed in the thalamus during development, particularly Sem F (Skaliora et al., 1998). It is conceivable that one or several of these molecules could make the thalamus and its surroundings a very axon-repulsive environment, which would have to be overcome by the appropriate incoming axons through the expression of a gene or genes under the control of Foxb1.

Foxb1 can be added to the still small number of transcription factors that have been shown to be involved in axon navigational decisions at choice points. Pax6 may play a role in the tract of the postoptic commissure (Anderson and Key, 1996; Mastick et al., 1997), and it has been proposed that Pax2 could be upstream of some of the surface molecules that regulate axonal traffic over the chiasm (Alvarez-Bolado et al., 1997; Macdonald et al., 1997; Torres et al., 1996).

**Is the apoptotic phenotype cell autonomous?**

Foxb1 mutants show two major phenotypical traits affecting the development of the diencephalon: (1) an axonal phenotype (failure of mammillothalamic axons to enter the thalamus), which appears to be non cell autonomous, and (2) an apoptotic phenotype (death of the medial mammillary cells, which originate the mammillothalamic axons). The original *fork head*...
mutation in Drosophila shows increased apoptosis in the embryonic gut (Weigel et al., 1989a,b), suggesting that deficiency in fkh could cause cell death by a cell autonomous mechanism. Forkhead proteins are involved in the activation of apoptotic mechanisms (Bernasconi et al., 1996; Brunet et al., 1999a,b; Tang et al., 1999). Therefore, the apoptotic phenotype found in Foxb1 mutants could be due to an alteration in the balance of factors that regulates the activation of apoptotic mechanisms. Alternatively, Foxb1 could act as a survival factor for the medial mammillary cells. Genes that have this role on different neuronal populations have recently been described. Phox2a and Phox2b are transcription factors known to promote the development of sympathetic neurons in vivo and in vitro (Stanke et al., 1999); c-ret is a proto-oncogene coding for a neurotrophic receptor that supports the survival and differentiation of specific central and peripheral neuronal cell types (Trupp et al., 1996; Tsujino et al., 1999).

A third possibility is that the navigational defect and the cell loss are linked by a neurotrophic effect. The neurotrophic hypothesis (Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980) suggests that the survival of developing neurons depends on the supply of a neurotrophic factor, which would be synthesized in limiting amounts by their targets. In the peripheral nervous system, many neurons survive this phase of development by acquiring target-derived factors for which they have to compete (reviewed in Cowan et al., 1984; Davies, 1996; Lewin and Barde, 1996; Oppenheim, 1991). It is not yet clear to what degree the neurotrophic hypothesis can be applied to the central nervous system (CNS; Davies, 1996). Indeed wherever the formation of CNS circuits has been rigorously examined from this point of view, the neurotrophic theory has been supported (chicken isthmo-optic nucleus; Primi and Clarke, 1996; von Bartheld et al., 1994), hamster retinotectal system (Ma et al., 1998), rat spinal cord (Wang and Tessier-Lavigne, 1999).

We are indebted to Dr Barbara Meyer and to Martina Daniel, Heike Fett, Sabine Geisendorf, Silke Schlott and Thomas Schulz for their help in the generation of mutants, to Ralf Altschaffel for his help with photography, and to Dr Gilbert Bernier for advice on the chimera help in the generation of mutants, to Ralf Altschaffel for his help with

REFERENCES


1038  G. Alvarez-Bolado and others


