Development 127, 5297-5307 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV1601

Control of the migratory pathway of facial branchiomotor neurones

Sonia Garel*, Mario Garcia-Dominguez and Patrick Charnay‡

Unité 368 de l'Institut National de la Santé et de la Recherche Médicale, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France

*Present address: Nina Ireland Laboratory, Department of Psychiatry, UCSF, 401 Parnassus Av., San Francisco, CA 94143, USA

‡Author for correspondence (e-mail: charnay@wotan.ens.fr)

Accepted 4 October; published on WWW 14 November 2000

SUMMARY

Facial branchiomotor (fbm) neurones undergo a complex migration in the segmented mouse hindbrain. They are born in the basal plate of rhombomere (r) 4, migrate caudally through r5, and then dorsally and radially in r6. To study how migrating cells adapt to their changing environment and control their pathway, we have analysed this stereotyped migration in wild-type and mutant backgrounds. We show that during their migration, fbm neurones regulate the expression of genes encoding the cell membrane proteins TAG-1, Ret and cadherin 8. Specific combinations of these markers are associated with each migratory phase in r4, r5 and r6. In *Krox20* and *kreisler* mutant mouse embryos, both of which lack r5, fbm neurones migrate dorsally into the anteriorly positioned r6 and adopt an r6-specific expression pattern. In embryos deficient for *Ebf1*, a gene normally expressed in fbm neurones, part of the fbm neurones migrate dorsally within r5. Accordingly, fbm neurones prematurely express a combination of markers characteristic of an r6 location. These data suggest that fbm neurones adapt to their changing environment by switching on and off specific genes, and that Ebf1 is involved in the control of these responses. In addition, they establish a close correlation between the expression pattern of fbm neurones and their migratory behaviour, suggesting that modifications in gene expression participate in the selection of the local migratory pathway.

Key words: *Ebf1*, *kreisler*, *Krox20*, Facial nucleus, Hindbrain development, Neuronal migration, Mouse

INTRODUCTION

During the formation of the vertebrate central nervous system (CNS), many cell populations migrate over long distances and follow complex trajectories before reaching their final destination. These migrations usually involve multiple changes in the direction of the movement and/or in the substrate on which the cells progress. The corresponding transitions may depend on changes in the environment itself, on responses elicited in migrating cells by the environment or on programmed, cell-autonomous mechanisms. In the past few years, many studies have demonstrated the existence of environmental cues that can act as contact or diffusible attractants or repellents to provide directional information to migrating cells through interactions with cell-surface receptors (Serafini et al., 1996; Ackerman et al., 1997; Robinson et al., 1997; Birchmeier and Gherardi, 1998; Artigiani et al., 1999; Bloch-Gallego et al., 1999; Yee et al., 1999; Alcantara et al., 2000; Brose and Tessier-Lavigne, 2000). However, the changes elicited in the migrating cell by the environment to adapt to a novel context, i.e. modifications in the sensitivity to a guiding cue or in the expression of an adhesion molecule, are only beginning to be investigated.

Facial branchiomotor (fbm) neurones undergo a complex and stereotyped migration in the mouse embryonic hindbrain (Auclair et al., 1996; Studer et al., 1996; McKay et al., 1997; Schneider-Maunoury et al., 1997; see Fig. 1A). This migration takes place between E10 and E14 and its initiation occurs while the hindbrain is transiently segmented along the anterior-posterior (AP) axis into metameric units called rhombomeres (r) (Lumsden and Keynes, 1989; Lumsden, 1990; Schneider-Maunoury et al., 1998). Fbm neurones are born in the basal plate of r4 between E9 and E11, and extend their axons dorsally towards the exit point of the VIIth nerve (Altman and Bayer, 1982; Ashwell and Watson, 1983; Auclair et al., 1996; Goddard et al., 1996; Studer et al., 1996; O. Voiculescu, S. Schneider-Maunoury and P. Charnay, unpublished data). From E10, their cell bodies start migrating tangentially along the ventral midline, reaching first r5 and then r6. In r6, these neurones begin a dorsal migration between the neuroepithelium and the mantle. Subsequently, approximately at the level of the alar/basal plate boundary, fbm neurones adopt a radial pathway towards the pial surface, where they finally settle to form the facial motor nucleus. The first fbm neurones reaching this final destination can be detected at E12. At this stage, some fbm neurones have still not left the territory derived from r4, which we will still call r4, although rhombomere boundaries cannot be morphologically identified any more (Marin and Puelles, 1995; Wingate and Lumsden, 1996; Mathis et al., 1999). These late fbm neurones follow the same migratory pathway as the early ones and the facial motor

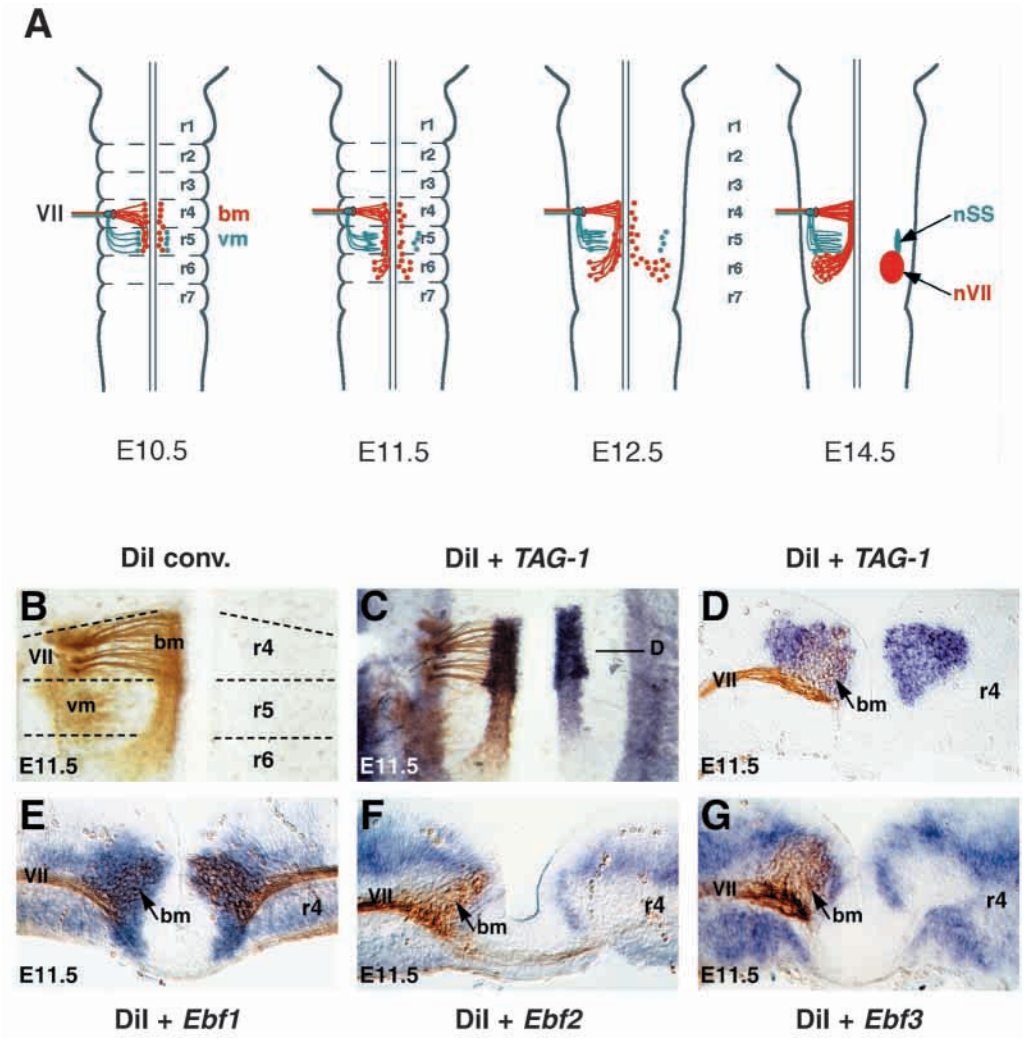


Fig. 1. Identification and migration of fbm neurones. (A) Schematic representation of the hindbrain showing the migration of fbm (red) and vm (grey) neurones. At each stage, both axons and cell bodies are represented on the left side and only cell bodies on the right side. fbm neurones migrate caudally from r4 to r6 and then dorsally (i.e. laterally) to form the facial motor nucleus. Vm neurones migrate dorsally within r5 and form the superior salivatory nucleus. (B) Flat-mounted E11.5 hindbrain labelled by a unilateral injection of DiI into the root of the VIIth nerve. DiI was photoconverted, and retrogradely labelled fbm and vm neurones appear in brown. (C) Flat-mounted E11.5 hindbrain showing combined retrograde labelling and *TAG-1* in situ hybridisation. (D) Transverse section at the level of r4 through the hindbrain presented in (C) indicating that retrogradely labelled fbm neurones express *TAG-1*. (E-G) Transverse sections at the level of r4 through E11.5 embryos subjected to bilateral (E) or unilateral (F,G) retrograde labelling and in situ hybridisation with the *Ebf1* (E), *Ebf2* (F) and *Ebf3* (G) probes. Whereas mature fbm neurones express *Ebf1*, *Ebf2* and *Ebf3* are expressed by fbm precursors located close to the ventricle. VII, facial nerve; bm, branchiomotor neurones; nSS, superior salivatory nucleus; nVII, facial motor nucleus; r, rhombomere; vm, visceromotor neurones.

nucleus migration is completed at around E14. Thus all fbm neurones execute a complex sequence of movements involving tangential progression through several rhombomeres followed by dorsal and then radial migrations. This migration pattern is atypical, since most motor neurones in the vertebrate hindbrain undergo a ventral to dorsal migration within a single rhombomere (Altman and Bayer, 1982; Lumsden, 1990). Furthermore, the caudal migration of fbm neurones is not observed in all vertebrate species. In the chick, these neurones perform their lateral and radial migrations within r4 (Lumsden and Keynes, 1989; Lumsden, 1990; Szekely and Matesz, 1993).

The analysis of several loss-of-function mutations have provided some information on the molecular mechanisms controlling fbm neuronal specification and migration. *Hoxb1* expression in the otic/preotic hindbrain is restricted to r4 (Murphy et al., 1989) and it has been shown to be involved in specifying the identity of this rhombomere (Studer et al., 1996; Bell et al., 1999). In *Hoxb1*$^{-/-}$ embryos, fbm neurones do not execute their normal caudal migration and directly progress laterally within r4, a behaviour similar to that of trigeminal branchiomotor neurones in r2 (Goddard et al., 1996; Studer et al., 1996). In addition, the inactivation of *Gata2*, *Gata3*, and *Phox2b* (*Pmx2b* – Mouse Genome Informatics), which are expressed in progenitors and precursors of fbm neurones, has been shown to impair fbm differentiation and to prevent their caudal migration out of r4 (Nardelli et al., 1999; Pata et al., 1999; Pattyn et al., 2000). Recent studies suggest that *Hoxb1* controls *Gata2*, which in turn regulates *Gata3* expression (Nardelli et al., 1999; Pata et al., 1999). These data suggest that the caudal migration of fbm neurones requires their correct AP specification and differentiation, which involves the *Hoxb1*, *Gata2*, *Gata3* cascade. In contrast, in embryos lacking normal r5, such as homozygous mutants for the genes *Krox20* (*Egr2* – Mouse Genome Informatics; Schneider-Maunoury et al., 1993; Swiatek and Gridley, 1993) or *kreisler* (*Mafb* – Mouse Genome Informatics; Frohman et al., 1993; McKay et al., 1994), caudal migration appears to occur, suggesting that the presence of r5 is not required for its initiation (McKay et al., 1997; Schneider-Maunoury et al., 1997; Manzanares et al., 1999). Taken together, these data suggest that the initiation of caudal migration of fbm neurones is mainly dependent on

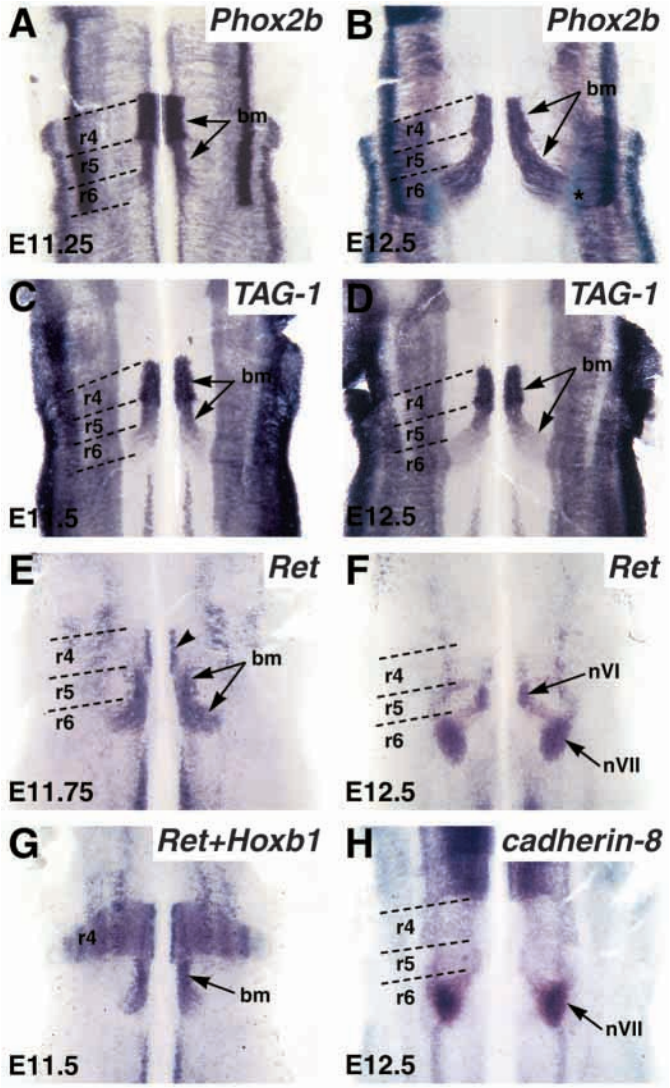


Fig. 2. Expression patterns of fbm neurone markers during the course of cell migration. In situ hybridisations were performed at as indicated (stage, probe) and the embryos were flat-mounted. (A) At E11.25 *Phox2b* is expressed in migrating fbm neurones located in r4, r5 and entering r6. (B) At E12.5 *Phox2b* expression is maintained in migrating facial bm neurones located in r4, r5 and r6. The facial nucleus forming on the pial surface can be observed by transparency (asterisk). (C) At E11.5 *TAG-1* is expressed in migrating fbm neurones located in r4, r5 and r6. (D) At E12.5 high level expression of *TAG-1* is still detected in fbm neurones located in r4 and r5, while the cells migrating in r6 have downregulated the gene. The forming facial nucleus is not labelled. (E) At E11.75, *Ret* transcripts in r4 are detected in a narrow stripe of cells (arrowhead), but not in the larger population of bm neurones. *Ret* is turned on in bm neurones when they reach r5. (F) A pial view of an E12.5 flat-mounted hindbrain shows *Ret* expression in fbm neurones located in r5 and r6, and in the abducens nucleus. (G) Double labelling with *Hoxb1* and *Ret* probes confirms that *Ret* expression is induced in fbm neurones immediately after exiting r4. (H) At E12.5 *Cdh8* (cadherin-8) expression is restricted to fbm neurones finishing their migration in r6 and forming the facial motor nucleus. bm, branchiomotor neurones; nVI, abducens nucleus; nVII, facial motor nucleus; r, rhombomere.

intrinsic properties of these cells or on local cues in r4. However, very little is known about the regulatory mechanisms that control their further progression through r5 and into r6.

To address the latter issue, we have searched for molecular markers specifically expressed during the different phases of fbm neuronal migration. We show that fbm neurones regulate the expression of three genes, *TAG-1* (*Cntn2* – Mouse Genome Informatics), *Ret* and cadherin 8 (*Cdh8*), during the different phases of their migration in r4, r5 and r6. We then studied the expression of these genes and the migration of fbm neurones in the context of the *kreisler*, *Krox20* and *Ebf1* mutations. *kreisler* and *Krox20* loss-of-function mutations lead to elimination of r5 and thus affect the environment through which cells migrate. We show that in both mutants, fbm neurones migrate dorsally into anteriorly positioned r6 and express a combination of genes characteristic of an r6 location. *Ebf1* encodes an HLH transcription factor (Hagman et al., 1991, 1993; Wang and Reed, 1993) expressed in differentiating fbm neurones in r4 and during the entire migratory process (Garel et al., 1997; Pattyn et al., 2000). The *Ebf1* loss-of-function mutation (Lin and Grosschedl, 1995) results in a subpopulation of fbm neurones adopting an r6-like pattern of migration within r5. Furthermore, these cells express a combination of markers normally characteristic of an r6 location. Altogether, these data show that the combination of r6-specific environmental cues and of cell-autonomous mechanisms involving Ebf1 elicit specific gene responses in fbm neurones and the subsequent selection of appropriate migratory pathway.

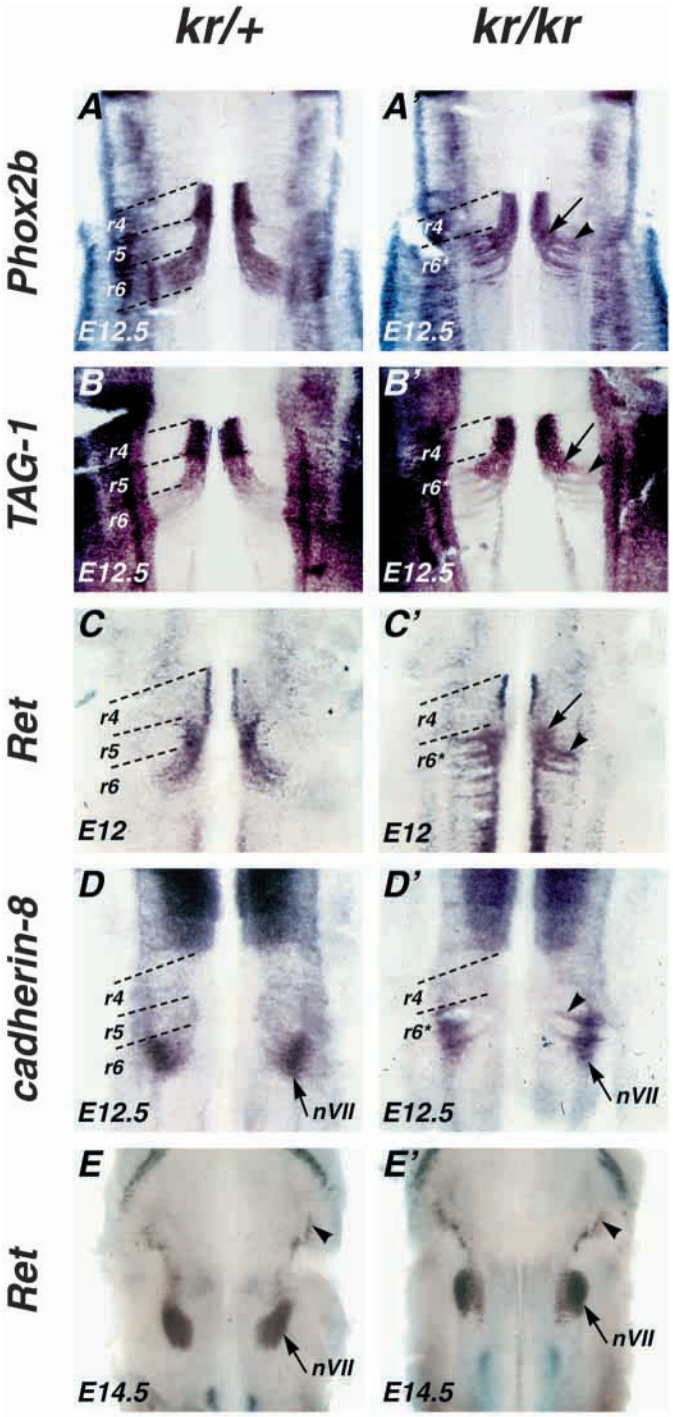
MATERIALS AND METHODS

Mouse lines and genotyping

Krox20/lacZ heterozygous mice (Schneider-Maunoury et al., 1993) were maintained in a mixed C57Bl6/DBA2 background and crossed to produce homozygous embryos. PCR genotyping of embryo yolk sac was performed as previously described (Schneider-Maunoury et al., 1993). *kreisler* mice (kindly provided by Dr Julian Lewis) were maintained in 129 background. Homozygous or heterozygous males were crossed with heterozygous females to produce homozygous embryos. These were identified by examination of the position of the inner ear or by PCR genotyping (Frohman et al., 1993). *Ebf1* heterozygous mice (Lin and Grosschedl, 1995) were maintained in C57Bl6 background and crossed to produce homozygous embryos. PCR genotyping was performed as described previously (Garel et al., 1999). For staging of embryos, midday of the vaginal plug was considered as embryonic day 0.5 (E0.5).

In situ hybridisation, immunohistochemistry and retrograde tracing

Embryos were fixed overnight in 4% paraformaldehyde (PFA) at 4°C. Whole-mount immunohistochemistry and in situ hybridisation on dissected hindbrains were performed as previously described (Schneider-Maunoury et al., 1997). Hindbrains were subsequently embedded in paraffin and sectioned (8-12 μm), or flat-mounted (Schneider-Maunoury et al., 1997). The following probes were used for in situ hybridisation: *Cdh8* (Korematsu and Redies, 1997); *Ebf1*, *Ebf2* and *Ebf3* (Garel et al., 1997); *EphA4* (Gilardi-Hebenstreit et al., 1992); *Hoxb1* (Wilkinson et al., 1989); islet 1 (*Isl1*; Nardelli et al., 1999); *Phox2b* (Pattyn et al., 1997); *Ret* (Pachnis et al., 1993); *TAG-1* (a kind gift of D. Karagogeos). For flat mounts of embryos between E9 and E12.5, the ventricular surface was positioned upwards except



for the *Ret* probe at E12.5. For E14.5 embryos, the pial surface was positioned upwards. DiI (Molecular Probes) injections in the root of the VIIth nerve were performed as previously described (Schneider-Maunoury et al., 1997; Helmbacher et al., 1998). Injected hindbrains were kept in the dark at room temperature for 3 to 7 days in 4% PFA to allow diffusion of the dye. They were then placed in a diaminobenzidine (DAB) solution (1.5 mg/ml of DAB, Tris pH 8.2 0.1 M) and illuminated on a fluorescence microscope for 30 minutes to 2 hours to obtain photoconversion of the DiI fluorescent signal, as previously described (Sandell and Masland, 1988; Marcus et al., 1995). After rinsing in PBS, the hindbrains were processed for whole-mount in situ hybridisation as above, cryoprotected in 30% sucrose and sectioned (20-40 μm) using a cryostat.

Fig. 3. Analysis of fbm gene expression and migration in *kr/kr* embryos. Embryos were flat-mounted after in situ hybridisation with the indicated probes. Heterozygous (left) and homozygous (right) mutant embryos are presented. (A,A') At E12.5 *Phox2b* detection shows that fbm neurones migrate dorsally within the r6* territory in *kr/kr* embryos. In r6*, fbm neurones constitute a compact group of cells close to the midline (arrow) and form several individual streams of cells during their dorsal migration (arrowhead). (B,B') At E12.5, in *kr/kr* embryos fbm neurones downregulate *TAG-1* when they migrate away from the ventral midline within r6* (arrowhead), a behaviour similar to that of fbm neurones in r6 in the heterozygous embryo. Fbm neurones located close to the midline in r6* (arrow) express high levels of *TAG-1*. (C,C') At E12 *Ret* expression is detected in fbm neurones exiting r4 in both heterozygous and homozygous embryos (arrow and arrowhead). (D,D') At E12.5, in *kr/kr* embryos fbm neurones express *Cdh8* (cadherin-8) when they migrate away from the ventral midline within r6* (arrowhead), similarly to fbm neurones in r6 in the heterozygous embryo. (E,E') At E14.5 *Ret* RNA detection labels the facial motor nucleus as well as part of the trigeminal nucleus (arrowhead). In *kr/kr* embryos the facial motor nucleus is anteriorly displaced. nVII, facial motor nucleus; r, rhombomere.

RESULTS

Facial branchiomotor neurones modulate gene expression during their migration

To perform a detailed study of the successive phases of migration of fbm neurones (Fig. 1A), we first searched for specific and informative markers. To identify fbm neurones unambiguously, we performed retrograde labelling experiments by injecting DiI into the root of the VIIth nerve at E11.5. This procedure labels fbm neurones migrating from r4 towards r6, and visceromotor (vm) neurones born in r5 and migrating dorsally within this rhombomere (Fig. 1A,B) (Auclair et al., 1996; Studer et al., 1996; McKay et al., 1997; Schneider-Maunoury et al., 1997). Photoconversion of the injected DiI combined with in situ hybridisation analyses allowed the characterisation of the patterns of expression of several genes in migrating fbm neurones (Fig. 1B-G and data not shown).

We confirmed that *Phox2b*, which encodes a homeodomain transcription factor, is expressed in progenitors and mature fbm neurones in r4 as well as during their migration through r5 and into r6 (Pattyn et al., 1997, 2000; Fig. 2A,B). Its expression was maintained in the forming facial motor nucleus at E14.5 (Pattyn et al., 1997; data not shown). In contrast to this expression pattern covering the entire migratory process, several genes were expressed by fbm neurones only during specific phases of the migration. *TAG-1*, which encodes a cell adhesion molecule of the immunoglobulin family (Dodd et al., 1988), was expressed in migrating fbm neurones in r4, r5 and r6 at E11.5 (Figs 1C,D, 2C). However, its expression was specifically downregulated in neurones migrating dorsally within r6 at E12.5 (compare Fig. 2C,D with 2A,B). *Ret*, which encodes a GDNF receptor subunit (Durbec et al., 1996a; Trupp et al., 1996), was expressed in r4 in a subset of ventral cells but not in fbm neurones at E11.5 (Fig. 2E and data not shown). However, *Ret* RNA was detected in fbm neurones located more caudally, in r5 and r6 (Fig. 2E). Double in situ hybridisation with an *Hoxb1* probe, which specifically labels r4 (Murphy et al., 1989), indicated that fbm neurones switch on *Ret* precisely

when they exit r4 to enter r5 (compare Fig. 2E and 2G). At E12.5, *Ret* expression was maintained in fbm neurones migrating in r5 and r6 where they form the facial motor nucleus (Fig. 2F). Finally, *Cdh8*, which encodes an adhesion molecule of the cadherin family (Tanihara et al., 1994) was first expressed at E12 in fbm neurones migrating away from the midline towards the dorsal region in r6 (Korematsu and Redies, 1997; data not shown). This expression was maintained from E12.5 to E14.5 in fbm neurones finishing their migration in r6 and forming the facial motor nucleus (Fig. 2H and data not shown).

In conclusion, fbm neurones display dynamic patterns of gene expression during their migration. They switch on *Ret* when they exit r4 and enter r5, downregulate *TAG-1* when they migrate dorsally in r6 and turn on *cadherin-8* during the final phase of their migration within r6 (see Fig. 8). The conservation of this precise position-specific pattern of expression between E11.5 and E12.5 indicates that early and late migrating neurones display the same expression profile during their migration. Furthermore, the sharp transitions observed in *TAG-1*, *Ret* and *Cdh8* expression suggest that fbm neurones may regulate the expression of these genes according to their position in the hindbrain, possibly in response to the rhombomeric environment. To test this hypothesis, we investigated how fbm neurones behave when this environment is affected.

r6 cues control the dorsal migration of fbm neurones and their pattern of gene expression

To analyse the influence of the environment on the migration of fbm neurones, we studied mouse mutants that modify the territories through or into which fbm neurones progress but presumably do not directly affect the migrating cells themselves. The *kreisler* (*kr*) mutation leads to the disappearance of r5 and, although the interpretation of the phenotype has been controversial (Frohman et al., 1993; Cordes and Barsh, 1994; McKay et al., 1994, 1997), recent results indicate that an r6-like (r6*) territory is maintained in *kr/kr* embryos (Manzanares et al., 1999). *Krox20*$^{-/-}$ embryos also lack r5, but have no apparent defects in r6 (Schneider-Maunoury et al., 1993; Swiatek and Gridley, 1993; Schneider-Maunoury et al., 1997; Seitanidou et al., 1997). It has been shown that in *kr/kr* embryos, vm facial neurones are not present and fbm neurones migrate dorsally within r6* (McKay et al., 1997; Manzanares et al., 1999). Using the *Phox2b* marker, we confirmed that fbm neurones migrated dorsally after exiting r4 (Fig. 3A,A'). However fbm neurones formed abnormal thick streams during this dorsal migration (Fig. 3A,A'), which may be due to the abnormality of r6*. Analysis of *Ret* expression at E14.5 showed that early dorsal migration resulted in anterior displacement of the facial motor nucleus (Fig. 3E,E').

As in *kreisler* mutants, in *Krox20*$^{-/-}$ embryos fbm neurones migrated dorsally within the r6 territory, immediately after exiting r4, as demonstrated by DiI injections into the root of the VIIth nerve at E12.5 (Fig. 4A,A'). This early dorsal migration led also to an anteriorly positioned facial motor nucleus (data not shown). In both *Krox20*$^{-/-}$ and *kr/kr* embryos, *Ret* was activated in fbm neurones as soon as they exited r4 and migrated into r6/r6* (Figs 3C,C', 4C,C'), *TAG-1* was downregulated during the dorsal migration (Figs 3B,B', 4B,B') and *Cdh8* was expressed in the final phase of the migration (Figs 3D,D', 4D,D').

In conclusion, although the two mutants lack r5, fbm neurones are able to migrate dorsally in the r6 territory after exiting r4 and express a combination of genes characteristic of r6. These results indicate that environmental cues in r6, rather than an internal

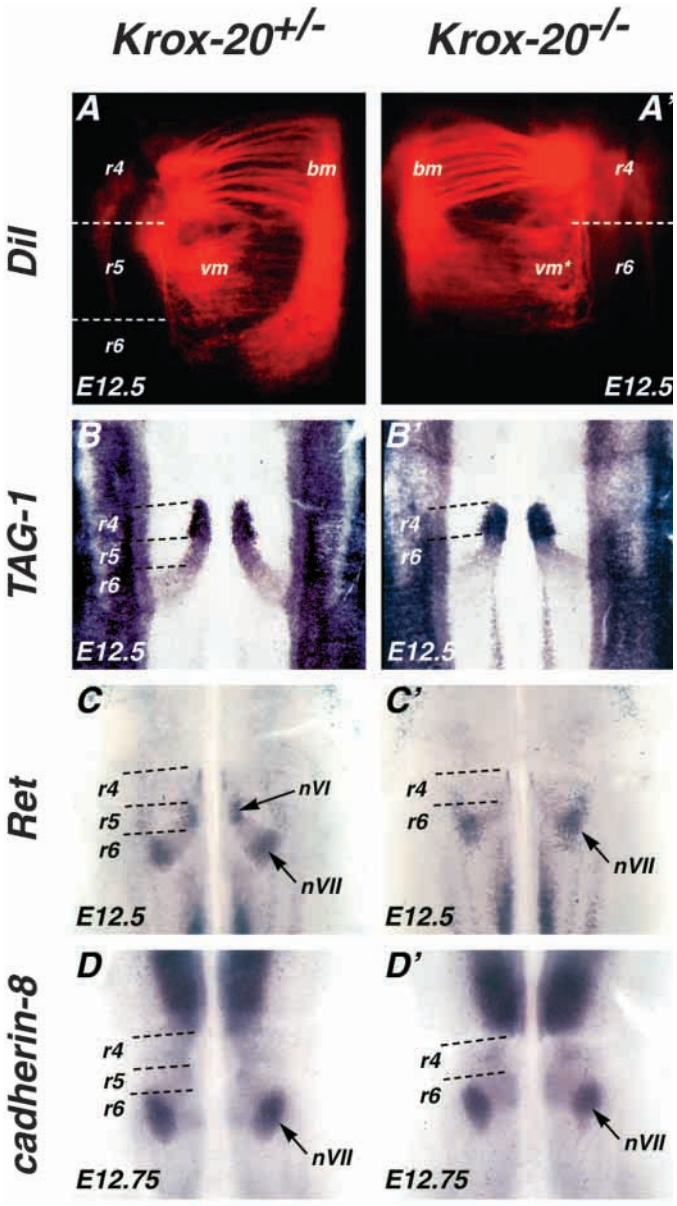


Fig. 4. Analysis of fbm migration and gene expression in *Krox20*$^{-/-}$ embryos. Heterozygous (left) and homozygous (right) mutant embryos are presented. (A,A') Flat-mounted E12.5 half hindbrains (mirror images) injected with DiI into the root of the VIIth nerve showing bm neurones migrating into the anteriorly positioned r6 in the homozygous mutant. This rhombomere contains inappropriately projecting vm neurones (vm*). (B-D') Flat-mounts of embryos subjected to in situ hybridisation as indicated (stage, probe). In both heterozygous and homozygous embryos, *TAG-1* expression is downregulated in fbm neurones migrating dorsally within r6 (B,B'), *Ret* expression is detected in cells leaving r4 (C,C') and *Cdh8* (cadherin-8) expression is detected in fbm neurones finishing their migration in r6 (D,D'). bm, branchiomotor neurones; nVI, abducens nucleus; nVII, facial motor nucleus; r, rhombomere; vm, visceromotor neurones.

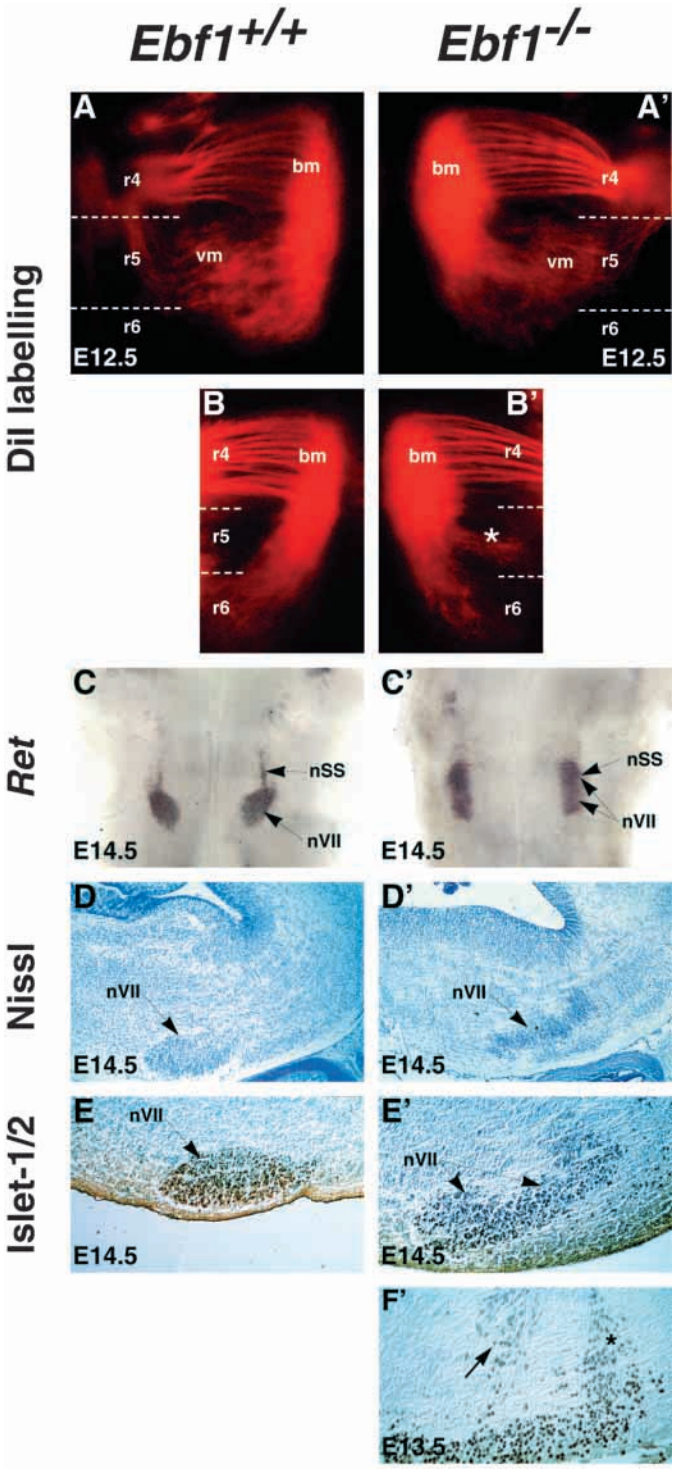


Fig. 5. Fbm neurones migration is specifically disturbed in *Ebf1*$^{-/-}$ embryos. Wild-type (left) and homozygous mutant (right) embryos are presented. (A,A', mirror images) Retrograde labelling of fbm and vm neurones at E12.5. The morphology, axonal pathway and lateral migration of vm neurones in r5 is not affected by the mutation, whereas in *Ebf1*$^{-/-}$ embryos fbm neurones appear to accumulate in the ventral region of r4 and an abnormal stream of cells migrating dorsally within r5 is observed. (B,B', mirror images) Specific labelling of fbm neurones confirms that a subpopulation of fbm neurones migrates dorsally within r5 (asterisk in B'). (C,C') Flat-mounted E14.5 hindbrains subjected to in situ hybridisation with the *Ret* probe. In *Ebf1*$^{-/-}$ embryos, the facial motor nucleus appears anteriorly elongated and overlaps with the superior salivatory nucleus. (D-E') E14.5 parasaggital sections, Nissl-stained (D,D') or processed for Islet 1/2 immunohistochemistry (E,E'), showing that the facial motor nucleus has an elongated shape in *Ebf1*$^{-/-}$ embryos. (F') Islet 1/2 immunohistochemistry performed at E13.5 indicates that this deformation results from the migration of fbm neurones in two streams, one located at the appropriate position (arrow) and the other more rostrally (asterisk). bm, branchiomotor neurones; nSS, superior salivatory nucleus; nVII, facial motor nucleus; r, rhombomere; vm, visceromotor neurones.

clock, control the dorsal migration of fbm neurones. Furthermore, they show that these cells regulate their pattern of gene expression in response to the changing environment.

Ebf1 inactivation affects the migration of fbm neurones

As indicated (see Introduction), *Ebf1* is expressed in fbm neurones in r4 and during their entire migratory process (Fig. 1E and data not shown; Garel et al., 1997; Pattyn et al., 2000). In contrast, the two other members of the mouse *Ebf* family, *Ebf2* and *Ebf3*, are expressed in the progenitors of fbm neurones, but not in mature neurones or during their migration (Fig. 1F,G and data not shown; Garel et al., 1997; Pattyn et al., 2000). In a previous study, we have shown that in the embryonic striatum, where *Ebf1* is the only family member expressed, it controls early neuronal differentiation (Garel et al., 1999). These data led us to analyse *Ebf1*$^{-/-}$ embryos (Lin and Grosschedl, 1995), to investigate whether *Ebf1* controls aspects of fbm neurone migration. We first performed retrograde labelling experiments involving DiI injection into the root of the VIIth nerve. At E11.5, both bm and vm populations were normally positioned in *Ebf1*$^{-/-}$ embryos (data not shown), suggesting that the migration of fbm neurones was not affected at this stage. In contrast, at E12.5, while the position of vm neurones was still normal, the migration of fbm neurones appeared severely affected (compare Fig. 5A and 5A'). We observed an accumulation of fbm neurones in the ventral region of r4 and an ectopic stream of cells progressing dorsally within r5 (Fig. 5A'), which was more clearly visible upon DiI injection into the facial nerve branch, which only labelled fbm projections (Auclair et al., 1996) (Fig. 5B,B'). Therefore the *Ebf1* mutation affects the migration of part of the fbm population, leading to abnormal dorsal migration within r5.

To study the consequences of this migratory defect, the morphology of the post-migratory facial motor nucleus was examined. At E14.5, in wild-type embryos, the facial motor nucleus forms a round structure, located caudally to the superior salivatory nucleus formed by the vm neurones that have migrated dorsally into r5 (Fig. 1A; Auclair et al., 1996; Studer et al., 1996; McKay et al., 1997; Schneider-Maunoury et al., 1997). Both nuclei are *Ret* positive at this stage (Fig. 5C). *Ret* in situ hybridisation in *Ebf1*$^{-/-}$ embryos indicated that the facial motor nucleus presented an elongated shape, with a large abnormal anterior expansion in the r5 derived territory, overlapping with the superior salivatory nucleus (Fig. 5C'). Parasagittal sections processed for Nissl staining or Islet 1/2 immunohistochemistry, which labels motoneurones (Ericson et

al.. 1992), confirmed this observation (Fig. 5D-E'). Moreover, Islet 1/2 immunohistochemistry performed earlier at E13.5 indicated that the elongated shape of the facial nucleus was due to the existence of two streams of fbm neurones, one located at the expected position and another one more rostrally (Fig. 5F'). Together these data indicate that in *Ebf1*$^{-/-}$ embryos the facial motor nucleus expands anteriorly, owing to the abnormal dorsal migration within r5 of a significant proportion of fbm neurones.

Hindbrain patterning and fbm neuronal differentiation appears unaffected in *Ebf1* mutants

In the hindbrain, the expression of *Ebf1* is not restricted to migrating fbm neurones and the gene is also transiently expressed in numerous populations of differentiating cells, including motoneurones and commissural neurones (Fig. 1E; Garel et al., 1997; Wang et al., 1997). Since in *Ebf1*$^{-/-}$ embryos a subpopulation of fbm neurones behave in r5 like wild-type fbm neurones in r6, we investigated whether this mutation would affect hindbrain patterning in the r4-r5 region or fbm cell differentiation. Expression of *Hoxb1*, a specific marker of r4 (Murphy et al., 1989), and of *Epha4*, which is specifically expressed in r2, r3 and r5 (Gilardi-Hebenstreit et al., 1992), were not modified in *Ebf1* null embryos (Fig. 6A-B'), suggesting that general hindbrain patterning was not affected.

To investigate whether *Ebf1* plays a role in fbm neuronal differentiation, we examined the expression of *Islet1* (*Isl1*), a general motoneurone marker (Ericson et al., 1992), and *Phox2b*, which labels differentiating fbm neurones (Pattyn et al., 1997, 2000). From E10.75 to E11.5, *Isl1* and *Phox2b* were not affected in *Ebf1*$^{-/-}$ embryos (Fig. 6C-D'). In addition, the expression of *Ebf2* and *Ebf3* was also not modified in *Ebf1*$^{-/-}$ embryos (data not shown). In conclusion, these data indicate that the *Ebf1* mutation does not grossly affect hindbrain patterning and early neuronal differentiation of fbm neurones.

Modifications of gene expression in fbm neurones correlate with migratory defects

To test whether the r5 fbm dorsal migration in the *Ebf1* mutant was related to abnormal gene expression, we examined the transcription of *Phox2b*, *TAG-1*, *Ret* and *Cdh8*. At E11.5, when fbm migration was not yet affected, *Ret* expression was normally induced in cells migrating in r5 (data not shown), while the level of *TAG-1* expression was slightly reduced in these cells, as compared with the wild type (Fig. 7A,A'). At E12.5, examination of *Phox2b* expression confirmed the existence of migration defects: two streams were observed progressing dorsally in r5 and r6. The reinforcement of the labelling in r4 suggested a possible delayed migration of fbm cells from this rhombomere (Fig. 7B,B'). At this stage, drastic anomalies in *TAG-1*, *Ret* and *Cdh8* expression were also observed in fbm neurones. *TAG-1* was prematurely downregulated in cells that exited r4 (Fig. 7C,C'). *Ret* was normally expressed in cells migrating in r5 and r6, but it was prematurely activated in some fbm neurones in r4 (Fig. 7D,D'). Finally, *Cdh8* was found to be expressed in cells located in ventral r4 and r5, in addition to cells migrating dorsally in r5 and r6 (Fig. 7E,E').

In conclusion, in *Ebf1*$^{-/-}$ embryos, a subpopulation of fbm neurones adopt an r5- or r6-specific expression pattern, while still located in r4 or r5 respectively. There is nevertheless a perfect correlation between gene expression pattern and migratory behaviour, since these cells perform an r6-like dorsal migration in r5 (Fig. 8). Furthermore, the molecular defects are likely to precede the modification in migration, since their first manifestations are observed in r4. Taken together, these data suggest that some fbm neurones in *Ebf1*$^{-/-}$ embryos are unable to interpret their local environment correctly and raise the possibility that the inappropriate expression of molecules like TAG-1, Ret and cadherin 8 may play a role in their abnormal migration.

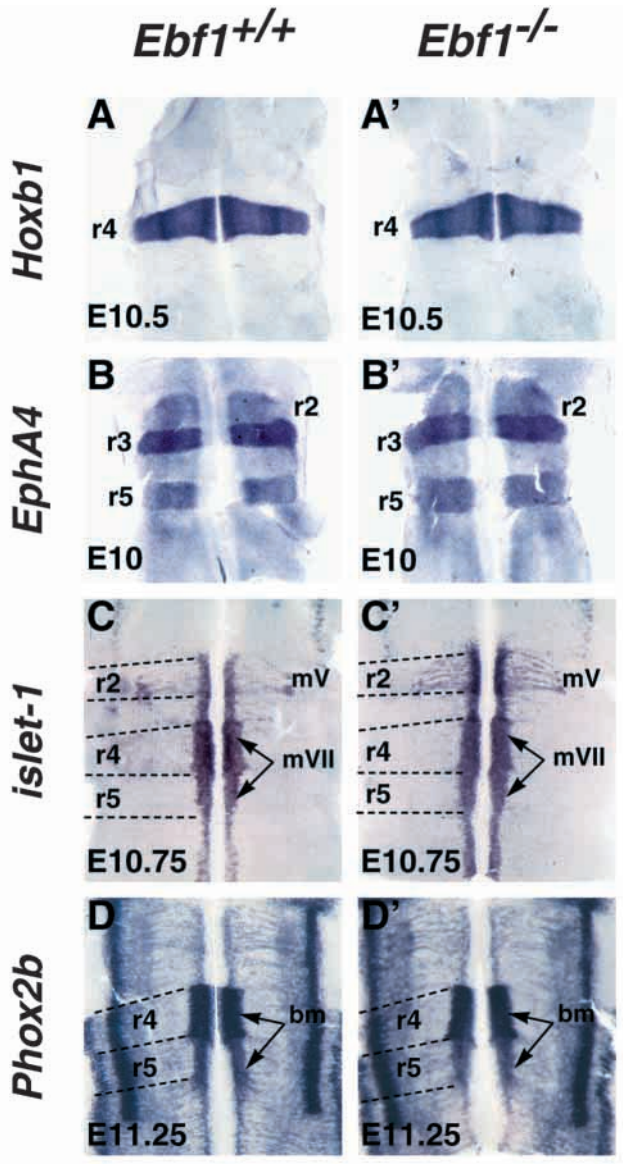


Fig. 6. Hindbrain segmentation and fbm neurones specification are not affected by *Ebf1* inactivation. Hindbrains from wild-type (left) and homozygous mutant (right) embryos were processed for in situ hybridisation as indicated (stage, probe) and flat-mounted. The expression patterns of *Hoxb1* (A,A'), *Epha4* (B,B'), *Isl1* (islet-1; C,C') and *Phox2b* (D,D') are not modified in *Ebf1*$^{-/-}$ embryos. bm, branchiomotor neurones; mV, trigeminal motor neurones; mVII, facial motor neurones; r, rhombomere.

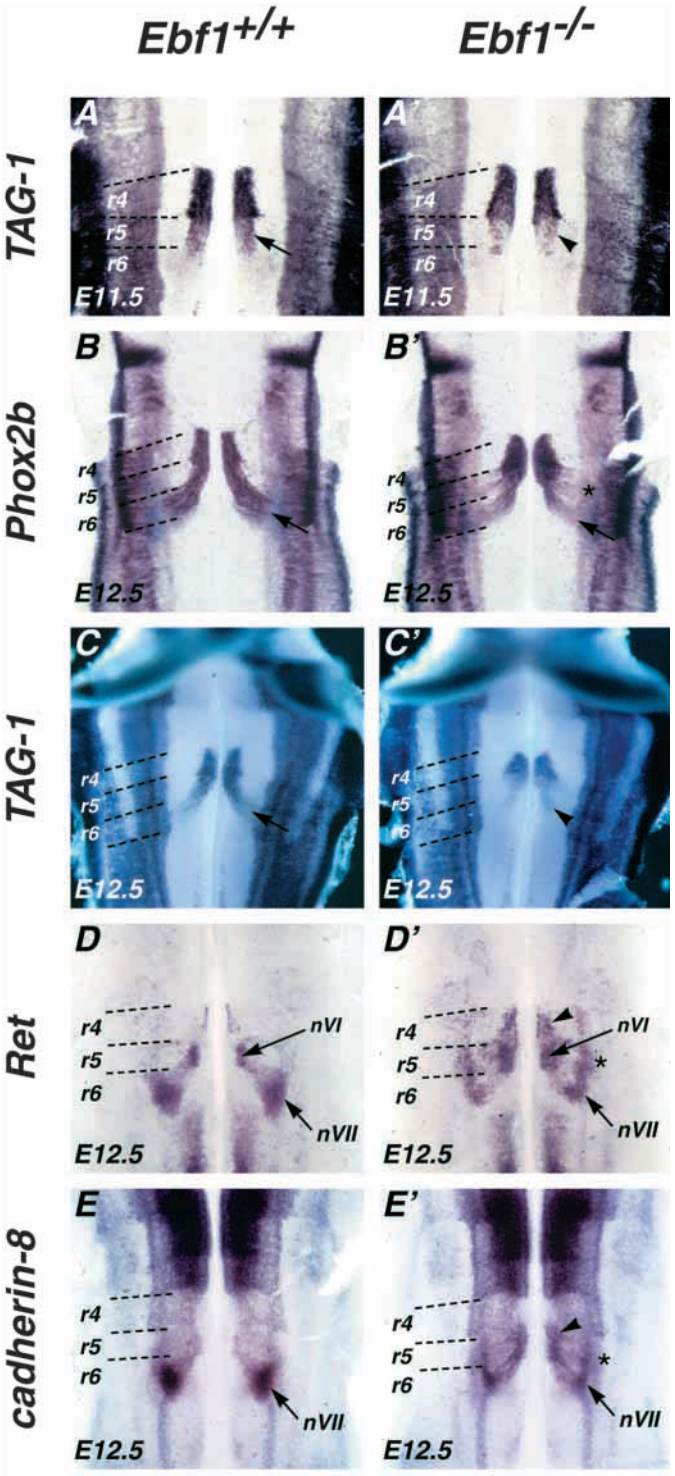


Fig. 7. The *Ebf1* mutation affects the pattern of gene expression in migrating fbm neurones. Wild type (left) and homozygous mutant (right) E11.5-12.5 embryos were processed for in situ hybridisation with the indicated probes. (A,A') At E11.5 *TAG-1* expression in migrating fbm neurones is slightly reduced in mutant (arrowhead) as compared with wild-type (arrow) embryos. (B,B') At E12.5 *Phox2b* labelling identifies a subpopulation of fbm neurones abnormally migrating in r5 in *Ebf1*$^{-/-}$ embryos (asterisk in B') as well as some fbm neurones normally migrating in r6 (arrow). In addition, a reinforcement of the labelling is observed in r4. (C,C') The level of *TAG-1* expression in cells migrating out of r4 (arrow in C) is clearly reduced in *Ebf1*$^{-/-}$ embryos (arrowhead in C'). (D,D') *Ret* expression, which is normally restricted to fbm neurones in r5 and r6, is observed in r4 in *Ebf1*$^{-/-}$ embryos (arrowhead in D'). In addition, the forming facial motor nucleus presents an elongated shape in homozygous mutant embryos, with cells mislocated at the level of r5 (asterisk in D'). (E,E') *Cdh8* (cadherin-8) is abnormally expressed in some fbm neurones located close to the ventral midline in r4 and r5 in *Ebf1*$^{-/-}$ embryos (arrowhead in E') and the forming facial motor nucleus shows an elongated shape (asterisk in E'). nVI, abducens nucleus; nVII, facial motor nucleus; r, rhombomere.

DISCUSSION

In this paper, we have investigated the migration of facial branchiomotor neurones and followed the expression of several migration markers in different mouse mutants. Our work demonstrates that these cells adapt their pattern of gene expression according to their local environment and supports the idea that these elicited responses of the migrating cells are required for the selection of the appropriate migratory pathways.

Migrating fbm neurones regulate gene expression in response to their environment

It has been proposed that cells undergoing complex migrations might respond to successive environmental cues by modifying some of their intrinsic properties, including their gene expression pattern, to follow the appropriate pathway. However, such an adaptation by migrating cells has not been investigated in detail in the vertebrate CNS so far. The migration of fbm neurones constitutes an attractive model to study this issue, since these cells progress through successive rhombomeres and adopt different trajectories within these well-defined territories. In the present study, we have shown that fbm cells express specific combinations of markers such as *Ret*, *TAG-1* and *Cdh8* during each phase of their migration (Fig. 8). Analysis of segmentation mutants lacking r5 (*Krox20*, *kreisler*) indicated that fbm neurones migrate dorsally after exiting r4 and express a combination of genes characteristic of r6 (Fig. 8). These data indicate that r6-specific cues control the dorsal migration and that no conditioning or change in gene expression that occurs in r5 is essential for further migration. Furthermore, they demonstrate that the r6-specific gene expression pattern is dictated by the location of migrating cells and not simply activated according to a prefixed chronological program. Overall, we show that fbm neurones adapt to their changing environment and respond to it by regulating their gene expression pattern.

Misinterpretation of the rhombomeric environment by fbm neurones in *Ebf1*$^{-/-}$ embryos

In *Ebf1*$^{-/-}$ embryos, a subpopulation of fbm neurones prematurely activate *Ret* expression in r4, which occurs normally only in r5 in wild-type embryos (Fig. 8). Similarly, these cells downregulate *TAG-1* and activate *Cdh8* in r5, while these subsequent modifications in gene expression normally occur only after the cells have reached r6 (Fig. 8). These data indicate that in *Ebf1* null embryos there is a shift between the pattern of gene expression of fbm neurones and their rhombomeric location as compared with wild-type embryos. In contrast, since this subpopulation of fbm neurones migrate

dorsally in r5 as if they were in r6, the association between the pattern of gene expression of fbm neurones and their migratory behaviour is maintained. Interestingly, the shift between location and gene expression is first detected in r4, before selection of an abnormal migratory pathway in r5. This suggests that modifications in gene expression in fbm cells reflects their interpretation (erroneous in *Ebf1*$^{-/-}$ embryos) of the local environment and influences their subsequent migratory pathway.

Role of Ebf1 in the control of fbm neurone migration

Since *Ebf1* expression is not restricted to fbm neurones in the hindbrain, the misinterpretation of their rhombomeric environment by fbm neurones in *Ebf1* null embryos raises the question of the cell autonomy of the phenotype: are fbm neurones affected in their capacity to appropriately evaluate their environment or is the environment modified, providing them with misleading cues? Although we cannot discard the second hypothesis, we favour the first one for the following reasons: (1) no modification in expression of regional markers indicative of a change of identity in r4 or r5 was observed; (2) we did not detect any other migratory defect in the hindbrain among vm, somatomotor (including the abducens nucleus) or other branchiomotor neurone populations, suggesting that only fbm neurones are affected; (3) the existence of an inappropriate response of fbm neurones is reminiscent of another phenotype associated with the *Ebf1* mutation in the embryonic striatum, where differentiating neurones do not downregulate the expression of early genes, leading to a discrepancy between the location of the cells and their pattern of gene expression (Garel et al., 1999).

The precise function of Ebf1 is still the object of speculations. The r6-like migratory behaviour of fbm neurones in r5 in *Ebf1*$^{-/-}$ embryos suggest that similar cues involved in guiding the neurones are present in r5 and r6. In wild-type embryos, these cues may not be recognised by migrating fbm neurones because these cells do not express the appropriate combination of genes. We propose that a function of Ebf1 is to prevent the establishment of the r6-specific pattern of expression in fbm cells until a signal provided in r6 releases this block. Therefore, in *Ebf1*$^{-/-}$ embryos, fbm cells express the r6 pattern prematurely, while still in r4 or r5, and engage into a lateral migration in r5. Since only a part of fbm cells migrate laterally in r5, the Ebf1-controlled blocking mechanism is likely to be partially redundant with another, unknown system. An alternative hypothesis is that Ebf1 plays a more general role in the control of neuronal differentiation of fbm neurones and that its mutation leads to premature differentiation. Although we cannot exclude this possibility, the absence of modification of *Phox2b*, *Isl1*, *Ebf2* and *Ebf3* expression suggests that at least the initial steps of fbm cell differentiation are not affected.

Possible involvement of TAG-1, Ret and cadherin 8 in the control of fbm neurone migration

The correlation between the patterns of expression of *TAG-1*, *Ret* and *Cdh8* in fbm neurones and their migratory behaviour is conserved in the mutant backgrounds that we have studied. This raises the possibility that the corresponding gene products may actually participate in establishing pathways of migration appropriate for each rhombomeric environment. Indeed, *TAG-1* and *Cdh8* encode cell-surface molecules that have been implicated in axonal outgrowth and cell adhesion (Furley et al., 1990; Redies and Takeichi, 1996). *Ret*, which encodes a GDNF-receptor subunit, is expressed by several populations of migrating cells (Pachnis et al., 1993; Durbec et al., 1996b) and may also be involved in migratory processes (Tang et al., 1998; Natarajan et al., 1999). During their migration in r5 and r6, fbm neurones are likely to interact successively with different substrates: they may first follow longitudinal fibre bundles present on both sides of the floor plate in r5, then progress through the mantle in r6 and finally interact with radial glia in r6. Such changes of migratory substrates are likely to require appropriate modifications in the adhesion properties of fbm neurones. TAG-1 might be involved in the tangential caudal migration of fbm cells within r5. It is expressed at the right location and it has been shown to interact with another cell adhesion molecule, L1, which is present on the longitudinal fibres mentioned above (Dodd et al., 1988; Kuhn et al., 1991) and has recently been shown to modulate the repulsion/attraction response to Sema3A, a specific guidance cue (Castellani et al., 2000). According to its site of expression, *Cdh8* might be involved in the radial

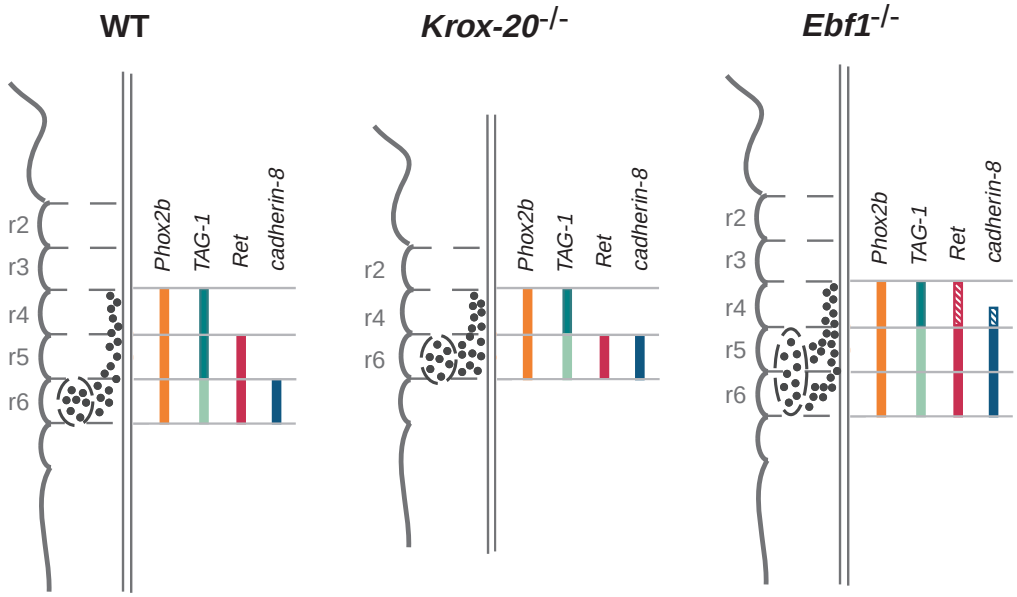


Fig. 8. The migration of fbm neurones and their correlated modifications in patterns of gene expression in the hindbrain of wild-type, *Krox20*$^{-/-}$ and *Ebf1*$^{-/-}$ embryos. The intensity of the colour (*TAG-1*) reflects the level of gene expression. Hatched pattern (*Ret*, *cadherin-8*) indicates that not all cells are positive within the territory.

migration within r6. Finally, in *Ebf1* mutants, the abnormal lateral migration of fbm neurones in a number of separate streams may reflect defective adhesion between the fbm neurones themselves. This could in turn affect the response of fbm neurones to guidance cues.

Conclusion

On the basis of the present work, we propose a model for the control of fbm neurone migration involving a constant dialogue between the migrating cells and their environment. Within each rhombomere, fbm neurones encounter specific cues, that they interpret according to a pre-established program. Their responses involve modifications in gene expression, including changes in adhesion properties, which in turn participate in the selection of the local migratory pathway. We provide evidence that *Ebf1* may contribute to the pre-established interpretation program, since its mutation affects the response of fbm neurones to the rhombomeric environment, and we propose that modifications in *TAG-1*, *Ret*, and *Cdh8* expression, which are part of the elicited responses, are actually involved in the elaboration of the migratory pathways. The existence of such a dialogue between local cues and elicited responses of the migrating cells is likely to be of general significance and to explain complex migratory patterns by successive adaptation steps.

We are grateful to R. Grosschedl for the kind gift of the *Ebf1* mutant mouse line. We thank members of the Charnay and Rubenstein labs for helpful comments on this work; and R. Grosschedl, F. Helmbacher, R. Hevner, F. Mechta, J. Nardelli, M. Studer, M. Wassef and K. Yun for stimulating discussions and critical reading of the manuscript. We are grateful to J.-F. Brunet, C. Goridis, D. Karagogeos, V. Pachnis, C. Redies, F. Sedel, M. Wassef and D. Wilkinson for the kind gift of probes, and to J. Lewis for providing the *kr* mouse line. S. G. was supported by fellowships from the ARC and the SSAS (Société de Secours des Amis des Sciences) and M. G.-D. was supported by a FEBS fellowship. This work was supported by grants from INSERM, MENRT, EC, ARC and AFM.

REFERENCES

Ackerman, S. L., Kozak, L. P., Przyborski, S. A., Rund, L. A., Boyer, B. B. and Knowles, B. B. (1997). The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. *Nature* **386**, 838-842.

Alcantara, S., Ruiz, M., De Castro, F., Soriano, E. and Sotelo, C. (2000). Netrin 1 acts as an attractive or as a repulsive cue for distinct migrating neurons during the development of the cerebellar system. *Development* **127**, 1359-1372.

Altman, J. and Bayer, S. A. (1982). Development of the cranial nerve ganglia and related nuclei in the rat. *Adv Anat. Embryol. Cell Biol.* **74**, 1-90.

Artigiani, S., Comoglio, P. M. and Tamagnone, L. (1999). Plexins, semaphorins, and scatter factor receptors: a common root for cell guidance signals? *IUBMB Life* **48**, 477-482.

Ashwell, K. W. and Watson, C. R. (1983). The development of facial motoneurones in the mouse – neuronal death and the innervation of the facial muscles. *J. Embryol. Exp. Morphol.* **77**, 117-141.

Auclair, F., Valdes, N. and Marchand, R. (1996). Rhombomere-specific origin of branchial and visceral motoneurons of the facial nerve in the rat embryo. *J. Comp. Neurol.* **369**, 451-461.

Bell, E., Wingate, R. J. and Lumsden, A. (1999). Homeotic transformation of rhombomere identity after localized Hoxb1 misexpression. *Science* **284**, 2168-2171.

Birchmeier, C. and Gherardi, E. (1998). Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. *Trends Cell Biol.* **8**, 404-410.

Bloch-Gallego E., Ezan, F., Tessier-Lavigne, M. and Sotelo, C. (1999). Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons. *J. Neurosci.* **19**, 4407-4420.

Brose, K. and Tessier-Lavigne, M. (2000). Slit proteins: key regulators of axon guidance, axonal branching, and cell migration. *Curr. Opin. Neurobiol.* **10**, 95-102.

Castellani, V., Chedotal, A., Schachner, M., Faivre-Sarrailh, C. and Rougon G. (2000). Analysis of the L1-deficient mouse phenotype reveals cross-talk between Sema3A and L1 signaling pathways in axonal guidance. *Neuron* **27**, 237-249.

Cordes, S. P. and Barsh, G. S. (1994). The mouse segmentation gene kr encodes a novel basic domain-leucine zipper transcription factor. *Cell* **79**, 1025-1034.

Dodd, J., Morton, S. B., Karagogeos, D., Yamamoto, M. and Jessell, T. M. (1988). Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron* **1**, 105-116.

Durbec, P., Marcos-Gutierrez, C. V., Kilkenny, C., Grigoriou, M., Wartiowaara, K., Suvanto, P., Smith, D., Ponder, B., Costantini, F., Saarma, M. et al. (1996a). GDNF signalling through the Ret receptor tyrosine kinase. *Nature* **381**, 789-793.

Durbec, P. L., Larsson-Blomberg, L. B., Schuchardt, A., Costantini, F. and Pachnis, V. (1996b). Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. *Development* **122**, 349-358.

Ericson, J., Thor, S., Edlund, T., Jessell, T. M. and Yamada, T. (1992). Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1. *Science* **256**, 1555-1560.

Frohman, M. A., Martin, G. R., Cordes, S. P., Halamek, L. P. and Barsh, G. S. (1993). Altered rhombomere-specific gene expression and hyoid bone differentiation in the mouse segmentation mutant, kreisler (kr). *Development* **117**, 925-936.

Furley, A. J., Morton, S. B., Manalo, D., Karagogeos, D., Dodd, J. and Jessell, T. M. (1990). The axonal glycoprotein TAG-1 is an immunoglobulin superfamily member with neurite outgrowth-promoting activity. *Cell* **61**, 157-170.

Garel, S., Marin, F., Mattei, M. G., Vesque, C., Vincent, A. and Charnay, P. (1997). Family of Ebf/Olf-1-related genes potentially involved in neuronal differentiation and regional specification in the central nervous system. *Dev. Dyn.* **210**, 191-205.

Garel, S., Marin, F., Grosschedl, R. and Charnay, P. (1999). Ebf1 controls early cell differentiation in the embryonic striatum. *Development* **126**, 5285-5294.

Gilardi-Hebenstreit, P., Nieto, M. A., Frain, M., Mattei, M. G., Chestier, A., Wilkinson, D. G. and Charnay, P. (1992). An Eph-related receptor protein tyrosine kinase gene segmentally expressed in the developing mouse hindbrain. *Oncogene* **7**, 2499-2506.

Goddard, J. M., Rossel, M., Manley, N. R. and Capecchi, M. R. (1996). Mice with targeted disruption of Hoxb-1 fail to form the motor nucleus of the VIIth nerve. *Development* **122**, 3217-3228.

Hagman, J., Travis, A. and Grosschedl, R. (1991). A novel lineage-specific nuclear factor regulates mb-1 gene transcription at the early stages of B cell differentiation. *EMBO J.* **10**, 3409-3417.

Hagman, J., Belanger, C., Travis, A., Turck, C. W. and Grosschedl, R. (1993). Cloning and functional characterization of early B-cell factor, a regulator of lymphocyte-specific gene expression. *Genes Dev.* **7**, 760-773.

Helmbacher, F., Pujades, C., Desmarquet, C., Frain, M., Rijli, F., Chambon, P. and Charnay, P. (1998). *Hoxa-1* and *Krox-20* synergize to control the development of rhombomere 3. *Development* **125**, 4739-4748.

Korematsu, K. and Redies, C. (1997). Restricted expression of cadherin-8 in segmental and functional subdivisions of the embryonic mouse brain. *Dev. Dyn.* **208**, 178-189.

Kuhn, T. B., Stoeckli, E. T., Condrau, M. A., Rathjen, F. G. and Sonderegger, P. (1991). Neurite outgrowth on immobilized axonin-1 is mediated by a heterophilic interaction with L1(G4). *J. Cell Biol.* **115**, 1113-1126.

Lin, H. and Grosschedl, R. (1995). Failure of B-cell differentiation in mice lacking the transcription factor EBF. *Nature* **376**, 263-267.

Lumsden, A. and Keynes, R. (1989). Segmental patterns of neuronal development in the chick hindbrain. *Nature* **337**, 424-428.

Lumsden, A. (1990). The cellular basis of segmentation in the developing hindbrain. *Trends Neurosci.* **13**, 329-335.

Manzanares, M., Trainor, P. A., Nonchev, S., Ariza-McNaughton, L., Brodie, J., Gould, A., Marshall, H., Morrison, A., Kwan, C. T., Sham, M. H. et al. (1999). The role of kreisler in segmentation during hindbrain development. *Dev. Biol.* **211**, 220-237.

Marcus, R. C., Blazeski, R., Godement, P. and Mason, C. A. (1995). Retinal axon divergence in the optic chiasm: uncrossed axons diverge from crossed axons within a midline glial specialization. *J. Neurosci*. **15**, 3716-3729.

Marin, F. and Puelles, L. (1995). Morphological fate of rhombomeres in quail/chick chimeras: a segmental analysis of hindbrain nuclei. *Eur. J. Neurosci.* **7**, 1714-1738.

Mathis, L., Sieur, J., Voiculescu, O., Charnay, P. and Nicolas, J. (1999). Successive patterns of clonal cell dispersion in relation to neuromeric subdivision in the mouse neuroepithelium. *Development* **126**, 4095-4106.

McKay, I. J., Muchamore, I., Krumlauf, R., Maden, M., Lumsden, A. and Lewis, J. (1994). The kreisler mouse: a hindbrain segmentation mutant that lacks two rhombomeres. *Development* **120**, 2199-2211.

McKay, I. J., Lewis, J. and Lumsden, A. (1997). Organization and development of facial motor neurons in the kreisler mutant mouse. *Eur. J. Neurosci.* **9**, 1499-1506.

Murphy, P., Davidson, D. R. and Hill, R. E. (1989). Segment-specific expression of a homoeobox-containing gene in the mouse hindbrain. *Nature* **341**, 156-159.

Nardelli, J., Thiesson, D., Fujiwara, Y., Tsai, F. Y. and Orkin, S. H. (1999). Expression and genetic interaction of transcription factors GATA-2 and GATA-3 during development of the mouse central nervous system. *Dev. Biol.* **210**, 305-321.

Natarajan, D., Grigoriou, M., Marcos-Gutierrez, C. V., Atkins, C. and Pachnis, V. (1999). Multipotential progenitors of the mammalian enteric nervous system capable of colonising aganglionic bowel in organ culture. *Development* **126**, 157-168.

Pachnis, V., Mankoo, B. and Costantini, F. (1993). Expression of the c-ret proto-oncogene during mouse embryogenesis. *Development* **119**, 1005-1017.

Pata, I., Studer, M., van, D. J., Briscoe, J., Kuuse, S., Engel, J. D., Grosveld, F. and Karis, A. (1999). The transcription factor GATA3 is a downstream effector of Hoxb1 specification in rhombomere 4. *Development* **126**, 5523-5531.

Pattyn, A., Morin, X., Cremer, H., Goridis, C. and Brunet, J. F. (1997). Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis. *Development* **124**, 4065-4075.

Pattyn, A., Hirsch, M., Goridis, C. and Brunet, J. F. (2000). Control of hindbrain motor neuron differentiation by the homeobox gene Phox2b. *Development* **127**, 1349-1358.

Redies, C. and Takeichi, M. (1996). Cadherins in the developing central nervous system: an adhesive code for segmental and functional subdivisions. *Dev. Biol.* **180**, 413-423.

Robinson, V., Smith, A., Flenniken, A. M. and Wilkinson, D. G. (1997). Roles of Eph receptors and ephrins in neural crest pathfinding. *Cell Tiss. Res.* **290**, 265-274.

Sandell, J. H. and Masland, R. H. (1988). Photoconversion of some fluorescent markers to a diaminobenzidine product. *J. Histochem. Cytochem.* **36**, 555-559.

Schneider-Maunoury, S., Topilko, P., Seitandou, T., Levi, G., Cohen-Tannoudji, M., Pournin, S., Babinet, C. and Charnay, P. (1993). Disruption of Krox-20 results in alteration of rhombomeres 3 and 5 in the developing hindbrain. *Cell* **75**, 1199-1214.

Schneider-Maunoury, S., Seitanidou, T., Charnay, P. and Lumsden, A. (1997). Segmental and neuronal architecture of the hindbrain of Krox-20 mouse mutants. *Development* **124**, 1215-1226.

Schneider-Maunoury, S., Gilardi-Hebenstreit, P. and Charnay, P. (1998). How to build a vertebrate hindbrain. Lessons from genetics. *C. R. Acad. Sci. Iii.* **321**, 819-834.

Seitanidou, T., Schneider-Maunoury, S., Desmarquet, C., Wilkinson, D. G. and Charnay, P. (1997). Krox-20 is a key regulator of rhombomere-specific gene expression in the developing hindbrain. *Mech. Dev.* **65**, 31-42.

Serafini, T., Colamarino, S. A., Leonardo, E. D., Wang, H., Beddington, R., Skarnes, W. C. and Tessier-Lavigne, M. (1996). Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* **87**, 1001-1014.

Studer, M., Lumsden, A., Ariza-McNaughton, L., Bradley, A. and Krumlauf, R. (1996). Altered segmental identity and abnormal migration of motor neurons in mice lacking Hoxb-1. *Nature* **384**, 630-634.

Swiatek, P. J. and Gridley, T. (1993). Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20. *Genes Dev.* **7**, 2071-2084.

Szekely, G. and Matesz, C. (1993). The efferent system of cranial nerve nuclei: a comparative neuromorphological study. *Adv. Anat. Embryol. Cell Biol.* **128**, 1-92.

Tang, M. J., Worley, D., Sanicola, M. and Dressler, G. R. (1998). The RET-glial cell-derived neurotrophic factor (GDNF) pathway stimulates migration and chemoattraction of epithelial cells. *J. Cell Biol.* **142**, 1337-1345.

Tanihara, H., Sano, K., Heimark, R. L., St John, T. and Suzuki, S. (1994). Cloning of five human cadherins clarifies characteristic features of cadherin extracellular domain and provides further evidence for two structurally different types of cadherin. *Cell Adhes. Commun.* **2**, 15-26.

Trupp, M., Arenas, E., Fainzilber, M., Nilsson, A. S., Sieber, B. A., Grigoriou, M., Kilkenny, C., Salazar-Grueso, E., Pachnis, V. and Arumae, U. (1996). Functional receptor for GDNF encoded by the c-ret proto-oncogene. *Nature* **381**, 785-789.

Wang, M. M. and Reed, R. R. (1993). Molecular cloning of the olfactory neuronal transcription factor Olf-1 by genetic selection in yeast. *Nature* **364**, 121-126.

Wang, S. S., Tsai, R. Y. and Reed, R. R. (1997). The characterization of the Olf-1/EBF-like HLH transcription factor family: implications in olfactory gene regulation and neuronal development. *J. Neurosci.* **17**, 4149-4158.

Wilkinson, D. G., Bhatt, S., Chavrier, P., Bravo, R. and Charnay, P. (1989). Segment-specific expression of a zinc-finger gene in the developing nervous system of the mouse. *Nature* **337**, 461-464.

Wingate, R. J. and Lumsden, A. (1996). Persistence of rhombomeric organisation in the postsegmental hindbrain. *Development* **122**, 2143-2152.

Yee, K. T., Simon, H. H., Tessier, L. M. and O'Leary, D. M. (1999). Extension of long leading processes and neuronal migration in the mammalian brain directed by the chemoattractant netrin-1. *Neuron* **24**, 607-622.