Development 128, 4635-4644 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV9780

The adhesion molecule TAG-1 mediates the migration of cortical interneurons from the ganglionic eminence along the corticofugal fiber system

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Accepted 31 August 2001

SUMMARY

Cortical nonpyramidal cells, the GABA-containing interneurons, originate mostly in the medial ganglionic eminence of the ventral telencephalon and follow tangential migratory routes to reach the dorsal telencephalon. Although several genes that play a role in this migration have been identified, the underlying cellular and molecular cues are not fully understood. We provide evidence that the neural cell adhesion molecule TAG-1 mediates the migration of cortical interneurons. We show that the migration of these neurons occurs along the TAG-1-expressing axons of the developing corticofugal system. The spatial and temporal pattern of expression of TAG-1 on corticofugal fibers coincides with the order of appearance of GABAergic cells in the developing cortex. Blocking the function of TAG-1, but not of L1, another adhesion molecule and binding partner of TAG-1, results in a marked reduction of GABAergic neurons in the cortex. These observations reveal a mechanism by which the adhesion molecule TAG-1, known to be involved in axonal pathfinding, also takes part in neuronal migration.

Key words: GABAergic interneurons, Tangential migration, Corticofugal fibers, Neocortex, Immunoglobulin superfamily, Adhesion molecules, TAG-1, L1, Rat, Mouse

INTRODUCTION

The two principal neuronal types of the mammalian cerebral cortex, the excitatory pyramidal cells and the inhibitory nonpyramidal neurons, are generated in distinct proliferative zones. Pyramidal neurons arise in the germinal ventricular zone (VZ) and migrate along the processes of radial glia to take up positions in the developing cortex (Rakic, 1990). Nonpyramidal cells, a diverse group of GABA-containing interneurons displaying a range of morphologies and molecular identities, originate in the ganglionic eminence (GE) of the ventral telencephalon and follow tangential migratory routes to reach the cortex (De Carlos et al., 1996; Anderson et al., 1997a; Anderson et al., 1997b; Anderson et al., 2001; Tamamaki et al., 1997).

Several lines of evidence suggest that the medial ganglionic eminence (MGE) is a major source of tangentially migrating interneurons to both the striatum and the cortex (Anderson et al., 1999; Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999; Parnavelas, 2000). On the other hand, the lateral ganglionic eminence (LGE) is mainly the source of olfactory bulb interneurons and striatal projection cells (Anderson et al., 1997a; Anderson et al., 1997b; Anderson et al., 2001; Sussel et al., 1999; Marin et al., 2000).

Genetic studies of mice deficient in transcription factors involved in regionalization or differentiation in the basal telencephalon, provided further information on the generation of tangentially migrating GABAergic neurons. Mice deficient in the homeobox genes *Dlx1/2* show no detectable cell migration from the subcortical telencephalon to the neocortex and retain only 25% of cortical GABAergic neurons (Anderson et al., 1997a; Anderson et al., 1999). Studies with mice deficient for the *Nkx2.1* (*Titf1* – Mouse Genome Informatics) or *Mash1* (Ascl1 – Mouse Genome Informatics) genes also show a significant reduction in cortical GABAergic neurons (Sussel et al., 1999; Casarosa et al., 1999).

Although the origin of the cortical GABAergic interneurons seems to be established, the mechanisms that underlie their migration are not fully understood. The chemotropic factor netrin is expressed at high levels in the GE (Metin et al., 1997), but mice deficient in netrin 1 or one of its receptors do not show a reduction in cortical interneurons (Serafini et al., 1996; Anderson et al., 1999). Recently, the chemorepellent Slit1, produced in the VZ of the LGE and MGE, has been proposed to play a role in the glia-independent tangential migration of GABAergic interneurons (Zhu et al., 1999). However, other guidance cues, distributed along the paths used by the migrating neurons, may also contribute to this process. Consistent with this hypothesis, a close association of tangentially oriented cells in the developing cortex and bundles of the corticofugal fiber system has been reported (Metin and Godement, 1996), suggesting that migrating interneurons may

use this fiber system as a scaffold for their migration into the neocortex.

In this report we provide evidence that the neural adhesion molecule TAG-1 (Cntn2 – Mouse Genome Informatics), a member of the immunoglobulin superfamily present on corticofugal fibers, serves as a substrate upon which GABAergic interneurons migrate. Blocking TAG-1 function in cortical slices with anti-TAG-1 antibodies or soluble TAG-1 protein results in a marked reduction of migrating GABAergic interneurons. However, L1, another immunoglobulin superfamily member and binding partner of TAG-1, is expressed along the path of the migrating interneurons but does not influence this process. These results render TAG-1 a cue for the tangential migration of cortical interneurons and suggest a novel role for this adhesion molecule, which has previously been shown to operate as a short-range axon guidance cue.

MATERIALS AND METHODS

Animals

Embryos from Sprague-Dawley albino rats (E1, day vaginal plug was found) as well as CBA×C57Bl/10 mice (E0.5, day vaginal plug was found) were used.

Female hemizygotes were crossed to wild-type C57Bl/10 males. Pregnant females resulting from these crosses were sacrificed at E12.5-14.5 and each embryo was sex genotyped and also genotyped by PCR for the normal and the L1-deficient allele (Dahme et al., 1997).

Immunohistochemistry

Immunohistochemical experiments were performed as described previously (Dodd et al., 1988) with some modifications. Briefly, 15 μm cryostat sections of E11.5-17.5 mouse brains were cut at the coronal plane, blocked in phosphate-buffered saline (pH 7.4; PBS), 1% bovine serum albumin (BSA), 0.1% Triton X-100, and incubated in primary polyclonal antibody against TAG-1 (1:3000) in PBS, 0.1% BSA, 0.1% Triton X-100 at 4°C overnight. After extensive washing, the sections were incubated with biotinylated anti-rabbit IgG (Amersham), processed with the avidin-biotin-peroxidase method using the ABC Elite kit (Vector) and reacted in DAB (Sigma). Immunohistochemistry on 70-100 μm Vibratome sections of rat cortical slices after two days in culture was performed as described previously (Dodd et al., 1988; Bailly et al., 1995) using the 1C12 monoclonal antibody against TAG-1 (Dodd et al., 1988) (1:10,000). A polyclonal antibody against GABA (1:1000; Sigma) and a polyclonal antibody against L1 (1:2000) (Rathjen and Schachner, 1984) were also used. Immunohistochemistry for radial glial fibers was performed using the RC2 monoclonal antibody (Developmental Studies Hybridoma Bank). Briefly, E14.5 mouse cortical slices were fixed after 2 days in culture, for 10 minutes in 4% paraformaldehyde (PFA), blocked in PBS, 1% BSA and incubated with RC2 monoclonal antibody (1/200) in PBS, 0.1%BSA at room temperature, overnight. Slices were then fixed again in 4% PFA and vibratome sections of 70-100 μm were cut. When indicated, immunohistochemistry for GABA on these vibratome sections was performed.

In situ hybridization

In situ hybridization experiments were performed on 18 μm cryostat sections according to the protocol of Scharen-Wiemers and Gefrin-Moser (Scharen-Wiemers and Gefrin-Moser, 1993). The TAG-1 specific probe was produced as follows: a 650 bp PCR product was obtained using as template P7 mouse cerebellar cDNA. The primers used start from position 2704 nucleotides to 2728 nucleotides and 3347 nucleotides to 3319 nucleotides of the rat TAG-1 published cDNA sequence (Furley et al., 1990). The fragment was subcloned into pBS and the antisense probe was obtained by *Hin*dIII digestion and T3 polymerase, while the sense with *Xho*I digestion and T7 polymerase.

DiI tracing

Corticofugal fibers were visualized by 1,1-dioctodecyl-3,3,3′,3′-tetramethylindocardocyanine (DiI) tracing in the cortex, according to Molnar and Cordery (Molnar and Cordery, 1999). Briefly, a small crystal of DiI (Molecular Probes) was inserted into the preplate on the convexity of the dorsal cortex of E13.5 mouse embryos, or in the marginal zone or intermediate zone of the dorsal cortex of E14.5 mouse (either in whole brain or in 400 μm cortical slices). After insertion of the crystal, the preparations were stored in PBS with 0.1% sodium azide at 37°C for maximum of 1 week. The preparations were resectioned and 100 μm sections were observed. After observation, immunohistochemistry for TAG-1 was performed as above without the addition of Triton X-100.

Cortical slice cultures

Cortical slice cultures of E15-16 rat or E13.5-14.5 mouse and DiI labeling were performed as described previously (Lavdas et al., 1999). Crystals of DiI were placed either in the MGE or LGE, as placement of the tracer in the latter labels passing MGE cells on their way to the cortex.

Function blocking experiments

Monoclonal antibodies against TAG-1, 4D7 (IgM) (Yamamoto et al., 1986) as well as polyclonal antibodies against either affinity-purified TAG-1 from embryonic mouse brains (Dodd et al., 1988) or against the baculovirus produced protein and their Fab fragments were used at concentrations 200-400 μg/ml. Fab fragments were prepared from polyclonal antibodies against TAG-1, as well as control polyclonal antibodies (raised against the long isoform of the tyrosine kinase receptor *Ret*) using the ImmunoPure Fab preparation kit (Pierce). To control further for the antibody blocking effect, antigen-depleted polyclonal antibodies were used in cortical slices. These were produced as follows: a stably expressing CHO-TAG-1 line (Buttiglione et al., 1998) was grown on 35 mm dishes to confluency. The antibody blocking solution (containing 300-400 μg antibody in 1.5 ml culture medium) was successively incubated five times for an hour each, changing to a new 35 mm dish each time. The complete absorption of TAG-1 immunoreactivity was checked, by using the antigen-depleted antibody on cryostat sections of E13.5 mouse cortex. In addition, control polyclonal and monoclonal antibodies of the same isotype (polyclonal antibodies against the HNF4 transcription factor and mouse IgM antibodies; the latter were purchased from Boehringer) were used in similar concentrations as the blocking antibodies to TAG-1. Fab fragments from polyclonal antibodies against L1 were used at concentrations ranging from 1 to 200 μg/ml (Rathjen and Schachner, 1984). Soluble TAG-1-Fc protein (TAG-1 Fc chimera) (Buttiglione et al., 1998) was used at a concentration of 200 μg/ml. MUC18-Fc soluble protein, another cell adhesion molecule containing Ig-like domains, was used as a negative control (Lehmann et al., 1989; Buttiglione et al., 1998). Both Fc-containing proteins were examined by SDS-PAGE and western blots using anti-human Fc antibody (1:15,000; Jackson Laboratories). Medium from COS7 cells transfected with the pIG vector alone was also used as a negative control.

Microscopy

Immunofluorescent sections were viewed and images were generated in a Leica TCS-NT Laser Scanning Confocal microscope using the 10× and 20× objectives. All other samples were viewed in a Zeiss Axioskop microscope and photographs were taken with a 35 mm camera using Fujichrome Sensia II 100ASA film.

Density measurements of GABA-labeled cells in the neocortex

Cultured slices were examined for the pixel density in the neocortex in response to GABA immunofluorescence, in order to assess the blocking effect that was due to the presence of antibodies against TAG-1 or TAG-1-Fc chimeric protein. Briefly, each slice was examined and the pixel density in all layers of the neocortex (starting from the internal capsule demarcation) was measured with the Scion Image Analysis program. Statistical analysis of the pixel density data was assessed using a one-factor ANOVA.

Western blot analysis

Cortical cell extracts were obtained by dissecting out the cortices of E14.5 rats, homogenized in 5mM Tris-HCl pH 7.2 with 1% NP-40, 1 mM PMSF, 5 mM EGTA, leupeptin 2 μg/ml and aprotinin 2 μg/ml. Samples were centrifuged for 20 minutes at 10,000 ***g*** at 4°C and the supernatants were isolated for further analysis by SDS-PAGE before blotting overnight (Dodd et al., 1988; Karagogeos et al., 1991). The ECL system (Amersham) was used for the revelation of the protein bands.

RESULTS

TAG-1 is expressed by the developing cortical efferents

Earlier reports (Wolfer et al., 1994; Fukuda et al., 1997; Kawano et al., 1999) have suggested that the neural adhesion molecule of the immunoglobulin superfamily, TAG-1 (Yamamoto et al., 1986; Dodd et al., 1988; Furley et al., 1990), is expressed by cortical efferent fibers. However, the precise details of the spatiotemporal expression of this molecule on cortical efferents have not been elucidated. We used TAG-1 immunohistochemistry and in situ hybridization in order to visualize the emergence of the corticofugal system in the developing cortex.

Immunohistochemical staining of cortical sections of E14 rat or E12.5 mouse embryos revealed the presence of labeled cells and their axons in the region of the plexiform primordium or preplate (PP) (Fig. 1A). Two days later, intense TAG-1 immunoreactivity was present in fibers of the intermediate zone (IZ), the cortical plate (CP) as well as the marginal zone (MZ) of the basolateral cortex (Fig. 1B). The TAG-1 immunopositive fibers of the IZ were tangentially oriented, projecting towards the internal capsule (IC), while distinct fascicles of radially oriented TAG-1 immunopositive axons were seen to cross the whole thickness of the CP. At later stages of corticogenesis (E17.5), TAG-1 immunostaining was still present in the MZ, IZ and CP but in the dorsomedial rather than the basolateral cortex. In the ventral telencephalon, TAG-1 immunoreactivity was restricted to the striatum (data not shown). After birth, TAG-1 immunoreactivity was not detected in any of the layers of the mouse cerebral cortex. Western blot analysis from E14 cortical extracts revealed a 135 kDa band as expected of the TAG-1 protein (Fig. 1D).

The expression pattern of TAG-1 mRNA during mouse cortical development has not been characterized. In situ hybridization experiments indicate that the pattern of expression of TAG-1 mRNA coincides with that of TAG-1 immunoreactivity at various stages of cortical development. For example, in cortical sections of E14.5 mouse brains, TAG-1 mRNA was observed in the MZ as well as in the IZ of the basolateral cortex (Fig. 1C). At later stages, TAG-1 mRNA signals were observed in the MZ and IZ of the dorsomedial cortex being absent from all cortical layers after birth (not shown). It is noteworthy that neither TAG-1 immunoreactivity nor TAG-1 mRNA signal on MGE migrating cells was observed (Fig. 1B,C).

The spatiotemporal expression pattern of TAG-1 mRNA and protein demonstrated here corresponds precisely with the development of the corticofugal projection system described in recent studies (Molnar and Cordery, 1999). To verify that TAG-1 marks the corticofugal fibers, we placed a crystal of DiI in the dorsal cerebral cortex of E13.5 or E14.5 mouse and performed immunohistochemistry for TAG-1. Fig. 1H,K shows extensive overlap of DiI and TAG-1 labeling (compare Fig. 1F with 1G, and 1I and 1J). In addition, the temporal expression pattern of TAG-1 corresponds with the order of appearance of migrating MGE cells in the different layers of the developing cortex. Double immunohistochemistry for TAG-1 and GABA at E12.5, when the migration of MGE cells has just started, showed the coincidence of expression of TAG-1 and GABA at the IC (Fig. 2B).

Migration of medial ganglionic eminence neurons occurs in close association with TAG-1 immunopositive axons

The migration of MGE-originating interneurons towards the cortex was visualized by using the fluorescent tracer DiI in organotypic slice culture preparations. We found that cells emerging from the MGE at the onset of corticogenesis (E14 in the rat, E12.5 in the mouse) were directed mostly towards the pial surface, probably contributing to the formation of the MZ, while at later stages they were also found in the forming IZ and CP (Fig. 2A) (Lavdas et al., 1999).

To investigate the possibility that TAG-1 expressed by the corticofugal fibers is involved in the migration of the MGE-derived interneurons, we combined DiI tracing with TAG-1 immunohistochemistry. In slice cultures from E15 rat or E13.5 mouse brains, we observed a number of tangentially oriented DiI-labeled cells in the MZ as well as in the newly forming IZ, with a tangential orientation amongst the TAG-1-labeled axonal bundles (Fig. 2C). In slices prepared from E16 rat or E14.5 mouse embryos, DiI-labeled cells were also seen in the CP with a leading process oriented perpendicularly to the pial surface and in close apposition to radially arranged bundles of TAG-1-containing axons (Fig. 2D).

Double-labeling experiments using antibodies to TAG-1 and GABA also showed numerous GABAergic neurons in the zones of the developing cortex replete with TAG-1-labeled axons. GABA immunostaining was used in these experiments to visualize the population of migrating MGE interneurons. In the MZ and IZ, these neurons often show a tangential orientation, whereas in the CP they tended to be intimately apposed to radially organized bundles of TAG-1-positive axons (Fig. 2E,F).

Although we have observed numerous migrating neurons (both with DiI tracing and GABA immunohistochemistry) in close association with radially oriented TAG-1-expressing fibers in the CP, it is possible that some of these neurons reach their final positions in the CP by using radial glial fibers, which at these developmental stages, traverse the whole cortex. In order to investigate this possibility, we have performed immunohistochemistry for RC2, a radial glial marker, followed

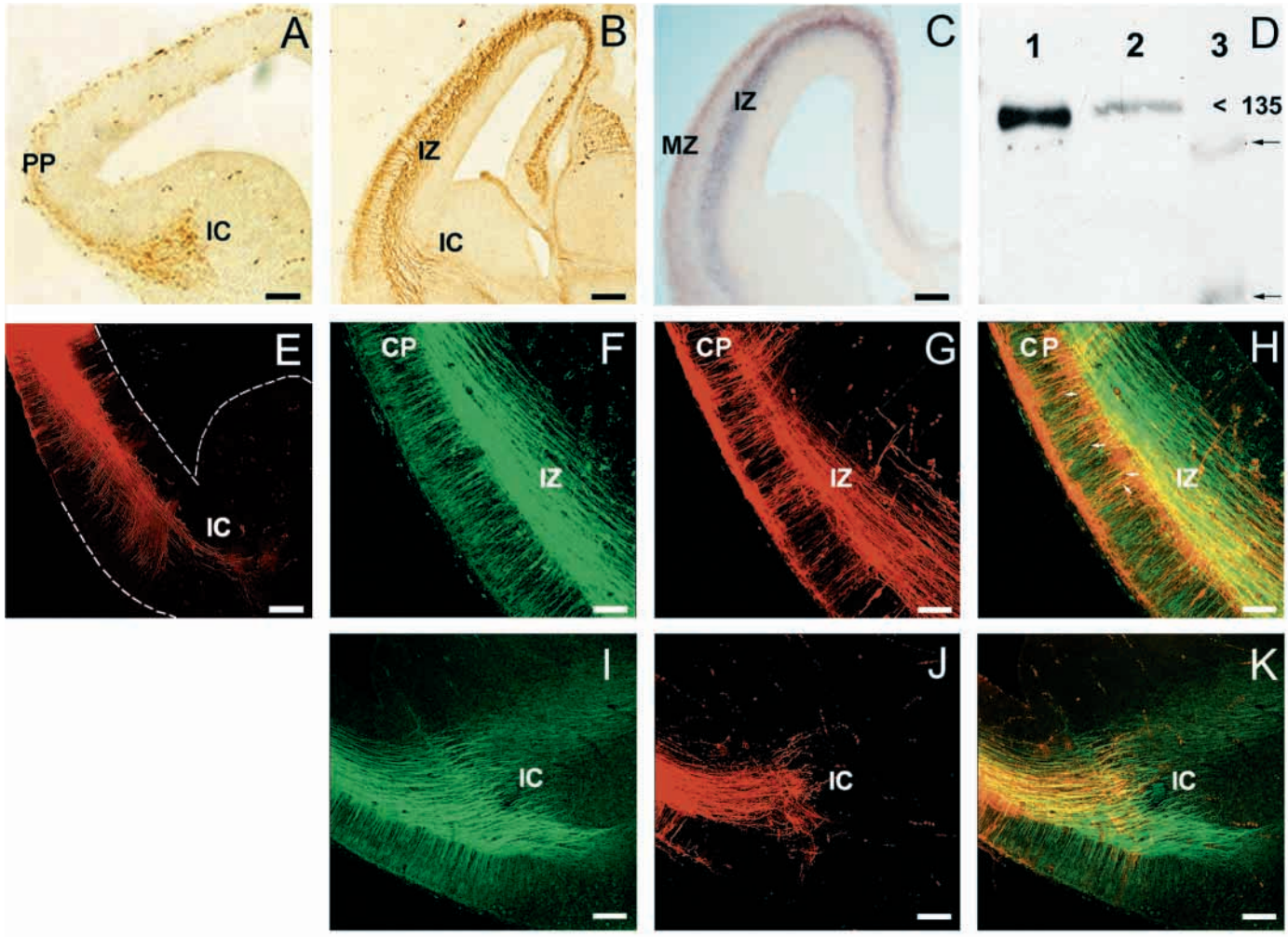

Fig. 1. TAG-1 is expressed by the developing corticofugal fibers. (A,B) Immunohistochemistry for TAG-1 in coronal sections of E12.5 (A) or E14.5 (B) mouse cortex. (A) Labeled cells in the PP project to the ventral telencephalon in the region of the IC. (B) Labeled axons are oriented tangentially in the MZ and IZ, and radially in the CP. (C) In situ hybridization for TAG-1 mRNA in coronal sections of E14.5 mouse cortex. The signal is detected predominantly in the IZ and MZ. (D) Western blot analysis of total lysates of E14.5 mouse spinal cord (control, lane 1) and cortex (lane 2) reveals a single band of 135 kDa, as expected of the TAG-1 antigen (arrowhead). The molecular weight standards shown (lane 3) are 111 and 83 kDa (arrows). (E) DiI crystal placement into the dorsal cerebral cortex of E14.5 mouse, labels corticofugal fibers descending via the IZ into the IC. (F-K) Immunohistochemistry for TAG-1 in coronal sections of E14.5 mouse cortex, after placement of DiI in the cortex of the whole brain, reveals numerous DiI- labeled corticofugal fibers that are immunopositive for TAG-1. DiI labeling (red, G,J) and immunohistochemistry for TAG-1 (green, F,I) in the IZ and CP of E14.5 cortical slices (F,G), as well as in the area of the IC (I,J). (H,K) Superimposed images of F,G and I,J, respectively. Arrows in H indicate DiI-labeled-TAG-1-immunopositive corticofugal fibers, radially oriented in the CP, cortical plate. IC, internal capsule; IZ, intermediate zone; MZ, marginal zone; PP, preplate. Scale bars: 100 μm in A,E,I-K; 50 μm in B,F-H; 60 μm in C.

by GABA immunohistochemistry in cortical slice cultures. In these double-labeling experiments, we have observed a number of migrating GABAergic cells in close association with RC2-immunopositive processes in the region of the CP (Fig. 2G). Similar experiments showed, however, that TAG-1 and RC2 immunoreactivities were not colocalized (Fig. 2H) in the CP. Thus, we concluded that the radial fibers that were stained for TAG-1 in the CP were not of radial glial origin.

Blocking TAG-1 function results in a marked decrease of migrating MGE neurons

To investigate whether cortical interneurons originating in the MGE actually use the TAG-1-expressing corticofugal system as a scaffold for their migration, slices of rat or mouse brains were cultured in the presence of blocking anti-TAG-1 antibodies or their Fab fragments. In these slices, we examined the migration of neurons labeled by placing a crystal of DiI in the MGE or LGE, as well as the density and disposition of GABAergic neurons in the cortex, by using immunohistochemistry. A marked decrease both in the number of DiI-labeled cells (compare Fig. 3A with 3B) and in the number of GABAergic neurons was observed in all layers of the developing cortex (compare Fig. 3C with 3D, also see 3I). Control polyclonal or monoclonal antibodies of the same isotypes as the anti-TAG-1 monoclonal antibodies did not reduce the number of labeled cells (Fig. 3B,D,I). In addition, antigen-depleted antibodies did not reduce the number or affect the density of GABA-containing neurons in the cortex (Fig.

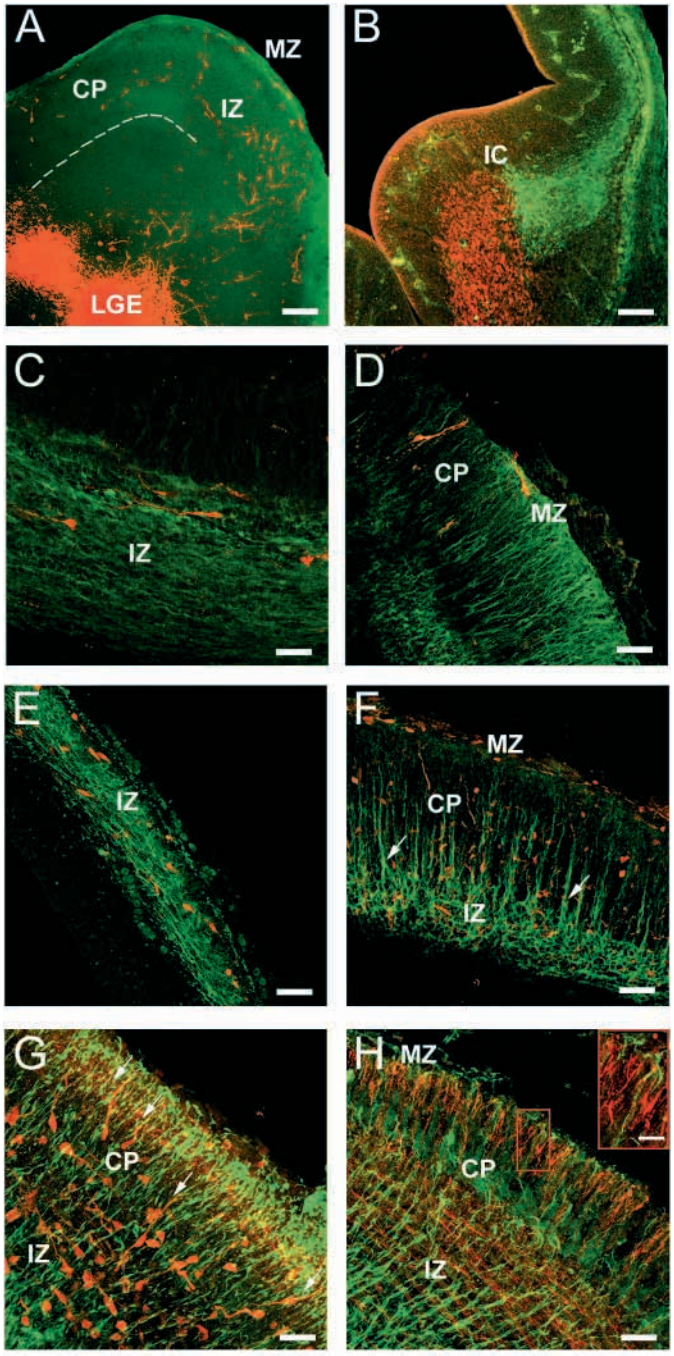

Fig. 2. Migratory routes of cortical interneurons along the TAG-1 immunopositive corticofugal fibers. CP, cortical plate; IC, internal capsule; IZ, intermediate zone; LGE, lateral ganglionic eminence; MZ, marginal zone. The broken line demarcates the ventricle. (A) Tangential migration of neurons to different layers of the developing cerebral cortex in cortical slice cultures of an E16 rat embryo, after placement of DiI into the ganglionic eminence. (B) Double immunohistochemistry against GABA (red) and TAG-1 (green) in coronal sections of E12.5 mouse cortex, shows the juxtaposition of TAG-1 immunoreactive fibers and GABAergic cells at the IC. (C,D) DiI tracing for tangentially migrating neurons (red) and immunohistochemistry against TAG-1 (green), in slice cultures of E15 (C) or E16 (D) rat cortex. (C) Tangentially migrating neurons in close association with TAG-1 immunoreactive fibers in the IZ. (D) DiI-labeled migrating neuron in the CP, with the leading process oriented perpendicular to the pial surface and in close apposition to the radially arranged bundles of the TAG-1 immunopositive axons. (E,F) Double immunohistochemistry against GABA (red) and TAG-1 (green), in slice cultures of E15 (E) or E16 (F) rat cortex. (E) Tangentially oriented GABAergic neurons are found dispersed between TAG-1-labeled axons, in the IZ. (F) GABAergic neurons and fibers in the IZ, CP and MZ. In IZ and MZ, cells show tangential orientation, whereas, in the CP, they tend to be radially organized in apposition to the TAG-1 positive axons. (G) Double immunohistochemistry with RC2 antibody (green) and anti-GABA (red) in slice cultures of E14.5 mouse cortex. Arrows point to migrating neurons in the CP with the leading process perpendicular to the pial surface and in close apposition to the radial glial fibers. (H) Double immunohistochemistry with RC2 antibody (green) and anti-TAG-1 (red) in coronal sections of E14.5 mouse cortex. TAG-1 and RC2 are not co-localized in the CP. Inset: higher magnification of the red box. Scale bars: 100 μm in A,B; 40 μm in C,F; 25 μm in G,H; 10 μm in inset.

3G), thus providing further support for the specificity of the anti-TAG-1 antibody blocking effect. Finally, we incubated cortical slices with the secondary antibody alone, after incubation with the blocking concentration of anti-TAG-1 antibodies, in order to examine further the specificity of the antibody-antigen binding (Fig. 3H). The appropriate expression pattern of TAG-1 was observed, indicating that there was sufficient binding of the primary antibodies to the antigen in culture and that the corticofugal system was histologically intact.

In parallel experiments, soluble protein in the form of TAG-1-Fc chimera (Buttiglione et al., 1998) included in the medium mimicked the blocking effects of the antibodies, thus providing an independent confirmation of the antibody blocking results (compare Fig. 3E with 3F, and see 3J). The blocking effect with the soluble protein was slightly stronger than that of antibodies (Fig. 3I,J). Although in our case this difference was not statistically significant, it has been mentioned in other studies as an effect that is due to binding to and saturating receptors for TAG-1, in addition to binding homophilically to TAG-1 (Stoeckli and Landmesser, 1995; Stoeckli et al., 1997). Control soluble Fc protein did not affect migration (Fig. 3F,J). It should be noted that in blocking experiments with antibodies or with soluble TAG-1, a plethora of GABAergic neurons (Fig. 3C,E) were seen accumulated in various regions of the ventral telencephalon, up to the area around the corticostriatal notch. This would be expected if the cues responsible for their migration were not operating. In addition, this observation indicated that blocking TAG-1 did not affect the differentiation of these cells, as they were able to express GABA.

The adhesion molecule L1, expressed by the thalamocortical axons, is not involved in cortical interneuron migration

The immunoglobulin superfamily member L1, a known ligand of TAG-1, is expressed by thalamocortical axons found in the IZ during cortical development (Fukuda et al., 1997). It has been reported that both efferent and afferent fibers are in contact with each other as they develop (Molnar and Blakemore, 1995; Auladell et al., 2000). As interactions between TAG-1 and L1 have been observed in vitro, it is conceivable that L1 may transduce the signal provided by

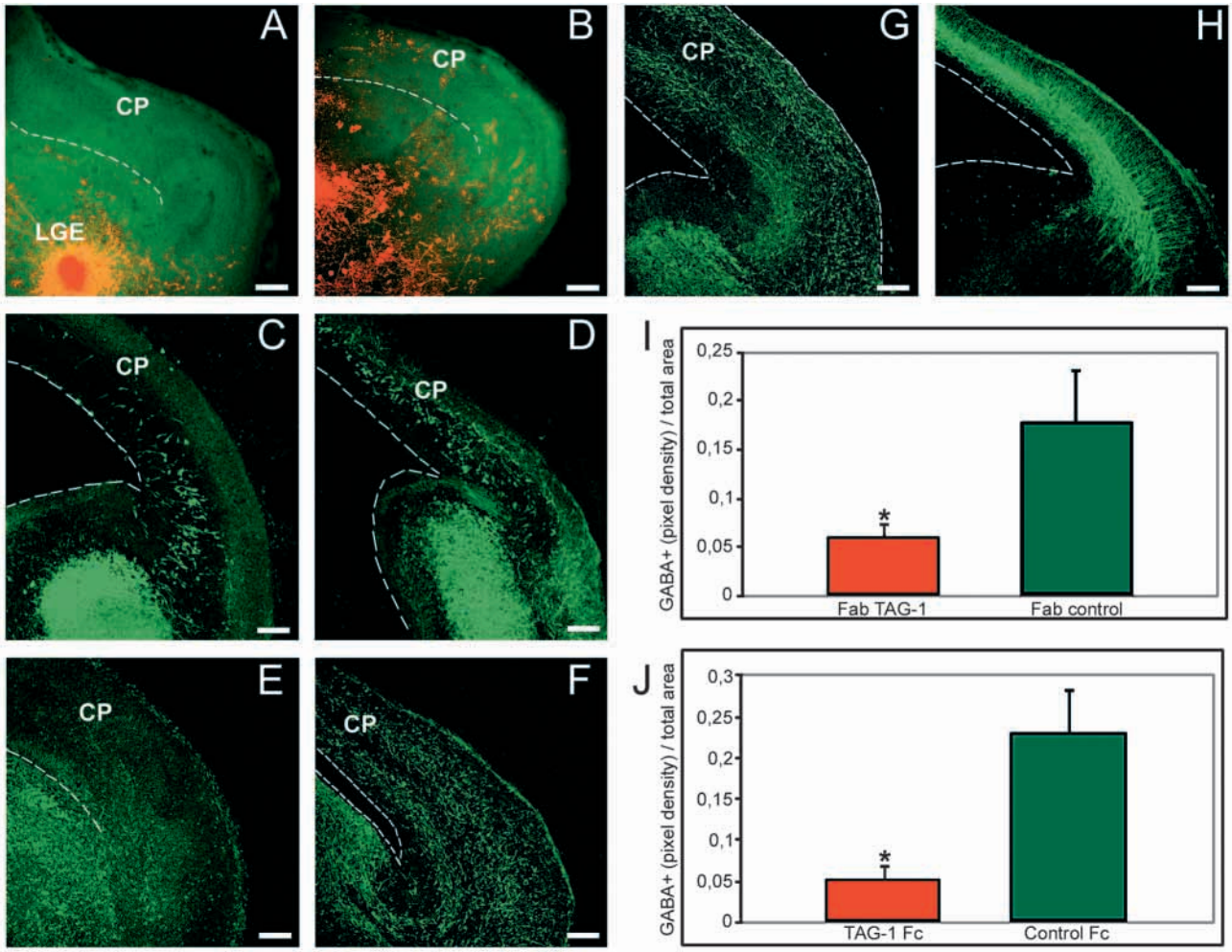

Fig. 3. TAG-1 mediates the tangential migration of MGE neurons along the fibers of the corticofugal system. CP, cortical plate; LGE, lateral ganglionic eminence. The broken line demarcates the ventricle and the edge of the slice. The migration of the MGE cells is examined in cortical slice cultures by placing a crystal of DiI in the LGE (A,B), as well as by using immunohistochemistry against GABA (C-F). Cortical slices were cultured for 2 days in the presence of monoclonal or Fab fragments of polyclonal antibodies against TAG-1 (A,C, respectively) or control antibodies (same isotype, Fab fragments of control antisera, see Materials and Methods) (B,D, respectively). Very few DiI-labeled migrating neurons (A) and GABAergic neurons (C) are detected in the presence of antibodies against TAG-1 in E15 rat (A) or E13.5 mouse (C), when compared with the controls in the cortex of E15 rat (B) or E13.5 mouse (D). A marked decrease in the number of GABAergic neurons in E16 rat slice cultures is observed in the presence of TAG-1-Fc protein (E). No effect is observed when control soluble Fc protein (MUC18) is added in the culture medium (F). Antigen depleted antibodies do not reduce the number or affect the density of GABA-containing neurons in the cortex (G). No differences in the expression pattern of TAG-1 is detected in cortical slices cultured in the presence of antibodies against TAG-1 and incubated with the secondary antibody alone (H). Quantification of the blocking effect of antibodies to TAG-1 (I) or TAG-1-Fc (J) protein on the migration of GABAergic neurons. Densitometry of GABA-expressing cells in slice cultures in the presence of Fab antibodies against TAG-1 (I) and control Fab fragments or TAG-1 Fc protein (J) and control MUC18 Fc protein (see also Materials and Methods). Values represent the ratio of the area where pixel densities were measured to the total area measured (i.e. all of neocortex). Value 1.00 would represent the entire length of the neocortex. Error bars represent the s.d.; *P<0.05, one-factor ANOVA. Values were collected from independent slice cultures (A, n=9; B, n=7) Scale bars: 100 μm in A-H.

TAG-1 (Felsenfeld et al., 1994; Buchstaller et al., 1996; Dhar-Mahotra et al., 1998). We, therefore, investigated whether L1 is also involved in this migration.

Double immunohistochemistry with antibodies to L1 and TAG-1 revealed that for the most part, the cortical afferents and efferents were intermingled in the IZ (Fig. 4A-C), although a difference in the intensity between L1 and TAG-1 immunolabeling was observed in the lower part of the IZ. As both types of fibers were found in the IZ, we hypothesized that L1 may bind to TAG-1 and thus influence the migrating interneurons.

We employed the same slice culture method to investigate whether blocking L1 function perturbed GABAergic migration in the IZ. Slices of E13.5 mouse cortices were cultured in the presence or absence of Fab fragments of polyclonal anti-L1 antibodies as shown in Fig. 5A,B, respectively. We did not observe any reduction in the number of migrating neurons at any concentration of anti-L1 Fab fragments used.

The phenotype of L1-deficient mice (Dahme et al., 1997) was then examined in terms of the corticofugal system, because the absence of L1 in the IZ may have influenced its development.

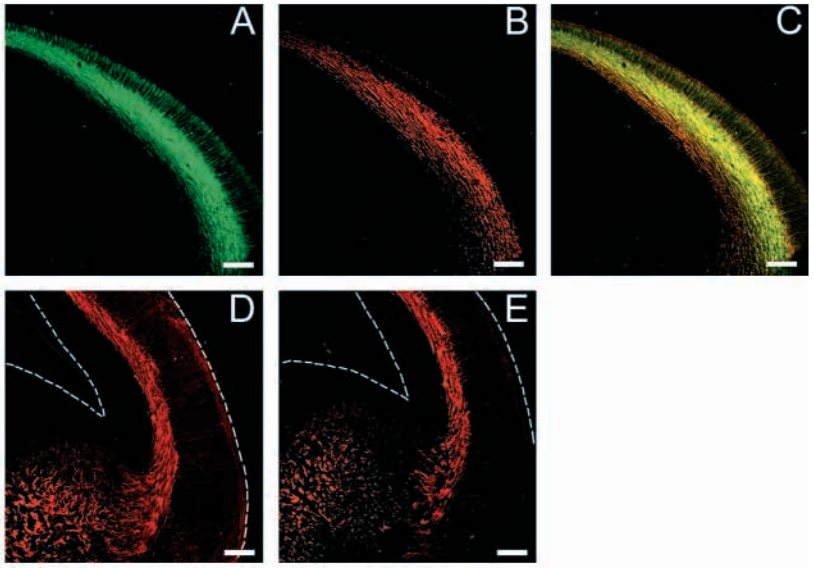

Fig. 4. L1 and TAG-1 immunopositive axons are both found in the intermediate zone. Double immunostaining against TAG-1 (green, A) and L1 (red, B) in cryostat sections of E14.5 mouse brain. (C) Superimposed image of A and B. No differences in the expression pattern of L1 are detected in cortical slices cultured in the presence of antibodies against TAG-1 (E) compared with control ones (D). Scale bar: 100 μm.

Fig. 5D shows that there was no change in TAG-1 immunostaining in E13.5 mutant cortices compared with wild-type mice (Fig. 5C). In addition, GABA immunostaining in the cortices of L1-deficient mice (Fig. 5F,H) revealed no apparent differences as compared to controls (Fig. 5E,G). Taken together, these results do not implicate L1 as a mediator of the TAG-1 effect on migration.

DISCUSSION

Earlier studies have suggested that axons can provide a substratum for non-radial neuronal migration in the developing central nervous system including the cerebral cortex (Rakic, 1985; Gray et al., 1990). In the case of the tangentially migrating GABAergic interneurons, this substratum has been suggested to be the developing axons of the corticofugal fiber system (Metin and Godement, 1996; Anderson et al., 1999; Parnavelas, 2000). However, this hypothesis, as well as the molecular components that either initiate or maintain the migration of cortical interneurons, remained to be elucidated.

The evidence presented here indicates that the neural adhesion molecule TAG-1, expressed by the corticofugal fibers, mediates the migration of neurons from the MGE to the neocortex. GABAergic interneurons migrate in close association with the axons of the corticofugal fiber system. Blocking the function of TAG-1 with antibodies or soluble TAG-1 protein reduces the number of migrating cells, suggesting that TAG-1 may act as a guidance cue for migrating interneurons. TAG-1 is not detected in migrating cells (Fig. 1A-C, Fig. 2B) suggesting that the TAG-1 effect is not mediated via homophilic interaction but via a yet unknown heterophilic mechanism.

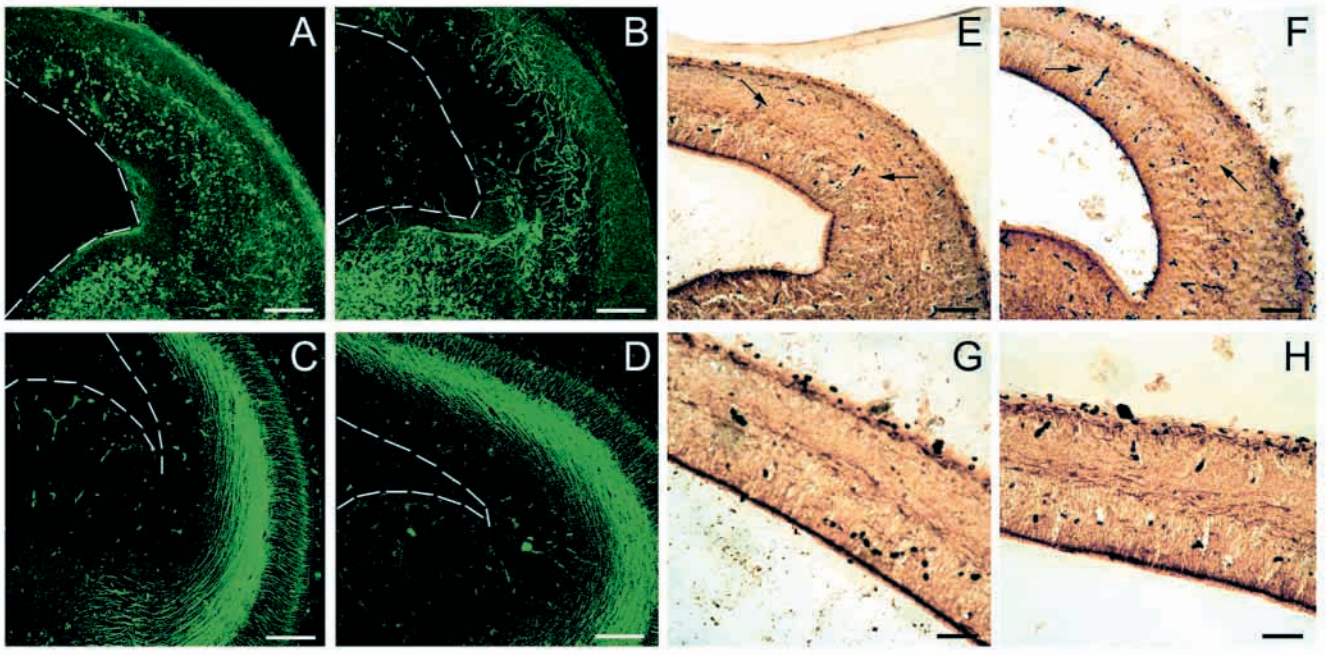

Fig. 5. L1 is not involved in the migration of cortical GABAergic interneurons. The broken line demarcates the ventricle. Arrows point to streams of GABA-immunopositive cells. The migration of MGE cells was examined in cortical slice cultures in the presence (A) or absence (B) of L1 Fab fragments. Immunostaining against TAG-1 in cryostat sections of E14.5 wild-type (C) and L1 mutant (D) mouse brains. Immunostaining against GABA in cryostat sections of E14.5 wild-type (E,G) and L1 mutant (F,H) mouse brains. Scale bars: 100 μm in A-D; 110 μm in E,F; 55 μm in G,H.

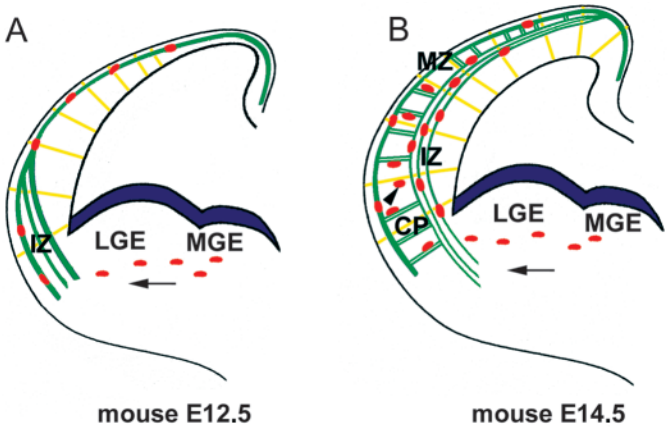

Fig. 6. Proposed mechanism for the migration of cortical interneurons from the MGE. The TAG-1 immunopositive fibers are depicted in green, migrating interneurons in red, radial glial fibers in yellow and *Slit1* mRNA in blue. MGE cells migrate away from the GE probably owing to a chemorepulsive effect of Slit1. They then use the TAG-1 immunopositive axons arranged tangentially in the MZ and IZ to migrate into the neocortex. They reach their positions in the CP by using the radially arranged bundles of efferent axons or radial glial fibers.

Recently, the TAG-1 deficient mouse phenotype has been published (Fukamauchi et al., 2001). The mice appear normal except for a significant elevation in adenosine A1 receptors in the hippocampus and enhanced seizure susceptibility to convulsant stimuli. Although no overt histological phenotype revealed by Nissl staining was observed in the brains of TAG-$1^{-/-}$ mice, no detailed analysis of GABAergic neurons and/or their development was mentioned in this publication. In light of our in vitro data, it would be interesting to study the generation and migration of this neuronal population in developing TAG-$1^{-/-}$ embryos.

We also investigated the putative role of the adhesion molecule L1, a known binding partner of TAG-1, in the migration of cortical interneurons. L1 has been shown to be involved in cell migration (Lindner et al., 1983), axon bundling and pathfinding (Fischer et al., 1986; Castellani et al., 2000), and is expressed by thalamocortical afferents (Fukuda et al., 1997). No reduction in GABA-immunopositive cells was observed either by blocking L1 function in vitro or by examining the phenotype of L1-deficient mice. These results do not implicate L1 in the cortical interneuron migration either as a binding partner or as a *trans*- or *cis*-interacting signal transducer of TAG-1 activity. We cannot formally exclude a putative role of the thalamocortical afferents in this migration. However, we consider such a role unlikely, owing to the fact that MGE neurons migrate to the cortex as early as E14 (in the rat), before thalamic axons invade (Molnar and Cordery, 1999). At that time, corticofugal fibers arising in the MZ have already grown down to the ventral telencephalon. In addition, blocking of TAG-1 function does not affect the architecture of the thalamocortical fibers, as revealed by L1 staining (compare Fig. 4E with 4D).

Evidence is accumulating to suggest that other known axon guidance cues, such as netrin 1, its receptor Unc5h3 and Slit1, are also involved in neuronal cell migration in various parts of the developing brain (Wu et al., 1999; Przyborski et al., 1998; Alcantara et al., 2000). Recent studies (Zhu et al., 1999) have suggested that Slit1, produced in the VZ of the LGE, acts as a chemorepellent on cells migrating out of LGE explants and also in their normal trajectory. Our evidence points to the presence of a contact dependent cue that operates along the pathway the migrating neurons follow after they have left the GE. Although our data strongly suggests that TAG-1 is acting as a cue for the migrating GABAergic interneurons, the presence of additional cues cannot be excluded. Reasonable candidates based on expression patterns that have been proposed in the literature are members of other axon guidance families such as the semaphorin family (Bagnard et al., 1998).

We provide evidence that the migration of GABAergic interneurons occurs along the corticofugal fiber system. How the MGE cells reach the terminal ends of the corticofugal projections in the region of the IC is unknown at present. It is possible that migrating cells 'fall' upon these fibers and may become 'hooked' onto the TAG-1 substrate. Then, they use the tangentially arranged axons in the MZ and IZ to reach the neocortex. Neurons may reach their positions in the CP by using the bundles of efferent axons that descend radially through the CP. Recent evidence (Soria and Fairén, 2000) suggests that these are the axons of pioneer cells located in the MZ. Our DiI tracing experiments and immunohistochemical preparations have clearly shown labeled cells in the CP oriented radially with leading processes pointing towards the pial surface. Our experiments cannot exclude the possibility that migrating cells also use radial glia (that are abundant in the CP), in addition to TAG-1-immunopositive, radially oriented fibers. Our proposed model (Fig. 6) attempts to bring together all these observations. MGE cells migrate away from the GE, possibly driven by the action of Slit1. At the IC, they become associated with the TAG-1-immunopositive corticofugal axons to migrate tangentially towards the cortex. Finally, they reach their positions in the CP by using the radially arranged TAG-1-immunopositive efferent axons or radial glia fibers.

All previous studies report that MGE-derived cells tangentially migrate into the cerebral cortex via the IZ, at least for the early period of cortical neurogenesis (E11.5-E14.5 in the mouse). However, there seems to exist an 'anatomical difference' in the migratory route of GABAergic interneurons between the rat and mouse. In the case of the rat, it is proposed that cortical interneurons migrate into the cortex specifically via the lower IZ (Tamamaki et al., 1997; Lavdas et al., 1999), while in the mouse there is no such specification (Anderson et al., 1997a; Sussel et al., 1999; Casarosa et al., 1999; Anderson et al., 2001). In our experiments, we used both mouse and rat tissues and we observed that TAG-1 is involved in the migration of GABAergic interneurons in both cases, as would be expected, as the TAG-1 protein is expressed in the entire width of the IZ, both in the mouse and rat, according to our and previous immunohistochemical studies (Wolfer et al., 1994; Fukuda et al., 1997; Kawano et al., 1999).

TAG-1 protein has been localized in many fiber systems, as well as in migrating neurons in the developing CNS, suggesting that it may be involved in adhesive processes required for migration to take place (Yamamoto et al., 1986; Dodd et al., 1988; Wolfer et al., 1994; Bailly et al., 1995). Moreover, TAG-1 is involved in the guidance of chick embryonic spinal commissural axons towards their

intermediate target, the floor plate (Stoeckli and Landmesser, 1995; Stoeckli et al., 1997). The present evidence that TAG-1 is also used as a substrate by migrating cortical interneurons demonstrates that the same molecule may mediate both axonal pathfinding and cell body migration in different systems. In addition, our results point to a novel role for this adhesion molecule.

In summary, we have provided evidence from tracing and immunohistochemical analyses as well as from functional experiments, in support of the hypothesis that the majority of the cortical interneurons derived from the MGE use the corticofugal system as a scaffold to reach their positions in different layers of the cortex. In addition, we have demonstrated that the adhesion molecule TAG-1, expressed by corticofugal axons, provides a substrate for the migrating neurons, lending support to the notion of common cues for axon guidance and neuronal migration.

We thank Tom Jessell, Susan Brenner-Morton, Fritz Rathjen, David Simmons and Iannis Talianidis for reagents, and Christine Metin, Vassilis Pachnis and Marion Wassef for critical reading of an earlier version of the manuscript. We thank the members of the Karagogeos laboratory for helpful discussions and Phaedra Xekardaki for help with figures. Supported by the following grants: the Greek Ministry of Education (EPEAEK 1092), the Greek Secretariat for Research and Technology (PENED 99ED329) and by the EU BIOTECHNOLOGY (Grant No. 980329) to D. K. and M. S. and by a Wellcome Trust project grant (No. 059370) to J. G. P.

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