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Connexin 43 expression in glial cells of developing rhombomeres of *Xenopus laevis*

Bharti Katbamna [a,*], Anna M. Jelaso [b], Charles F. Ide [b]

[a] *Department of Speech Pathology and Audiology, Western Michigan University, Kalamazoo, MI 49008-5355, USA*
[b] *Environmental Institute, Western Michigan University, Kalamazoo, MI 49008, USA*

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Abstract

Connexin 43 is a gap junctional protein found predominantly in astrocytes. In the mammalian nervous system, it appears to play an organizational role during neural development. In the current study, conducted on the frog, *Xenopus laevis*, we found that connexin 43 occurs in glial cells during development of rhombomeres and that its expression is spatially and temporally regulated. We used neural (2G9) and cell proliferation (BrdU) markers to identify the overall organization of *Xenopus* rhombomeres and then tracked expression of connexin 43 and glial fibrillary acidic protein, an intermediate filament protein known to mark glia during rhombomeric development. 2G9 was expressed in rhombomeric centers (ventricular concavities) and outlying neuropil regions, whereas BrdU-labeled cells marked boundary regions (ventricular convexities), as early as stage 35/36. These labeling patterns persisted through premetamorphic stages of hindbrain development. At stage 47, 2G9-labeled profiles were highlighted by the presence of connexin 43, and at stage 49/50, connexin 43-labeled profiles, i.e., rhombomeric centers and neuropil, as well as rhombomeric boundaries, not labeled by connexin 43, became immunoreactive to glial fibrillary acidic protein. Cells of rhombomeric center regions and their processes in the outlying neuropil co-expressed glial fibrillary acidic protein and connexin 43 at a time that is characterized by the emergence of hindbrain auditory neural circuitry. Glial fibrillary acidic protein positive glial cells that appeared at rhombomeric boundaries never expressed connexin 43, but rather appeared to physically bisect ventricular convexities into adjacent rhombomeric regions. Thus, glial cells that express connexin 43 in developing rhombomeric centers may be similar to radial glia, assisting in formation of neural circuitry, while glial cells that do not express connexin 43, situated at rhombomeric boundaries, may be involved in demarcating adjacent rhombomeres.

Keywords: Rhombomeres; Connexin 43; Gap junction proteins; Glia; Glial; Neural and cell proliferation markers; Auditory neural circuitry

1. Introduction

During development of the vertebrate hindbrain, the neuroepithelium becomes transiently organized into seven distinct segments called rhombomeres (Vaage, 1969; Lumsden, 1990; Trainor and Krumlauf, 2000). As development proceeds, rhombomeres and adjoining boundary regions become defined by specialized glial cells. In the chick hindbrain, as rhombomeres emerge, boundary regions become strongly immunoreactive to vimentin, an intermediate filament protein, indicating that at least some of the boundary cells mature into glia or glial precursors (Heyman et al., 1995).

In the developing hindbrain of *Xenopus laevis* frog, boundary regions between adjacent rhombomeres are also defined by specialized glial cells (Yoshida and Colman, 2000). However, unlike the boundary cells of the chick hindbrain, these glial cells co-express vimentin and glial fibrillary acidic protein (GFAP), another intermediate filament protein, during early stages of hindbrain development. Furthermore, expression patterns of these intermediate filament proteins change during later development such that vimentin remains localized in the boundary glial cells, but GFAP becomes spatially restricted to rhombomere center regions (Yoshida and Colman, 2000; Yoshida, 2001). Such regional differences in intermediate filament protein expression indicate that glia in the rhombomere centers are morphologically and biochemically different from those in the boundary regions. However, the role of glial cells in both locations remains unclear.

In the developing mammalian nervous system radial glial cells express connexin 43 (Cx43), a gap junction protein

Abbreviations: Cx43, connexin 43; GFAP, glial fibrillary acidic protein; 2G9, neural marker; BrdU, 5-bromo-2′deoxyuridine; NGS, normal goat serum; NHS, normal horse serum; PBS, phosphate buffered saline; DAB, 3′3′ diaminobenzidine

* Corresponding author. Tel.: +1-269-387-8037; fax: +1-269-387-8044.
E-mail address: katbamna@wmich.edu (B. Katbamna).

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found predominantly in astrocytes, during a period that correlates with neuronal migration (Nadarajah et al., 1997; Nadarajah and Parnavelas, 1999). In the neocortex of Cx43 null mutant mouse embryos, neuronal migration is significantly delayed (Fushiki et al., 2003). Thus, in mammals Cx43 appears to play a critical organizational role in the development of cortical circuitry.

The purpose of the present study was to determine if glial cells found in rhombomeres of the amphibian hindbrain express Cx43 and if expression occurs during formation of hindbrain circuitry. In *Xenopus*, the framework for cranial nerve III through XI is set up by stage 45/46. Thus, hindbrain circuit formation is expected to occur concomitantly and/or following the emergence of cranial nerves at stage 45/46. This notion is apparent at least in the developmental timetable of the auditory system and its hindbrain circuitry. All sensory organs of the peripheral hearing system differentiate at stage 47, with their afferent projections and the first order octaval nucleus (dorsolateral nucleus) appearing at stage 50 (Fritzsch, 1990). If Cx43 plays an organizational role during hindbrain circuit formation, its expression should occur during this developmental period. Moreover, since glial cells of *Xenopus* rhombomeres show regional specialization, Cx43 might show unique expression patterns. Thus, in the present study, we also determined if Cx43 expression patterns were spatially segregated to glial cells of rhombomeric center or boundary regions. Furthermore, since neurons arise from the neuroepithelial cells of the ventricular zone in the *Xenopus* hindbrain, we assessed the overall relationship of the specialized glia to newborn neurons.

2. Experimental procedures

2.1. Animals

All animals were treated in accordance with an animal use protocol approved by the Institutional Animal Care and Use Committee at Western Michigan University. Embryos were obtained by injecting adult *Xenopus laevis* frogs with human chorionic gonadotropin (Sigma). Fertilized eggs were separated and maintained in spring water (Absopure). Embryos were staged according to Nieuwkoop and Faber (1994) and collected for immunocytochemical analysis from 2 days through 67 days post-fertilization at the following stages: 32/33, 35/36, 40/41, 45/46, 47, 47/48, 48, 49, 49/50, 50/51 and 57. Five animals were examined at each of these stages with each of the antibodies listed below. All animals were anesthetized in MS-222 (dilution 1:2000; 3-aminobenzoic acid ethyl ester; Sigma) and fixed overnight at 4 °C in Bouin's solution.

2.2. BrdU labeling

In order to understand the relationship between newly born cells and 2G9/Cx43 expression, proliferating cells were identified using a BrdU labeling protocol adapted from Sandeman and Sandeman (2000). Three groups of tadpoles, i.e., stages 35/36, 47 and 57, were exposed to 5-bromo-2′-deoxyuridine (BrdU; Sigma) by immersion in 4 mg/ml BrdU/10% Steinberg's solution for 24 h. BrdU-exposed animals from each of the three age groups were collected every day for three consecutive days after 24 h of exposure, along with non-exposed control animals. Thus, animals exposed at stage 35/36 were collected at stages 40/41, 45/46 and 47, whereas those exposed at stage 47 were collected at stages 47/48, 48 and 49. Animals exposed at stage 57 were collected 24, 48 and 72 h post-BrdU exposure and were gauged to be at the same developmental stage, i.e., stage 57.

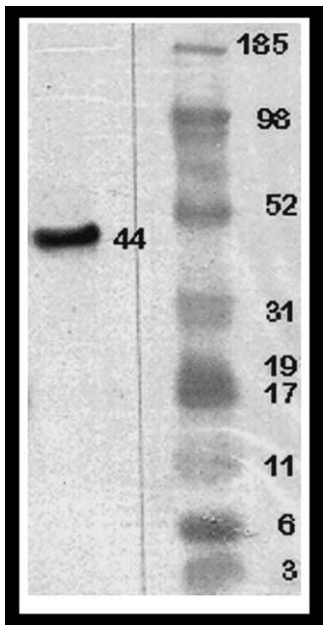

Fig. 1. Western blot analysis of protein loaded in the left lane shows a single 44 kDa band representing the putative *Xenopus* Cx43 protein. A molecular weight marker appears in the right lane.

2.3. Antibodies

An affinity isolated rabbit anti-Cx43 antibody (Sigma) was used to identify Cx43 expression. The specificity of the antibody in *Xenopus laevis* was confirmed by Western blotting (Fig. 1). A single band with a molecular weight of approximately 44 kDa was detected using protein isolated from 28-day post-fertilization tadpoles (see methods described below).

A monoclonal antibody, 2G9 (gift from E. A. Jones), developed against the adult *Xenopus* brain and spinal cord, was used as a neural marker. 2G9 is expressed throughout the nervous system from stage 29 through adulthood in *Xenopus laevis* (Jones and Woodland, 1989).

BrdU-labeled S-phase nuclei were detected using either a monoclonal mouse (Dako) or a polyclonal rabbit (Megabase Research) anti-BrdU antibody.

Chicken anti-GFAP antibody characterized by Dahl et al. (1985) was used to confirm the identity of Cx43-labeled glial cells.

2.4. Immunocytochemistry

Immunocytochemistry for all antibodies was performed on paraffin sections, except for the anti-GFAP antibody that reacted optimally on vibratome sections. Vibratome sections (160 μm) were cut horizontally at a plane that showed both the hindbrain ventricle and the surrounding rhombomeres clearly. All other specimens were embedded in paraffin, sectioned (20 μm) in the horizontal or transverse plane and mounted on positively charged slides. Paraffin sections were deparaffinized and rehydrated before quenching. All tissue sections were quenched in 3% hydrogen peroxide for 5 min. For BrdU-exposed specimens, the sections were treated with 2N HCl for 20 min at 37 °C for DNA denaturation followed by rinses with borate buffer. All sections were subsequently incubated in blocking solution (3% normal goat serum [NGS] or 3% normal horse serum [NHS], 0.4% Triton X-100 in phosphate buffered saline [PBS]) for 2 h. Sections were incubated in primary antibodies (1:100 for anti-BrdU and anti-Cx43; undiluted for 2G9; 1:5 for anti-GFAP) overnight at 4 °C. Control sections processed simultaneously were incubated with NGS or NHS instead of primary antibodies. The following day, all sections were treated for 1 h with biotinylated mouse or rabbit secondary antibody (1:100 for paraffin sections; 1:50 for vibratome sections) and then with avidin-horseradish peroxidase-biotin complex (Vector). Antibody staining was visualized with 3′3′ diaminobenzidine (DAB) chromogen (0.05% DAB-H_2O_2 in 0.05M Tris–HCl, pH 7.6). For double-label immunocytochemistry (anti-Cx43/2G9; anti-Cx43/anti-BrdU; 2G9/anti-BrdU; anti-GFAP/anti-Cx43), the second label was detected with red alkaline-phosphatase substrate (Vector). The sections were dehydrated, cleared in xylene and coverslipped with D.P.X. neutral mounting medium (Sigma-Aldrich). Vibratome sections were placed in a depression slide filled with PBS and visualized as whole mounts.

Immunoreactivity in paraffin and vibratome sections was assessed with a Nikon Eclipse light microscope, equipped with epifluorescent filters and/or a Nikon SMZ-U stereomicroscope. The presence and distribution of immunoreactivity for all antibodies within the hindbrain were assessed in each animal, except negative control sections that showed no staining. Figures were composed with the Adobe Photoshop software system.

2.5. Western blotting

Twenty 28-day post-fertilization tadpoles (stage 49/50) were anesthetized in MS-222 and homogenized in an extraction buffer containing 20% SDS, 10 mM EDTA and 0.1 M Tris–HCl, pH 6.8 (Karasinski et al., 2000). The homogenate was clarified by centrifugation and protein concentration was determined by Lowry assay (Lowry et al., 1951). Protein samples (12 and 30 μg) were diluted with sample buffer, electrophoresed through 4–12% Bis–Tris–HCl polyacrylamide gels in a Novex NuPage mini-gel system (Invitrogen), and transferred onto a nitrocellulose membrane. The blots were treated with 5% BSA/0.1% Tween-20 solution overnight at 4 °C and incubated in anti-Cx43 antibody (dilution 1:250; Sigma) for 1 h, followed by peroxidase conjugated anti-rabbit secondary antibody (dilution 1:20,000; Amersham-Pharmacia) for 1 h. Specific bands were visualized with enhanced chemiluminescence according to the manufacturer's instructions (ECL; Amersham-Pharmacia). The blots were scanned into Adobe Photoshop and imported into SigmaGel analysis software programs (Jandel Scientific) for molecular weight determination.

3. Results

3.1. Specificity of Cx43 antibody

The specificity of the commercial Cx43 antibody in *Xenopus laevis* was determined by Western blot analysis. Analysis of the blot showed a single protein with a molecular weight of approximately 44 kDa (Fig. 1), indicating that this antibody recognizes the putative *Xenopus* Cx43.

3.2. 2G9 appears in rhombomere centers

We used 2G9, a neural marker, to identify the overall organization of rhombomeres in the developing *Xenopus* hindbrain. 2G9 first appears throughout the hindbrain at stage 35/36 (Fig. 2A). By stage 40/41, 2G9 immunoreactivity is present in clusters of cells, at the ventricular surface, and outlying neuropil regions (Fig. 2B). Subsequently, 2G9 immunoreactivity becomes more defined in cells that appear to be localized to rhombomeric centers (ventricular concavities), but not in cells at rhombomeric boundaries (ventricular convexities; Fig. 2C and D). Furthermore, the processes from rhombomeric center regions appear to reach out into the outlying neuropil regions. Fig. 2C and D demonstrate this pattern of immunostaining in the hindbrain of a stage 50 tadpole at low and high magnifications, respectively. This pattern of immunostaining in rhombomeric centers and outlying neuropil regions emerges after stage 40/41 and persists through stage 57 of tadpole development (∼10 weeks, the last developmental stage assayed in this report).

3.3. BrdU appears in rhombomere boundaries

In the chick hindbrain, boundary regions at the ventricular surface are marked by the presence of S-phase nuclei (Guthrie et al., 1991). In *Xenopus*, 2G9 labels rhombomeric centers (Fig. 2C and D). Therefore, we used BrdU to identify S-phase nuclei predicted to be associated with rhombomeric boundaries. After 24 h BrdU exposure, we examined the

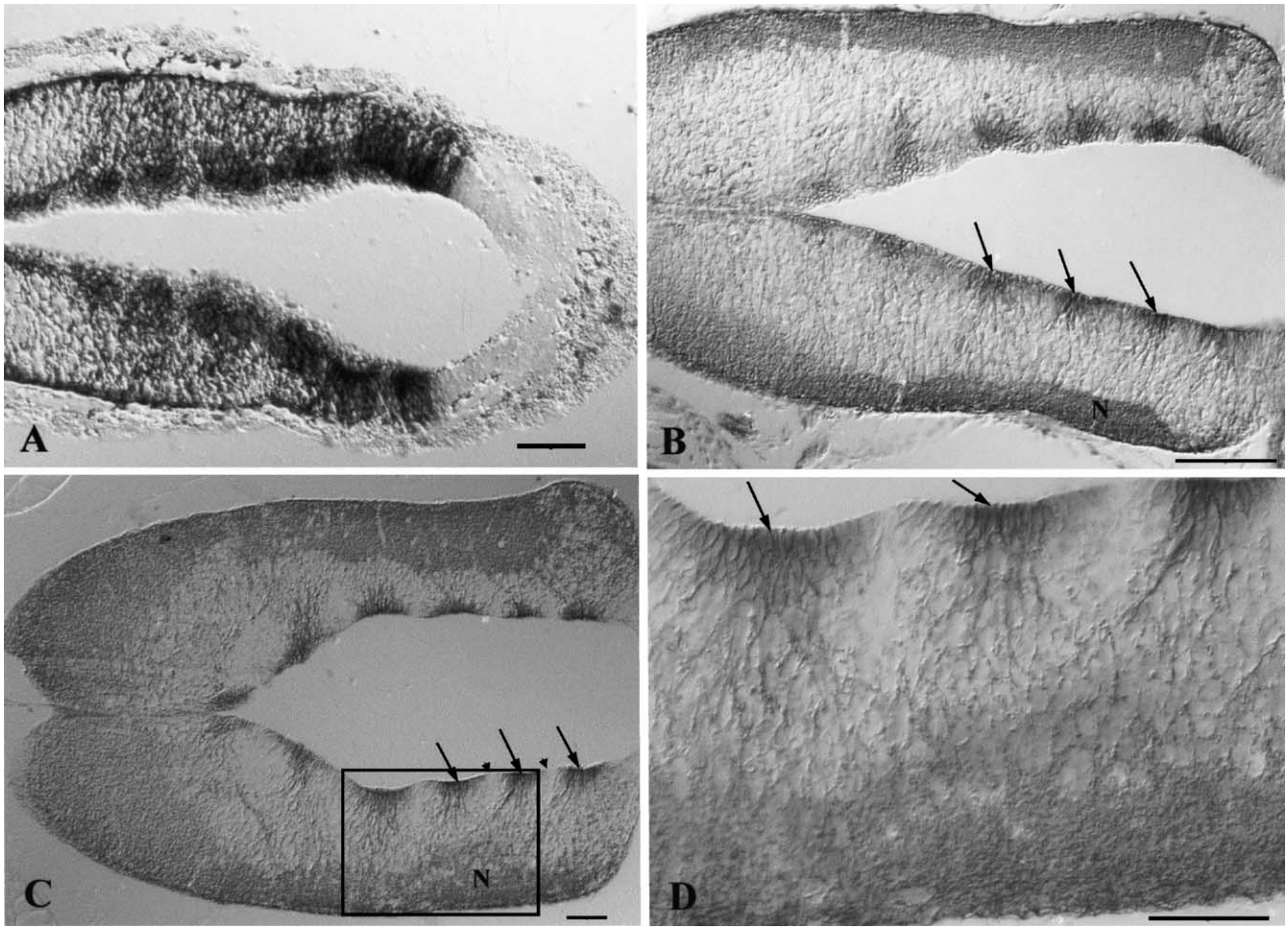

Fig. 2. Developmental changes in 2G9 expression showing: (A) emerging segmental organization of 2G9 stained neural tissue in the hindbrain of stage 35/36 tadpole; (B) definition of 2G9 clusters at the ventricular surface (arrows) and outlying neuropil regions (N) at stage 40/41 of hindbrain development; (C) localization of 2G9 clusters to rhombomere centers (ventricular concavities; arrows), but not boundary regions (ventricular convexities; arrow heads), and outlying neuropil (N) at stage 50; and (D) high magnification of the inbox in (C) depicting 2G9 stained cell processes emanating from the clusters (arrows) and reaching into the outlying neuropil (N). All figures show horizontal sections, where posterior is to the left. Magnification bars: 50 μm.

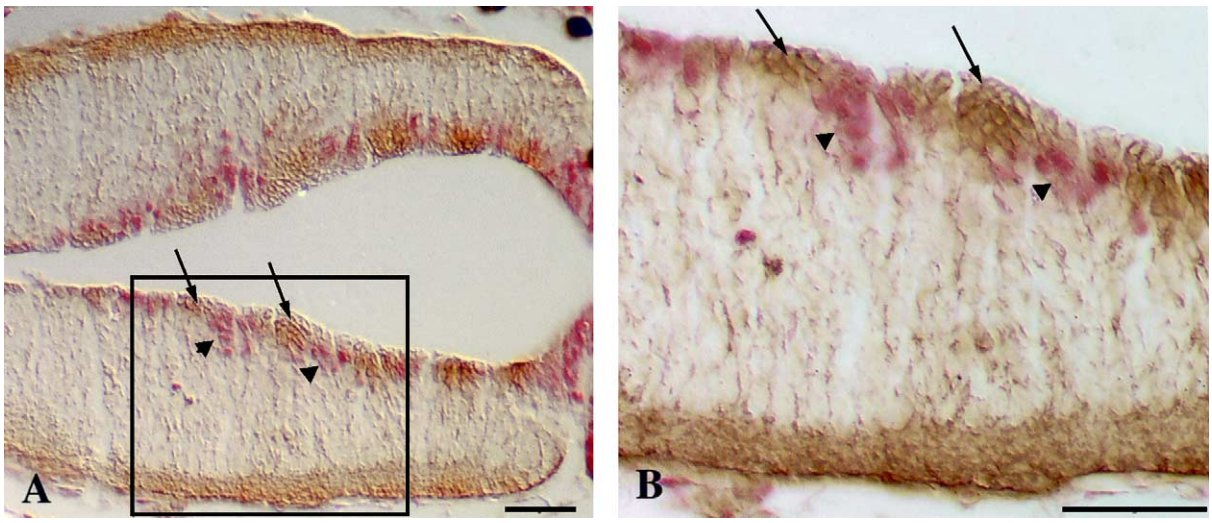

Fig. 3. Results of double labeling portraying: (A) alternating 2G9 clusters (brown; arrows) and groups of dividing cells (red; arrow heads) at the ependymal surface in the hindbrain of a stage 40/41 tadpole; (B) high magnification image of the inbox in (A) demonstrating almost no overlap between the relatively quiescent cells of the 2G9 clusters (arrows) and the BrdU labeled proliferating cells (arrows heads). All figures show horizontal sections, where posterior is to the left. Magnification bars: 50 μm.

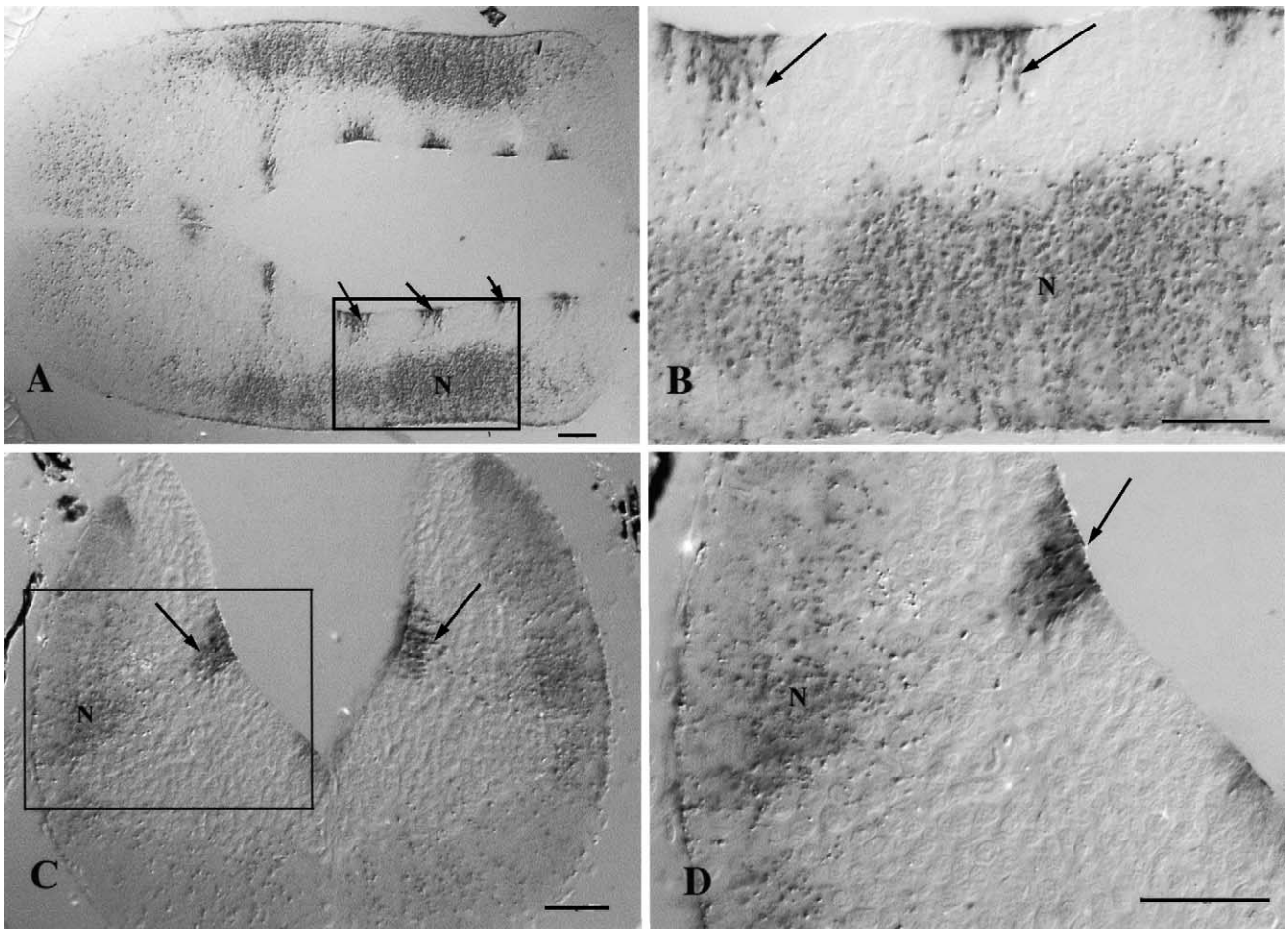

Fig. 4. Developmental changes in Cx43 expression showing: (A) the appearance of Cx43 clusters (arrows) at the ependymal surface and in the outlying neuropil (N) of a stage 50 tadpole; (B) high magnification view of the inbox in (A) depicting the punctate and fibrous characteristics of Cx43 immunostaining in the clusters (arrows) and the neuropil (N), as well as in cell processes emanating from the clusters and reaching into the neuropil; (C) similar Cx43 immunostaining viewed in the clusters (arrows) and the neuropil (N) of a transverse section at the level of the fourth rhombomere; and (D) high power image of the inbox in (C) displaying characteristic punctate Cx43 staining within clusters (arrows) and associated neuropil. (A) and (B) show horizontal sections, where posterior is to the left; (C) and (D) show transverse sections, where dorsal is up. Magnification bars: 50 μm.

hindbrain of tadpoles at stages 40/41, 45/46 and 47 for the presence of both 2G9 and BrdU labels. Double labeling with these antibodies showed that 2G9 clusters alternated with groups of dividing cells at the ventricular surface (see color plate for Fig. 3A and B), although an occasional BrdU-labeled nucleus was detected within the 2G9 clusters. This alternating pattern of 2G9 and BrdU-labeling was observed at all of the above developmental stages indicating that elaboration of rhombomeres occurs via cell division between the 2G9 clusters and that the 2G9 clusters occupy rhombomere center positions throughout development.

3.4. Cx43 expression appears in rhombomeric centers

Although Cx43 expression was first detected in stage 35/36 tadpoles as longitudinal columns in the ventral most regions of the floor plate of the hindbrain (not shown), Cx43 immunoreactivity in the ventricular zone and neuropil regions of the hindbrain appeared at stage 47 and persisted through the last stage assayed (stage 57). Fan-shaped clusters of Cx43 labeled cells occurred along the ventricular surface and a band of Cx43 immunoreactivity was detected in the neuropil extending towards the pial surface (Fig. 4 A–D). At high magnification, Cx43 labeled clusters and neuropil profiles appeared to show punctate immunostaining in both longitudinal and transverse sections (Fig. 4B and D, respectively), indicating the presence of Cx43 in both longitudinally and radially oriented fibers. Moreover, punctate staining associated with clusters at the ventricular surface appeared to project into the outlying neuropil (Fig. 4B and D).

3.5. Cx43 and 2G9 are co-expressed in cells located in rhombomeric centers

To determine if Cx43 was associated with the 2G9-defined rhombomere center cells, we performed double label

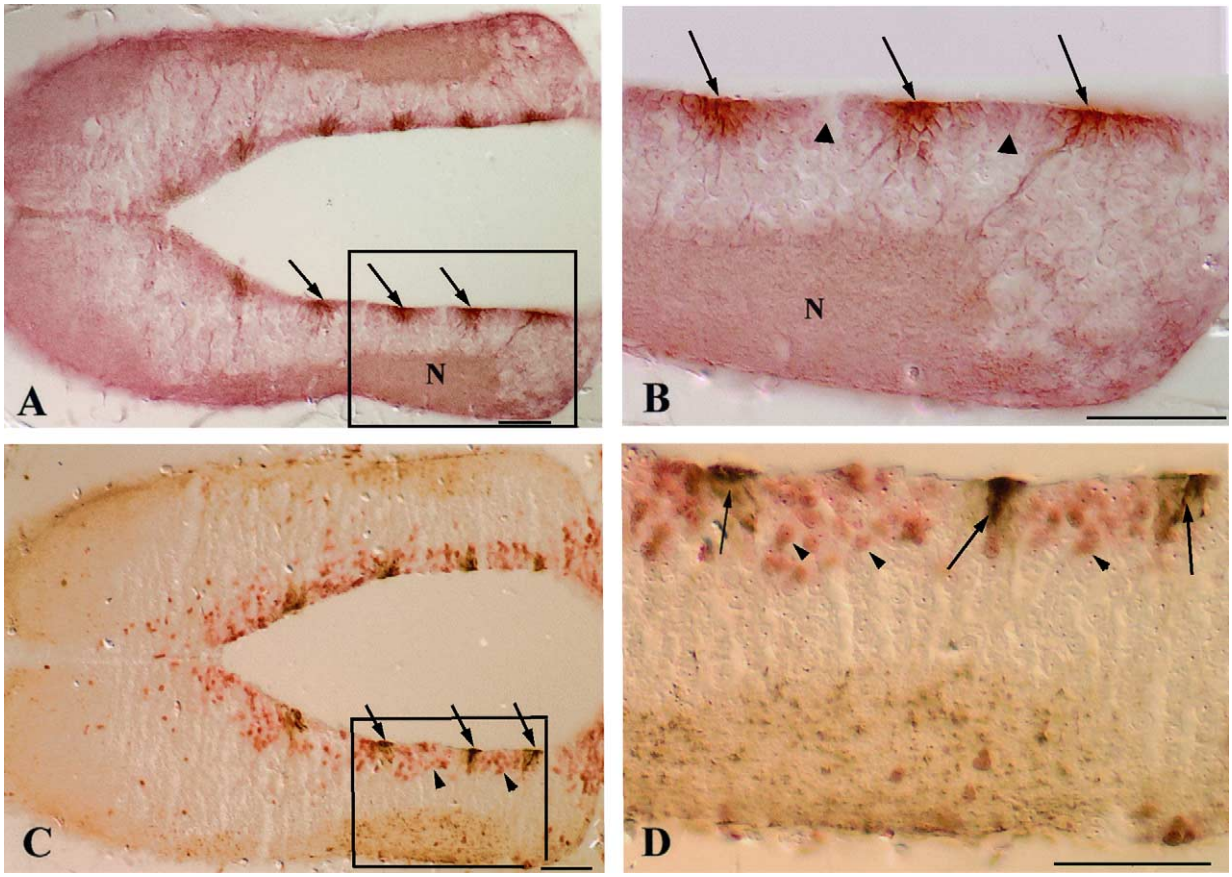

Fig. 5. Results of double labeling portraying: (A) co-localization of 2G9 (red) and Cx43 (brown) expression within clusters of cells at rhombomeric centers (arrows) and the outlying neuropil region (N) in the hindbrain of a stage 47 animal; (B) high magnification of the inbox in (A) demonstrating 2G9-Cx43 labeled profiles within ventricular clusters (arrows) and processes reaching into the outlying neuropil (N); rhombomeric boundary regions show no labeling above background (arrow heads); (C) BrdU-labeled S-phase nuclei (red; arrow heads) alternating with Cx43 clusters (brown; arrows) at the ependymal surface of the hindbrain seen 72 h post-BrdU exposure in stage 49 tadpole; (D) high power view of the inbox in (C) portraying minimal overlap between the BrdU (ventricular convexities; arrow heads) and Cx43 labeled clusters (ventricular concavities; arrows). All figures show horizontal sections, where posterior is to the left. Magnification bars: 50 μm.

immunocytochemistry with 2G9 and Cx43 antibodies. Cx43 immunostaining co-localized with 2G9 immunostaining in clusters of cells at rhombomeric centers and in their processes that extended into the outlying neuropil (see color plate for Fig. 5A and B).

3.6. Cx43 and BrdU labels are not co-localized

Double labeling with Cx43 and BrdU antibodies showed that Cx43 clusters alternated with groups of dividing cells at the ventricular surface (see color plate for Fig. 5C and D), although S-phase nuclei were occasionally detected within Cx43 clusters. Thus, Cx43 clusters were characterized by cells with reduced rates of cell division and were flanked on either side by bands of proliferating cells. Similar to 2G9-BrdU immunostaining patterns, Cx43 expression patterns occurred in rhombomeric centers, whereas BrdU labeling occurred at rhombomeric borders.

3.7. Cx43 marks a subpopulation of GFAP-labeled glial cells

Work in *Xenopus* showed that GFAP is expressed in both rhombomeric center and boundary cells during early development, although in later developmental stages GFAP staining became restricted to rhombomeric centers (Yoshida and Colman, 2000). We performed immunocytochemistry with an anti-GFAP antibody to define the spatial distribution of this glial cell marker with respect to Cx43 expression in the developing hindbrain. GFAP immunoreactivity first appeared at stage 49/50 in cells in rhombomeric centers and boundary regions, and in outlying neuropil regions (Fig. 6A). This GFAP expression pattern, however, was different from Cx43 immunoreactivity, which localized to rhombomeric center and outlying neuropil regions only (Fig. 6B; also shown earlier in Fig. 4A–D). Thus, double staining revealed that Cx43 is expressed by GFAP-positive glial cells residing within rhombomeric centers and outlying neuropil regions,

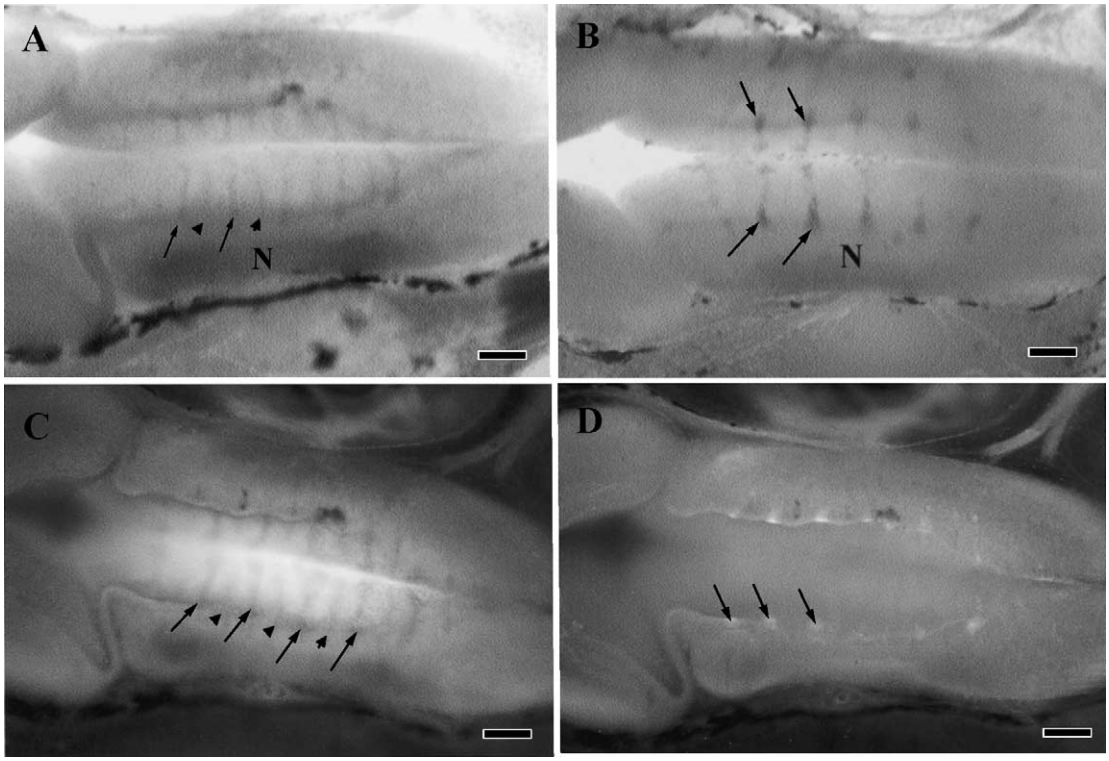

Fig. 6. Results of single and double labeling in vibratome sections displaying: (A) GFAP immunoreactivity in rhombomeric centers (ventricular concavities; arrows), rhombomeric boundaries (ventricular convexities; arrow heads) and the outlying neuropil (N) of a stage 49/50 tadpole; (B) Cx43 expression in rhombomeric centers (ventricular concavities; arrows) and outlying neuropil regions (N); (C) an image of the same vibratome section that was labeled with GFAP (shown in A), now double stained with Cx43 and photographed with a combination filter (DAPI/FITC/TRITC) to highlight DAB-stained GFAP as described in (A), and subsequently rephotographed with a Texas red filter to show (D) red alkaline phosphatase labeled Cx43 as described in (B). All figures show horizontal sections, where posterior is to the right. Magnification bars: 100 μm.

and not by the GFAP-positive cells of rhombomeric boundary regions (Fig. 6C and D). Fig. 6C shows an image of a double-labeled vibratome section that was photographed with a combination filter (DAPI/FITC/TRITC) to highlight DAB-stained GFAP-positive cells. Fig. 6D shows a picture of the same section photographed with a Texas Red filter to delineate Cx43 labeling with red alkaline phosphatase. These findings indicate that cells within rhombomeric centers and outlying neuropil regions become specialized at stage 47, expressing Cx43 just prior to their maturation into GFAP immunoreactive glial cells at stage 49/50 (Fig. 6A–C).

4. Discussion

4.1. 2G9 and Cx43 are co-expressed in rhombomeric center cells, whereas BrdU-labeled cells appear at rhombomeric boundaries

2G9 is a neural marker that is expressed throughout the nervous system from stage 29 through adulthood in *Xenopus laevis* (Jones and Woodland, 1989). In the present study, 2G9 expression localized to rhombomeric center and outlying neuropil regions from early development (stage 40/41) through premetamorphic stages (stage 57). Moreover, these 2G9-labeled profiles co-localized with Cx43 expression at stage 47 and Cx43-labeled profiles subsequently became immunoreactive to GFAP at stage 49/50. Thus, 2G9 appears to label, at a minimum, presumptive glial cells or glial precursors during early developmental stages in the *Xenopus* hindbrain.

Interestingly, ventricular zone cells undergo mitotic activity at rhombomeric boundaries, but not in the rhombomeric center regions, as indicated by BrdU labeling patterns. During all developmental stages where BrdU labeling was performed, BrdU-labeled cells appeared throughout boundary region convexities. Moreover, at stage 49/50, a population of GFAP-positive cells appeared to bisect boundary region convexities, separating cells from adjacent rhombomeres. These GFAP-positive boundary marker cells never expressed Cx43 at any examined developmental stage up to stage 57, indicating that these glial cells are different from those residing within rhombomeric center regions. The strategic location of GFAP-positive boundary glia, in fact, suggests that they may serve as barriers segregating growing cells to respective rhombomeres.

4.2. Cx43 and GFAP are also co-expressed in rhombomeric centers and outlying neuropil

GFAP immunoreactivity was also identified within ventricular zone concavities corresponding to rhombomeric centers and outlying neuropil regions. These GFAP immunolabeled profiles co-localized with Cx43 expression patterns. Labeled cell bodies appeared at the ventricular surface with their labeled processes extending to the pial surface with some elaboration in the neuropil. Thus, these cells appear to resemble radial glial cells of the mammalian nervous system.

4.3. Functional significance of Cx43 in the mammalian nervous system

In the developing mammalian brain, radial glial cells reside in the subventricular zone, and extend their processes to ventricular and pial surfaces; neurons born in the ventricular zone use radial glial processes as guides to migrate to their final destination in the cortical plate (Rakic, 1981, 1988). Thus, radial glial cells facilitate neuronal migration during corticogenesis.

Radial glial cells have recently been shown to express Cx43 during neuronal migration (Nadarajah et al., 1997). Cx43 is the predominant gap junction protein of the adult mammalian brain that is expressed abundantly by astrocytes and accounts for the majority of intercellular coupling in the adult brain (Dermietzel et al., 1989). A significant increase in Cx43 in radial glia during a period that coincides with neuronal migration suggests that radial glial cells may establish gap junctional communication as they form the scaffolding that neurons use to migrate into the cortical plate (Nadarajah et al., 1997). Such a notion is further endorsed by the outcomes of a recent study that traced neuronal migration in the neocortex of Cx43 null mutant mice (Fushiki et al., 2003). These investigators found that BrdU-labeled cells accumulated in the intermediate zone or in the inner part of the cortical plate, indicating that neocortical neuronal migration in Cx43 null mutant mice is significantly delayed (Fushiki et al., 2003). The primary focus of the Cx43 knockout may reside in disruption of the radial glia network, neuronal-glial communication, or in both.

4.4. Functional significance of Cx43 in the vertebrate hindbrain

The results of our investigations indicate that glial cells of the *Xenopus* hindbrain show spatially and temporally regulated expression of Cx43 and GFAP during rhombomeric development. These Cx43–GFAP expressing cells always appear in the rhombomeric centers (ventricular concavities) and are not associated with mitotic cells of rhombomeric convexities that include a boundary between adjacent rhombomeres. Furthermore, the Cx43–GFAP expressing glial cells of the ventricular zone reach out into the outlying neuropil of the *Xenopus* hindbrain. In light of the studies in mammals, it may be that Cx43–GFAP expressing cells of the *Xenopus* hindbrain serve as radial glial cells, helping guide newly born neurons, and/or their processes to positions in forming hindbrain nuclei and related neuropil during hindbrain circuit formation. This idea is particularly evident in the developmental timetable of the auditory system and its hindbrain circuitry. In *Xenopus*, all sensory organs of the peripheral auditory system differentiate and emerge at stage 47, the time of appearance of Cx43, but their afferent projections and the first order octaval nucleus, dorsolateral nucleus, do not appear until stage 50 (Fritzsch, 1990). The finding that Cx43 is expressed in the hindbrain neuropil region that is directly across from the VIII cranial nerve roots (rhombomere 5/6), where afferents of the VIII cranial nerve terminate, and that Cx43 expression persists through premetamorphic stages of development supports the idea that Cx43 may play such a role in hindbrain circuit formation. Thus, Cx43-glial networking may play an essential organizational role during differentiation of rhombomeric tissue throughout neural development.

Acknowledgements

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