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Differential expression and cellular localization of doublecortin in the developing rat retina

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Abstract

Doublecortin is 40 kDa microtubule-associated phosphoprotein required for neuronal migration and differentiation in various regions of the developing central nervous system. We have investigated the expression and cellular localization of doublecortin in the developing rat retina using immunocytochemistry and Western blot analysis. The expression of doublecortin was high from embryonic day 18 (E18) until E20 and was low during the postnatal period. The doublecortin immunoreactivity first appeared in a few radially orientated cells in the mantle zone of the primitive retina at E15. From E16 onward, the immunoreactivity appeared in two different regions: the inner part of the retina and middle of the neuroblastic layer. In the inner part, the somata of cells in the ganglion cell layer, in the distal row of the neuroblastic layer and profiles in the inner plexiform layer showed doublecortin immunoreactivity up to postnatal day 1 (P1). Afterwards, the doublecortin immunoreactivity persisted in the inner plexiform layer until P15, although the intensity decreased gradually with the maturation of the retina. In the middle of the neuroblastic layer, doublecortin immunoreactivity appeared in the radially orientated cells. These cells transformed into horizontal cells. The doublecortin immunoreactivity persisted in these cells up to P21. Given these results, doublecortin may play an important role in the migration and differentiation of specific neuronal populations in developmental stages of the rat retina.

Introduction

Doublecortin (DCX) is 40 kDa microtubule-associated phosphoprotein that contains a consensus AbI phosphorylation site and other sites of potential phosphorylation (Francis *et al*., 1999; Gleeson *et al*., 1999). Differential phosphorylation and dephosphorylation by various kinases and phosphatases are thought to be involved in intracellular signalling (Hannan *et al*., 1999). A recent study strongly suggests that signalling processes through nonreceptor tyrosine kinases are important for neuronal migration to the cortex, and it appears that DCX, homologous to the amino terminus of the nonreceptor tyrosine kinases, may function in this signalling pathway (Ware *et al*., 1997). DCX is also known to have regions that are homologous to the putative Ca^{2+}/calmodulin-dependent protein kinase, that may be involved in neuronal migration through Ca^{2+}-dependent signalling pathways (Sossey-Alaoui *et al*., 1998).

A previous study that examined the developing mouse nervous system using immunocytochemistry has shown that high levels of DCX immunoreactivity are present in the cerebral cortex, lateral ganglionic eminence, thalamus, midbrain, hindbrain, cerebellum, spinal cord and retina (Gleeson *et al*., 1999). Several studies have also shown that expression of DCX is found in migrating neuroblasts associated with radial glial fibers and horizontally orientated cells that are present in the subventricular zone (Gleeson *et al*., 1999), suggesting that DCX could be involved in radial and tangential migration (Francis *et al*., 1999; Gleeson *et al*., 1999). In addition, DCX immunoreactive cells have been found in the adult rostral migratory stream, that is formed by neuroblasts migrating from the subventricular zone to the olfactory bulb (Sossey-Alaoui *et al*., 1998; Gleeson *et al*., 1999).

Despite the important roles of DCX in cells that migrate in the brain, little is known regarding the temporal and spatial expression pattern of DCX protein in the developing retina, except for DCX immunoreactivity observed in ganglion cell layer at embryonic day 14 (E14) (Gleeson *et al*., 1999). Therefore, we investigated the expression and cellular localization of DCX in the developing rat retina to obtain a better understanding of the functional role of DCX. Specifically, we conducted immunocytochemistry and Western blot analysis using an anti-DCX antibody in the developing rat retina. Additionally, we characterized DCX-expressing cells by double immunocytochemistry using an antibody for anti-β-tubulin, a specific marker for neuronal cells, or anticalbindin, a common marker for horizontal cells.

Materials and methods

Animals

Eight litters of Sprague–Dawley rats were used for assessment of diverse developmental stages. All experimental procedures performed on the animals were conducted with the approval of the Catholic Ethics Committee of the Catholic University of Korea and were consistent with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23, revised 1996).

Tissue preparation

Five pregnant rats and their litters of embryos at E15, 16, 17, 18, and 20, pups (five of each age) at postnatal day 1 (P1), 3, 5, 7, 10, 15, and 21,

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and adult rats and their litters were killed by an i.p. injection of 10% chloral hydrate (10 mL/100 g body weight). The eyes of the embryos and pups were enucleated, and they were then cut along the anterior border of the ora serrata. The posterior segments of the eyes were processed according to established methods (Wässle *et al*., 1998).

Immunocytochemistry

The posterior segments of the eyes were immersed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 30 min. The retinas were carefully dissected from the choroid and placed in the same fixative for 2 h at 4 °C. The retinas were then washed through several changes of PB, and transferred to 30% sucrose in PB for 5 h at 4 °C. They were frozen in liquid nitrogen, thawed and rinsed in 0.01 M phosphate buffered saline (PBS), pH 7.4. Subsequently, 50-μm-thick vibratome sections were made from the central region of the retina. Before immunostaining, the retinal sections were incubated in 10% normal donkey serum in PBS for 1 h at room temperature to block non-specific binding activity. The sections were incubated in a guinea-pig polyclonal antiserum directed against DCX (Chemicon, Temecula, CA, USA; dilution 1 : 3000) for 1 day at 4 °C. After washing in PBS for 45 min (3 × 15 min), the sections were incubated with peroxidase conjugated goat anti-guinea-pig IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA; dilution 1 : 100) in PBS for 2 h at room temperature. The sections were then rinsed in three changes of 0.05 M Tris/HCl buffer (TB; pH 7.6) for 5 min each at room temperature and incubated in 0.05% 3,3′-diaminobenzidine tetrahydrochloride in TB for 10 min. Hydrogen peroxide was added to the incubation medium to a final concentration of 0.01%, and the container was shaken gently as the reaction proceeded. The reaction was stopped with several washes of TB or PB after 1–2 min, as determined by the degree of staining. The retinas were mounted using glycerol.

Western blot analysis

For Western blot analysis, retinal tissues were dissected, frozen in liquid nitrogen and stored at −70 °C. Western blot analysis was performed on the retinal extracts which were homogenized in 10 vols of 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 0.02% sodium azide, 1 mM phenymethylsulphonyl fluoride and 5 μg/mL leupeptin. Protein concentration in each sample was assayed by the Lowry method (Lowry *et al*., 1951; Peterson, 1979) in duplicate and the result averaged. Duplicate sets of protein standards containing 0, 1, 3, 5, 10, 20, 40 or 60 μg bovine serum albumin were assayed by the same method and the results averaged and graphed to give a linear equation that was used to estimate protein content of the retinal extracts. Optical density of each sample was measured at 660 nm using a spectrometer (Spectronic 20; Bausch and Lomb, Rochester, NY, USA). Aliquots of tissue samples corresponding to 25 μg total protein were heated at 100 °C for 10 min with an equivalent volume of 2 × sample buffer (containing 4% sodium dodecyl sulphate and 10% mercaptoethanol) and loaded onto 10% polyacrylamide gels. The proteins were electrotransferred to a nitrocellulose membrane in Tris-glycine-methanol buffer. The membrane was blocked for 1 h at room temperature in a blocking solution containing 5% nonfat dry milk, 0.05% Tween-20, and PBS (pH 7.4). The membrane was then incubated for 15 h at 4 °C with a guinea-pig polyclonal antiserum directed against DCX (Chemicon; dilution 1 : 3000) in the blocking solution. The membrane was rinsed with 0.05% Tween-20 in PBS for three washes of 10 min and incubated for 1 h at room temperature in a 1 : 2000 dilution of peroxidase-conjugated goat anti-guinea-pig IgG (Vector Laboratories). The blot was washed three times for 10 min each and then processed for analysis using an enhanced chemiluminescence detection kit (Amersham, Arlington Heights, IL, USA).

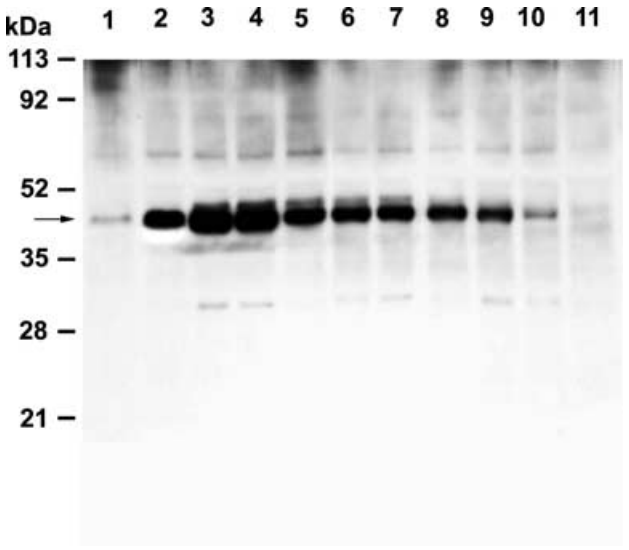

FIG. 1. Differential expression of doublecortin (DCX) protein during development of the rat retina. Retinal extracts of all experimental development ages were prepared and 25 μg of each lysate was immunoblotted with anti-DCX antibody. Lane 1, E15; lane 2, E 16; lane 3, E 18; lane 4, E 20; lane 5, P1; lane 6, P 3; lane 7, P 5; Lane 8, P 7; lane 9, P 10; lane 10, P 15; lane 11, P 21.

Double immunocytochemistry

To test whether DCX immunoreactivity was present in the migrating neuronal cells and horizontal cells, double immunocytochemistry was performed using the indirect fluorescence method. The retinal sections were incubated in PBS containing 10% normal donkey serum. The sections were then incubated in a mixture of a polyclonal antibody against DCX and a mouse monoclonal antibody against β-tubulin (Sigma, Saint Louis, MO, USA; dilution 1 : 500) or a monoclonal antibody against calbindin (Sigma; dilution 1 : 3000) for 1 day at 4 °C. After being washed in PBS for 45 min, the sections were incubated in a mixture of fluorescein isothiocyanate (FITC)-conjugated donkey anti-mouse IgG (Jackson; dilution 1 : 50) and Cy3-conjugated donkey anti-guinea-pig-IgG (Jackson; dilution 1 : 500) for 2 h at room temperature. The sections were then rinsed in three changes of PB, cover-slipped, and observed with a Bio-Rad Radiance Plus (Bio-Rad, Hemel Hempstead, UK) confocal scanning microscope (Nikon, Tokyo, Japan). FITC labelling was activated by using the 488-nm line of the Argon ion laser and detected after passing a HQ513/30 (Bio-Rad) emission filter. For the detection of the Cy3 signal, the 543-nm line of the green HeNe laser was used in combination with the 605/32 (Bio-Rad) emission filter. Images were imported into Adobe PHOTOSHOP and graphed onto slide film (Kodak Ektachrome 100; Eastman Kodak, Rochester, NY, USA).

Results

DCX expression during development

Western blot analysis demonstrated a single band of 40 kDa during all developmental periods (Fig. 1). At E15, a weak band was noticed, but a high amount of the protein was seen from E16–20. From P1 onward, DCX protein level gradually decreased as the retina matured. Very little DCX expression was detected at P21, consistent with the suggestion that DCX probably exerts its major effects during early developmental stages (Gleeson *et al*., 1999).

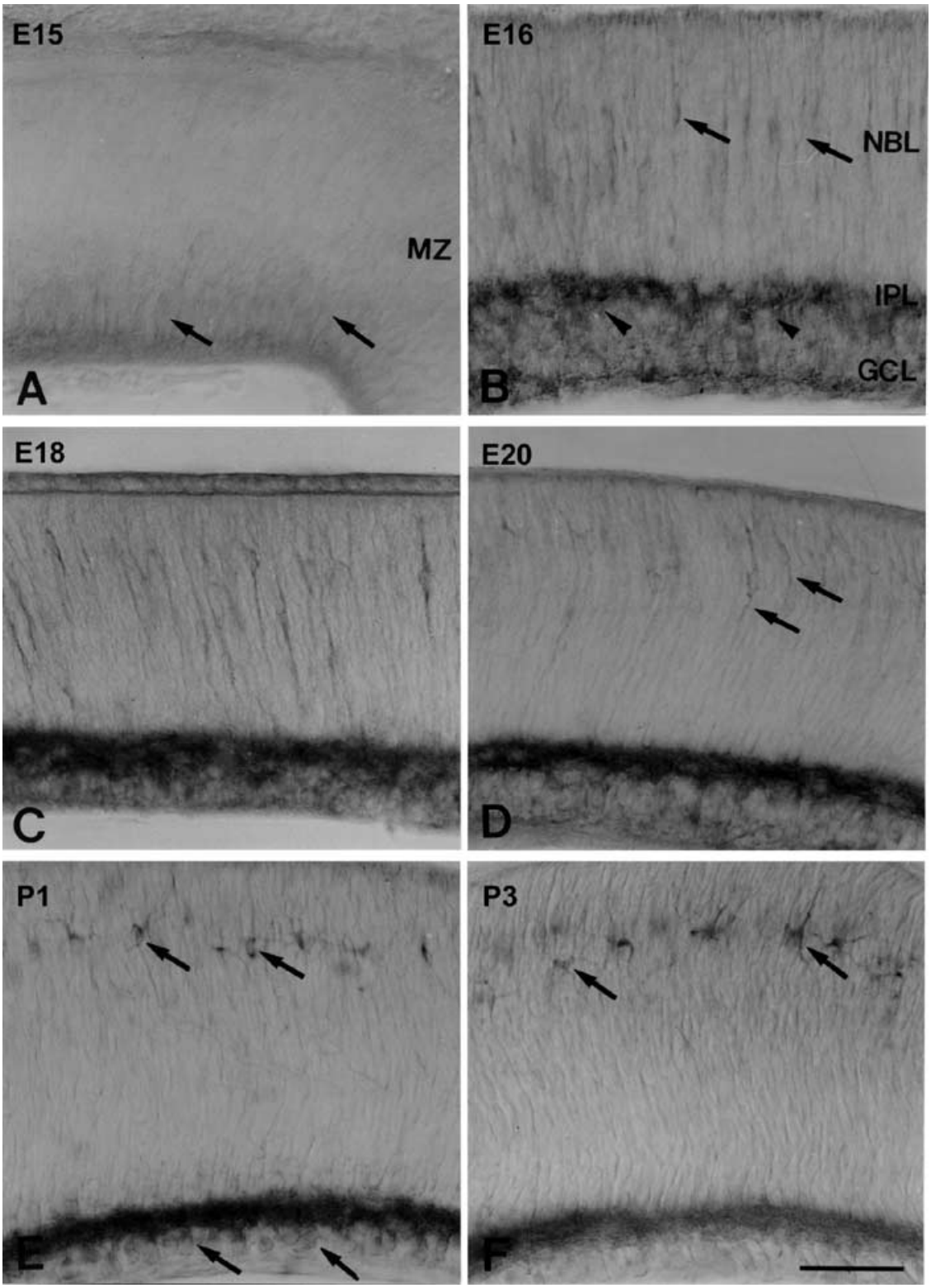

FIG. 2. Light microphotographs of 50-μm-thick vibratome sections processed for doublecortin (DCX) immunoreactivity at E15 (A), E16 (B), E18 (C), E20 (D), P1 (E) and P 3 (F). In A, several neuroblasts (arrows) in the mantle zone of the retina show weak DCX immunoreactivity. In B, DCX immunoreactivity is visible in the somata (arrowheads) of the inner retina and in the inner plexiform layer (IPL). Note DCX labelled neuroblasts (arrowheads) with long processes (arrows) in the outer part of the neuroblastic layer (NBL). In C and D, the immunoreactivity of the inner retina is similar to that shown in B. However, in the outer part, DCX immunoreactivity is more prominent, and labelled neuroblasts have retracted leading processes (arrows) in D. In E, in the inner part, labelled somata (arrows) are clearly visible in the ganglion cell layer and in the distal row of the neuroblastic layer. In the outer part, labelled neuroblasts are multipolar in shape (arrows). In F, the immunoreactivity is decreased compared with that shown in E. Scale bar in F, 50 μm (A–F).

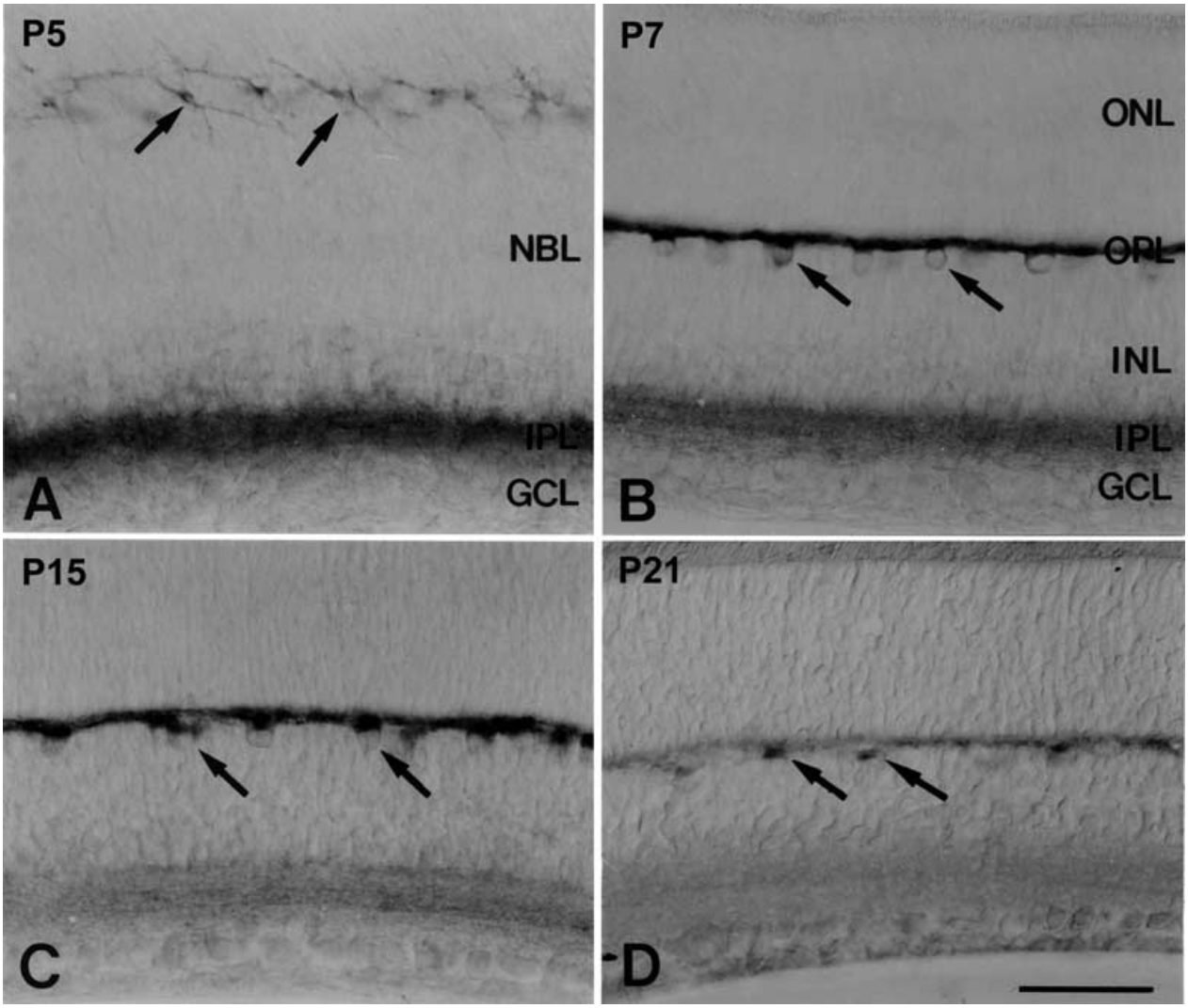

FIG. 3. Light microphotographs of 50-μm-thick vibratome sections processed for doublecortin (DCX) immunocytochemistry at P5 (A), P7 (B), P15 (C) and P21 (D). In A, labelled neuroblast horizontally orientated processes (arrows) are visible in the outer part of the neuroblastic layer (NBL). In B, DCX-labelled cells (arrows) are visible in the outer margin of the inner nuclear layer (INL). Note that the immunoreactivity is markedly reduced in the inner plexiform layer (IPL). In C, the labelled neurons (arrows) are similar to those in shown in B. However, the immunoreactivity is hardly seen in the IPL. In D, DCX profiles (arrows) are visible as puncta in the outer margin of the INL and in the outer plexiform layer (OPL). ONL, outer nuclear layer; GCL, ganglion cell layer. Scale bar in D, 50 μm (A–D).

DCX immunoreactivity during development

During developmental periods, DCX immunoreactivity appeared in two different regions of the retina; the inner and outer parts.

In the inner part of the developing retina, DCX immunoreactivity was first noted at E15 in a few neuroblasts in the inner parts of the mantle zone (Fig. 2A). At E16, strong immunoreactivity was seen in the presumptive inner plexiform layer (IPL). Most cells beneath the vitreal surface of the retina showed weak immunoreactivity (Fig. 2B). At E18, the IPL showed strong DCX immunoreactivity (Fig. 2C). At E20, cell bodies showing DCX immunoreactivity were clearly visible in the inner margin of the neuroblastic layer (NBL) and in the ganglion cell layer (Fig. 2D). The immunoreactivity of the IPL was similar to that shown at E18 (Fig. 2E). These immunoreactive patterns in the inner part of the retina remained until P1. After P1, DCX immunoreactivity in the inner retina became less intense with increasing development, and DCX immunoreactivity was hardly seen in the IPL at P21 (Figs 2F and 3).

In the outer part of the developing retina, DCX immunoreactivity was first visible in the cells that formed a single row in the middle of the NBL. These cells were bipolar in shape, their outer processes reached the outer limiting membrane, and their inner processes reached the IPL (Fig. 2B). At E18, more strongly stained migrating neuroblasts could be identified. DCX labelled cells were seen at E20 in the outer one-third of the NBL, but their inner processes could not reach the IPL (Fig. 2D). From P1–5, labelled cells in the outer one-third of the NBL were multipolar in shape. They had three to four branched processes, that did not reach the outer limiting membrane (Figs 2E and F, and 3A). By P7, the dense networks of strongly labelled processes were seen in the outer plexiform layer (OPL), and labelled cell bodies were located at the outer margin of the inner nuclear layer (INL) (Fig. 3B). At P10, the immunoreactivity patterns of the labelled cells located at the outer margin of the INL were similar to those of the retina at P7. From P15 onward, DCX immunoreactivity decreased, and labelled profiles appeared as puncta in the OPL at P21 (Fig. 3C and D). On the basis of position and morphology, labelled cells that appeared

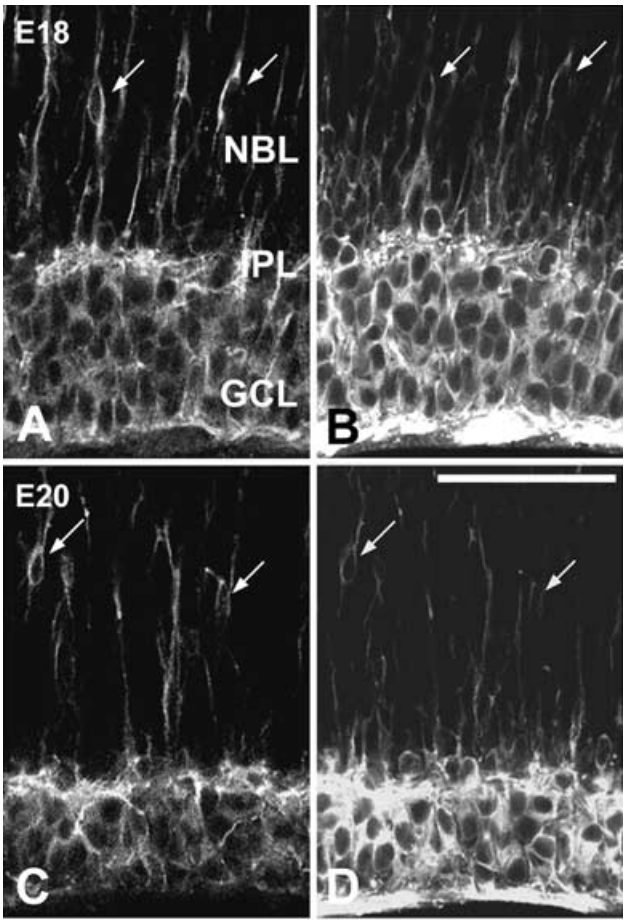

FIG. 4. Confocal micrographs taken from a vertical vibratome section processed for doublecortin (DCX) (A, C) and β-tubulin (B, D) immunoreactivities at E18 (A, B) and E20 (C, D). DCX immunoreactivity was visualized using a Cy3-conjugated secondary antibody; β-tubulin immunoreactivity was visualized using a FITC-conjugated secondary antibody. DCX immunoreactive cells (arrows) in the neuroblastic layer (NBL) also express β-tubulin immunoreactivity. Scale bar, 50 μm.

in the outer part of the NBL from E16–P5 were considered immature horizontal cells.

Co-expression of DCX and β-tubulin

Double-labelling experiments were performed in developing retinas using antisera against DCX and β-tubulin, that have previously been shown to be present in migrating and differentiating neurons in the developing cortex (Lee *et al*., 1990). DCX immunoreactivity (Fig 4A and C) was localized to the same cells that showed β-tubulin immunoreactivity (Fig. 4B and D). These results clearly demonstrate that DCX immunoreactivity was intense in migrating neurons of the neuroblastic layer during the embryonic period.

Co-expression of DCX and Calbindin

To identify the type of cells in the outer one-third of the NBL that were DCX immunoreactive, double-labelling using antiserum against DCX and antiserum against calbindin, a specific marker for the horizontal cells (Röhrenbeck *et al*., 1987), was performed. The coexpression of DCX and calbindin was evident in the cells positioned in the outer one-third of the NBL at P1 (Fig. 5A and B) and P5 (Fig. 5C and D), and in the cells located in the outer margin of the INL at P7 (Fig. 5E and F) and P 15 (Fig. 5G and H). These results demonstrate clearly that DCX

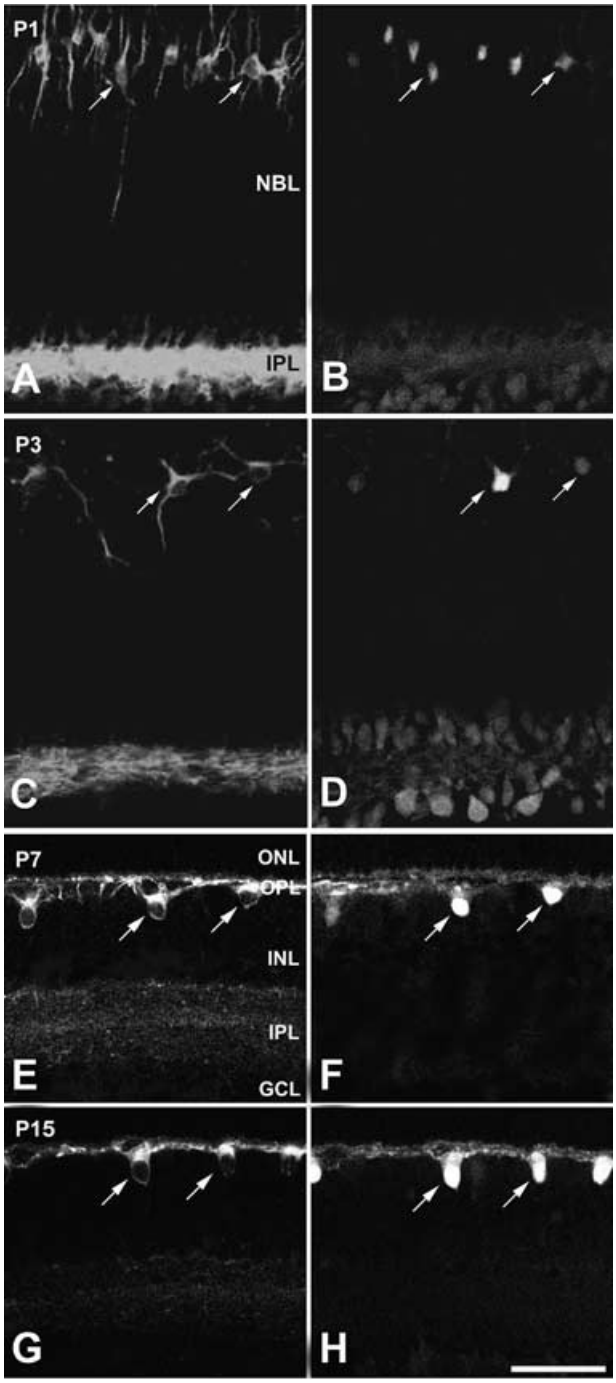

FIG. 5. Confocal micrographs taken from a vertical vibratome section processed for doublecortin (DCX) (A, C, E and G) and calbindin (B, D, F and H) immunoreactivities at P1 (A, B); P5 (C, D) P7 (E, F) and P15 (G, H). DCX immunoreactivity was visualized using a Cy3-conjugated secondary antibody; calbindin immunoreactivity was visualized using a FITC-conjugated secondary antibody. DCX immunoreactive cells (arrows) show calbindin immunoreactivity. Scale bar in H, 50 μm (A–H).

labelled cells, that appeared in the middle of the NBL from early developmental periods, are horizontal cells.

Discussion

In an initial attempt to characterize the role of DCX in retinal development, we have investigated the expression of DCX and the

temporal and spatial distribution of DCX-labelled cells in the developing rat retina using Western blot analysis and immunocytochemistry.

Western blot analysis showed that DCX was expressed highly in the embryonic period and lower during the postnatal period. Very little DCX expression was detected at P21, that is consistent with the suggestion that DCX exerts its effects during early development (Francis *et al*., 1999). The reason for the weak DCX immunoreactivity at P21 appears to be independent of neuronal migration, and the presence of low levels is possibly due to residual DCX, which remains in the somata and processes of neurons.

DCX expression appeared initially at E15 in a few neuroblasts in the mantle zone of the primitive retina. This finding concurs with a previous report by Gleeson *et al*. (1999), saying that cells in the ganglion cell layer of the mouse retina showed DCX immunoreactivity at E14. From E16 onward, cells in two different regions, the inner parts of the retina and middle of the NBL, began to express DCX immunoreactivity. All DCX-labelled cells also showed immunoreactivity for an anti-β-tubulin antibody, a marker of neuronal cells that is present in migrating and differentiating neurons in the developing cortex (Lee *et al*., 1990). Thus, DCX-labelled cells are developing retinal neurons. Studies of neurogenesis in the mammalian retina have demonstrated that the neurogenesis of cell types follows a specific temporal sequence. Ganglion cells, cone cells and horizontal cells are the first-born. Amacrine cells are separate, highly diverse cells with some cells differentiating prenatally, and others postnatally. Bipolar cells differentiate postnatally (Carter-Dawson & Lavail, 1979; Johns *et al*., 1979; Polley *et al*., 1986; Zimmermann *et al*., 1988; LaVail *et al*., 1991). It was reported that differentiation of the inner retina in the developing rat retina is completed mostly during the first postnatal week and synaptogenesis of the IPL during the second postnatal week (Horsburgh & Sefton, 1987). Based on their location, at late embryonic stages and early postnatal stages, retinal neurons showing DCX immunoreactivity in the inner part of the retina are thought to be ganglion/displaced amacrine cells in the ganglion cell layer and amacrine cells in the inner margin of the NBL. These cells did not show any migrating features. Thus, DCX seems to be expressed in these cells after migration ceases. In addition, DCX immunoreactivity in the IPL persisted until P15. Thus, the expression of DCX in the inner retinal neurons appears to be independent from migration. It has also been shown that DCX immunoreactivity appears in the granular and perglomerular neurons of the olfactory bulb and granular neurons of the dentate gyrus that have completed their migration (Nacher *et al*., 2001). In addition, DCX has been shown to participate in microtubule reorganization (Francis *et al*., 1999; Gleeson *et al*., 1999), that is a necessary event in the formation of new neurites (Gordon-Weeks, 1991; Williamson *et al*., 1996). Taken together, DCX might play a role in the differentiation of the inner retina neurons and in synaptogenesis of the IPL in the developing rat retina, as suggested by Nacher *et al*. (2001). However, more deailed studies are clearly needed to clarify the possible mechanisms responsible for the expression of DCX in the differentiating neurons.

In the present study, DCX labelled neurons in the middle of the NBL had long leading processes that were maintained throughout the embryonic days, suggesting they were actively migrating. From our results using double-labelling techniques, it was apparent that these cells became horizontal cells, suggesting that DCX might be involved in horizontal cell migration. This suggestion is supported by previous studies (Gleeson *et al*., 1998; des Portes *et al*., 1998; Nacher *et al*., 2001) that have shown that in the developing and adult mouse brain, DCX immunoreactivity is localized to migrating neurons. DCX immunoreactivity persisted in horizontal cells up to P21 after their migration ceased. These findings also suggest that DCX might be involved in differentiation and synaptogenesis of horizontal cells as well as in those of inner retinal neurons.

The colocalization of DCX with calbindin has also been shown in mouse cerebelar Purkinje cells during periods of migration (Gleeson *et al*., 1999) and implies that DCX might be correlated with Ca^{2+}-dependent signalling pathways during development of the retina. Calbindin is enriched in horizontal cells (Rabie *et al*., 1985; Röhrenbeck *et al*., 1987; Versaux-Botteri *et al*., 1989). Calbindin has buffering functions to regulate the intracellular free Ca^{2+} concentration, and Ca^{2+} sensing functions to modulate activities of enzymes, ion channels, and cell surface receptors (Ikura, 1996). Wässle *et al*. (1998) suggested that calbindin may control the level of intracellular calcium levels within the first 100 ms following calcium influx in retinal horizontal cells, as shown in cerebellar Purkinje cells (Airaksinen *et al*., 1997). Biochemical data from a previous study suggests that the presence of Ca^{2+}/calmodulin-dependent kinase in horizontal cells of fish retinas has an important function in the spinule plasticity at dendrites of retinal horizontal cells (Weiler *et al*., 1996). The presence of Ca^{2+}/calmodulin-dependent kinase has also been detected in rat horizontal cells using immunocytochemistry (Ochiishi *et al*., 1994). DCX contains several potential phosphorylation sites that could regulate the transduction of signals within the neurons, thereby controlling neuronal migration activity during nervous system development (Sobel, 1991). Thus, DCX might be involved in the migration of horizontal cells during the development of retina by putative Ca^{2+}/calmodulin-dependent protein kinase.

In previous studies, calbindin was expressed in rat horizontal cells as early as E17 (Pasteels *et al*., 1990; Uesugi *et al*., 1992). The expression of calbindin, however, was almost the same as that of DCX within the horizontal cells during development, except for the intensity of immunoreactivity. Therefore, anti-DCX antibody could be used as a good marker for rat horizontal cells.

Acknowledgements

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Abbreviations

DCX, doublecortin; E, embryonic day; FITC, fluorescein ispthiocyanate; INL, inner nuclear layer; IPL, inner plexiform layer; NBL, neuroblastic layer; OPL, outer plexiform layer; PB, phosphate buffer; PBS, phosphate-buffered saline.

References

Airaksinen, M.S., Eilers, J., Garaschuk, O., Thoenen, H., Konnerth, A. & Meyer, M. (1997) Ataxia and altered dendritic calcium signalling in mice carrying a targeted null mutation of the calbindin D28k gene. *Proc. Natl Acad. Sci. USA*, **94**, 1488–1493.

Carter-Dawson, L.D. & Lavail, M.M. (1979) Rods and cones in the mouse retina. II. Autoradiographic analysis of cell generation using tritiated thymidine. *J. Comp. Neurol.*, **188**, 263–272.

Francis, F., Koulakoff, A., Boucher, D., Chafey, P., Schaar, B., Vinet, M., Friocourt, G., McDonnell, N., Reiner, O., Kahn, A., McConnell, S.K., Berwald-Netter, Y., Denoulet, P. & Chelly, J. (1999) Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron*, **23**, 247–256.

Gleeson, J.G., Allen, K.M., Fos, J.W., Lamerti, E.D., Berkovic, S., Scheffer, I., Cooper, E.C., Dobyns, W.B., Minnerath, S.R., Elizabeth Ross, M. & Walsh, C.A. (1998) Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell*, **92**, 63–72.

Gleeson, J.G., Lin, P.T., Flanagan, L.A. & Walsh, C.A. (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron*, **23**, 257–271.

Gordon-Weeks, P.R. (1991) Evidence for microtubule capture by filopodial actin filaments in growth cones. *Neuroreport*, **2**, 573–576.

Hannan, A.J., Henke, R.C., Seeto, G.S., Capes-Davis, A., Dunn, J. & Jeffrey, P.L. (1999) Expression of doublecortin correlates with neuronal migration and pattern formation in diverse regions of the developing chick brain. *J. Neurosci. Res.*, **55**, 650–657.

Horsburgh, G.M. & Sefton, A.J. (1987) Cellular degeneration and synaptogenesis in the developing retina of the rat. *J. Comp. Neurol.*, **263**, 553–566.

Ikura, M. (1996) Calcium binding and conformational response in EF-hand proteins. *Trends Biochem. Sci.*, **21**, 14–17.

Johns, P., Ruffet, A. & Dubin, M.W. (1979) Postnatal neurogenesis in the kitten retina. *J. Comp. Neurol.*, **187**, 545–556.

LaVail, M.M., Rapaport, D.H. & Rakic, P. (1991) Cytogenesis in the monkey retina. *J. Comp. Neurol.*, **309**, 86–114.

Lee, M.K., Tuttle, J.B., Rebhun, L.I., Cleveland, D.W. & Frankfurter, A. (1990) The expression and posttranslational modification of a neuron-specific β-tubulin isotype during chick embryogenesis. *Cell Motil. Cytoskel.*, **17**, 118–132.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

Nacher, J., Crespo, C. & McEwen, B.S. (2001) Doublecortin expression in the adult rat telencephalon. *Eur. J. Neurosci.*, **14**, 629–644.

Ochiishi, T., Terashima, T., Sugiura, H. & Yamauchi, T. (1994) Immunohistochemical localization of Ca^{2+}/calmodulin-dependent protein kinase II in the rat retina. *Brain Res.*, **634**, 257–265.

Pasteels, B., Rogers, J., Blachier, F. & Pochet, R. (1990) Calbindin and calretinin localization in retina from different species. *Visual Neurosci.*, **5**, 1–16.

Peterson, G.L. (1979) Review of the folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. *Anal. Biochem.*, **100**, 201–220.

Polley, E.H., Zimmerman, R.P. & Fortney, R.L. (1986) Interaction of a temporal sequence of cell birthdays and a spatial gradient of morphological maturation in the mammalian retina. *Invest. Ophthalmol. Vis. Sci.*, **27**, 326.

des Portes, V., Francis, F., Pinard, J.M., Desguerre, I., Moutard, M.L., Snoeck, I., Meiners, L.C., Cusmai, R., Ricci, S., Motte, J., Echenne, B., Ponsot, G., Dulac, O., Chelly, I. & Beldjord, C. (1998) Doublecortin is the major gene causing X-linked subcortical laminar heterotopia (SCLH). *Hum. Mol. Genet.*, **7**, 1063–1070.

Rabie, A., Thomasset, M., Parkes, C.O. & Clavel, M.C. (1985) Immunocytochemical detection of 28000-MW calcium-binding protein in horizontal cells of the rat retina. *Cell Tiss. Res.*, **240**, 493–496.

Röhrenbeck, J., Wässle, H. & Heizmann, C.W. (1987) Immunocytochemical labelling of horizontal cells in mammalian retina using antibodies against calcium-binding proteins. *Neurosci. Lett.*, **77**, 255–260.

Sobel, A. (1991) Stathmin: a relay phosphoprotein for multiple signal transduction. *Trends Biochem. Sci.*, **16**, 301–305.

Sossey-Alaoui, K., Hartung, A.J., Manchester, D.K., Posar, A., Puche-Mira, A., Andermann, E., Dobyns, W.B. & Srivastava, A.K. (1998) Human doublecortin (DCX) and the homologous gene in mouse encode a putative Ca^{2+}-dependent signaling protein which is mutated in human X-linked neuronal migration defects. *Human Mol. Genet.*, **8**, 1327–1333.

Uesugi, R., Yamada, M., Mizuguchi, M., Baimbridge, K.G. & Kim, S.U. (1992) Calbindin D-28k and parvalbumin immunohistochemistry in developing rat retina. *Exp. Eye Res.*, **54**, 491–499.

Versaux-Botteri, C., Pochet, R. & Nguyen-Legros, J. (1989) Immunohistochemical localization and GABA-containing neurons during postnatal development of the rat retina. *Invest. Ophthalmol. Vis. Sci.*, **30**, 652–659.

Ware, M.L., Fox, J.W., Gonzalez, J.L., Davis, N.M., Lambert de Rouvroit, C., Russo, C.J., Chua, S.C., Goffinet, A.M. & Walsh, C.A. (1997) Aberrant splicing of a mouse disabled homolog, mdab1, in the scrambler mouse. *Neuron*, **19**, 1–20.

Wässle, H., Peichl, L., Airaksinen, M.S. & Meyer, M. (1998) Calcium-binding proteins in the retina of a calbindin-null mutant mouse. *Cell. Tissue Res.*, **292**, 211–218.

Weiler, R., Schultz, K. & Janssen-bienhold, U. (1996) Ca^{2+}-dependency of spinule plasticity at dendrites of retinal horizontal cells and its possible implication for the functional role of spinules. *Vision Res.*, **36**, 3891–3900.

Williamson, T., Gordon-Weeks, P.R., Schachner, M. & Taylor, J. (1996) Microtubule reorganization is obligatory for growth cone turning. *Proc. Natl Acad. Sci. USA*, **93**, 15221–15226.

Zimmermann, R.P., Polley, E. & Fortney, R.L. (1988) Cell birthdays and rate of differentiation of ganglion and horizontal cells of the developing cat's retina. *J. Comp. Neurol.*, **274**, 77–90.