

# Expression of the *Emx-1* and *Dlx-1* homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes

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## SUMMARY

Homologies between vertebrate forebrain subdivisions are still uncertain. In particular the identification of homologs of the mammalian neocortex or the dorsal ventricular ridge (DVR) of birds and reptiles is still a matter of dispute. To get insight about the organization of the primordia of the main telencephalic subdivisions along the anteroposterior axis of the neural tube, a fate map of the dorsal prosencephalon was obtained in avian chimeras at the 8- to 9-somite stage. At this stage, the primordia of the pallium, DVR and striatum were located on the dorsal aspect of the prosencephalon and ordered caudorostrally along the longitudinal axis of the brain. Expression of homeobox-containing genes of the *Emx*, *Dlx* and *Pax* families were

used as markers of anteroposterior developmental subdivisions of the forebrain in mouse, chick, turtle and frog. Their expression domains delineated three main telencephalic subdivisions in all species at the onset of neurogenesis: the pallial, intermediate and striatal neuroepithelial domains. The fate of the intermediate subdivisions diverged, however, between species at later stages of development. Homologies between forebrain subdivisions are proposed based on the conservation and divergence of these gene expression patterns.

Key words: Evolution, Forebrain, Telencephalon, Brain, Vertebrates, Mouse, Chick, Turtle, Frog, Neural tube, *Emx-1*, *Dlx-1*, *Pax-6*

## INTRODUCTION

Several features of brain organization have been conserved during vertebrate evolution, in particular in the midbrain and hindbrain of higher vertebrates. Structures like the optic tectum or the cerebellum that have phylogenetically conserved position, cytoarchitecture, connections, function and neurochemistry are considered as homologous in different species. In contrast, the organization of the forebrain is more divergent and there is still no consensus regarding the homology of the different telencephalic subdivisions in mammals, birds and reptiles. This question has led, since the beginning of the century to a wide variety of interpretations and controversies (reviewed in Striedter, 1997).

The telencephalon of adult mammals comprises two major subdivisions: the cerebral cortex, dorsally, and the deeply located basal ganglia (composed of striatum and pallidum). The telencephalon of living birds and reptiles (sauropsids) is subdivided into three major domains, a dorsal cortical-like pallium, a subpallial formation called the dorsal ventricular ridge (DVR), which bulges into the lateral ventricle and a

basally located striatal domain. At the beginning of this century, the location and extent of the basal ganglia in birds and reptiles were a matter of dispute. These uncertainties are still reflected in the nomenclature of telencephalic subdivisions and in their variations between species in both birds and reptiles (see Fig. 1). More recent findings (Reiner et al., 1984; Medina and Reiner, 1995; Northcutt and Kaas, 1995) have shown that there is a great similarity between the neuronal populations that form the basal ganglia in living species of birds, reptiles and mammals with regard of neurotransmitter contents, physiology and connections. Therefore, most modern comparative neuroanatomists now agree that the evolution of basal ganglia has been more conservative than previously believed.

The six-layered neocortex or isocortex of mammals is a recent evolutionary acquisition not found in birds and reptiles. It has been claimed that, in the synapsid line (mammal-like reptiles and mammals), the isocortex is derived from the ancestral dorsal pallium comparable to that of living amphibians (Herrick, 1948; Northcutt, 1981; Northcutt and Kaas, 1995). An alternative hypothesis proposes that the lateral portion of the isocortex and the anterior part of the DVR (ADVR) have a common origin and

are thus homologous (Karten, 1991; Butler, 1994). This hypothesis is based on evidence that the ADVR is not, as previously thought, a striatal formation, but shares many neurochemical, connectional and physiological similarities with the mammalian isocortex. In contrast, the posterior basal part of the DVR (BDVR) displays characteristics that resemble those of the amygdala (Ulinski, 1983, Streidter, 1997 for review).

These theories of an isocortical ADVR have not gone unchallenged (Lohman and Smeets, 1991; Smeets and Gonzalez, 1994; Northcutt and Kaas, 1995) and the ADVR has been considered as related to various mammalian non-cortical structures: claustrum (Filimonoff, 1964; MacLean, 1990), lateral amygdala (Bruce and Neary, 1995) and endopiriform nucleus (Streidter, 1997).

It is now well established that the structure and cellular functions of many *Drosophila* developmental genes have been conserved during evolution. In addition, it has been realized that the arrangement along the main body axes of the expression domains of many of these genes, notably the *Hox* genes but also genes of the *Otx*, *Emx*, *Dlx* and *Pax* families, has been maintained to a large extent during evolution. This finding was unexpected because of the great morphological diversity of adult animals. At early stages, these developmental genes are supposed to demarcate relative positions in animals rather than to specify particular structures (Carroll, 1995). This early role seems to have been conserved in vertebrates, in particular in the developing neural tube. In the embryonic mouse brain, the expression of homologs of *Drosophila* developmental genes have been shown to delineate a map comprising potential morphogenetic units that correspond in part to the neuromeres of earlier authors (Puelles and Rubenstein, 1993; Rubenstein et al., 1994).

The aim of the present study was to try to understand better the formation of divergent telencephalic structures in higher vertebrates by taking advantage of the similarity of early developmental processes and gene expression between species. Using quail-to-chick transplantation, we first determined that the primordia of the three main subdivisions of the avian telencephalic vesicle are aligned along the anteroposterior axis of the early neural tube. We then compared the telencephalic expression of *Emx-1*, *Dlx-1* and *Pax-6* at successive embryonic stages in a series of species belonging to different tetrapod taxa: mammals (*Mus*), birds (*Gallus*), reptiles (*Emys*) and amphibians (*Xenopus*). We found that the developing mouse telencephalic vesicle is similar at early developmental stages to those of the other species studied in that its germinative neuroepithelium contains transiently an intermediate third subdivision. We discuss the possible homologies between telencephalic subdivisions in light of these observations, which make some of the previous interpretations unlikely.

## MATERIALS AND METHODS

### Embryos

OF1 mouse (Iffa Credo) embryos were considered E0.5 the day where vaginal plug was detected, White Leghorn chick embryos (Morizeau) and Japanese quail embryos (La Caille de Chanteloup, Corps-Nuds, France) were staged according to Hamburger and Hamilton (1951, designed HH), turtle embryos (*Emys orbicularis*) according to Pieau and Dorizzi (1981), and frog embryos (*Xenopus laevis*) according to Nieuwkoop and Faber (1956).

### BrdU administration

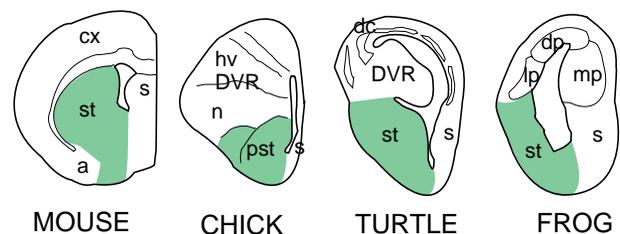
Pregnant mice were injected intravenously (20 mg/kg) with a solution of 5-bromo-2'-deoxyuridine (BrdU) (Sigma) in 0.9% NaCl on embryonic days (E) 10.5 to E14.5. They were killed at E18.5 and the fetuses were fixed in paraformaldehyde 4% in sodium phosphate buffer 0.12M pH 7.4 overnight and washed in PBS. The prosencephalon was dissected out, and coronal sections (100 µm) were cut using a vibrating slicer (Campden Instruments).

### Fate map of the telencephalic vesicle

The quail-to-chick transplantations were performed as described (Bally-Cuif and Wassef, 1994). The same portion of the telencephalic vesicle was cut out from a 7- to 9-somite quail embryo and transported to a stage-matched chick host with a glass pipette, to replace the ablated portion. The egg was then closed with tape and returned to the incubator for 3 to 6 days, after which time the embryos were fixed with 4% paraformaldehyde and processed for QCPN immunocytochemistry. The present study is based on a series of 180 grafts 65 of which survived and were successfully immunostained.

### Immunocytochemistry

The quail-specific QCPN monoclonal antibody, developed by B. and J. Carlson, was obtained from the Developmental Studies Hybridoma Bank maintained at Johns Hopkins University, Baltimore and University of Iowa, Iowa City under contract NO1-HD-2-3144 from the NICHD. For BrdU detection, sections were preincubated for 45 minutes in 2 N HCl, followed by washes in PBS and then treated with proteinase K at 20 mg/ml. After several rinses in PBS, the sections were postfixed in paraformaldehyde 4% glutaraldehyde 0.2% in sodium phosphate buffer 0.12 M pH 7.4. In toto dissected neural tubes or 200 µm thick vibratome sections were incubated overnight in QCPN (1/50 dilution) or anti-BrdU antibody (Becton Dickinson, 1/400 dilution) in PBS-0.2% gelatin-1% Triton. After washing, they were incubated with a biotinylated secondary antibody (Jackson Lab 1/200) followed by a streptavidin-biotin-peroxidase complex (Amersham 1/400) and revealed with diaminobenzidine (DAB).



**Fig. 1.** Telencephalic subdivisions in representative species of mammals (mouse), birds (chick), reptiles (turtle) and amphibians (frog). Schematic representation of transverse sections at comparable anteroposterior levels of the rostral telencephalon in adult mouse, chick, turtle and frog brains. The mouse telencephalon contains two main subdivisions the cortex (cx) and striatopallidum (st); the septum (s) and amygdala (a) regions are also indicated. The putative homologs of the mouse striatopallidum (st) in the other species are depicted in green. This region is known as paleostriatum (pst) in the chick. The DVR of reptiles is a large structure of the lateral telencephalic wall which bulges into the ventricle. It separates the dorsal cortex (dc) from the striatum (st). The complex formed by the neostriatum (n) and the hyperstriatum ventrale (hv) in birds is considered to be the homolog of the reptilian DVR. a, amygdala; cx, cerebral cortex; dc, dorsal cortex; dp, dorsal pallium; DVR, anterior dorsal ventricular ridge; hv, hyperstriatum; lp, lateral pallium; mp, medial pallium; n, neostriatum; pst, paleostriatum; s, septum; st, striatum.

### Cloning of a fragment of mouse, chick and turtle *Dlx-1* cDNAs

*Dlx-1* probes were isolated by RT-PCR using the following oligonucleotides, designed by comparing the mouse *Dlx-1* (Price et al., 1991) and the *Xenopus Xdll* (Asano et al., 1992) cDNA sequences.

Oligo a: 5' GCGAGGTGCGCTTTAACGG 3'

Oligo b: 5' CTGCATAGCTTCTTGGTGCG 3'

Oligo c: 5' CATCAGTTGAGGCTGCTGCAT 3'

They amplified a 401 bp fragment from mouse and chick *Dlx-1* and a 416 bp fragment from *Emys orbicularis Dlx-1* including the homeobox domain. These fragments were subcloned in the PCR II vector (Invitrogen) and sequenced.

### Cloning of a fragment of the chick and turtle and *Xenopus* orthologs of *Emx-1*

*Emx-1* probes were isolated by PCR using the following oligonucleotides, already used to clone the *Xenopus* cognate (Patarnello et al., 1997):

emx1F: CAGGTAAGGTTTGGTTTCA

emx1R: TAATCGTCTGAGGTGACGTC

The amplified 376 bp fragments were subcloned and sequenced (Patarnello et al., 1997).

### Probes for *in situ* hybridization

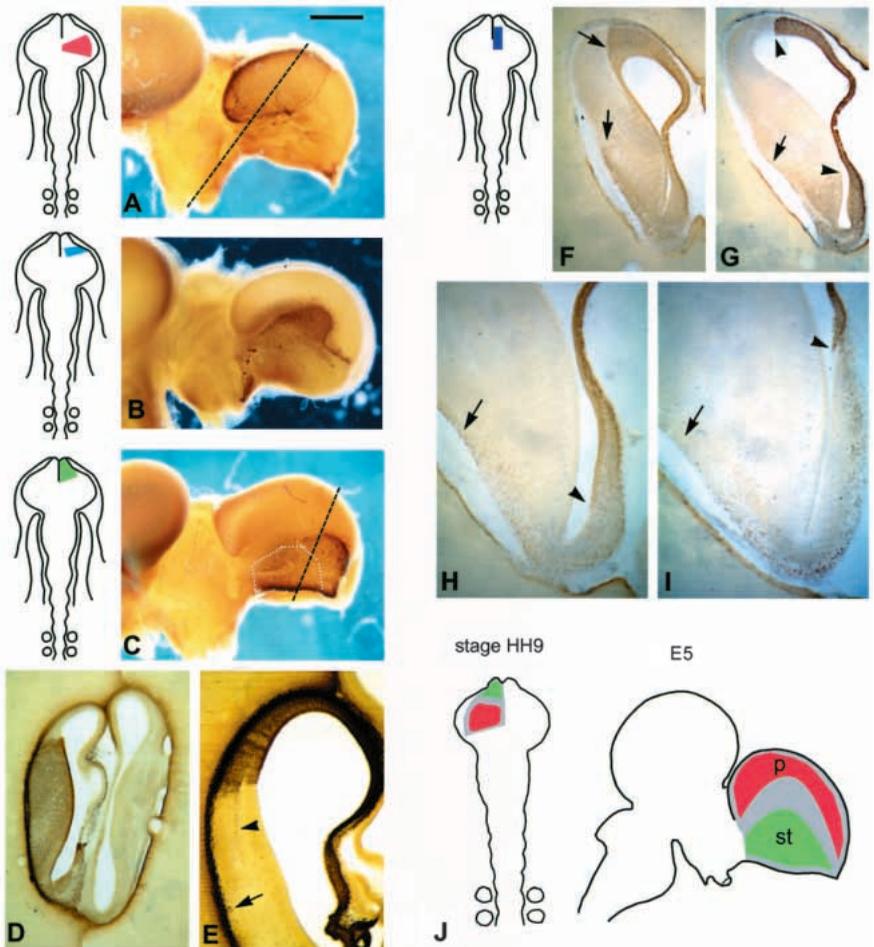
The chick and turtle *Dlx-1* subclones were linearized with *Xba*I and transcribed using SP6 RNA polymerase or linearized with *Hind*III and transcribed with T7 to generate the antisense and sense probes, respectively (*Xba*I, SP6: antisense; *Hind*III, T7: sense). The other subclones were used as follows: mouse *Dlx-1* subclone (*Hind*III, T7: antisense; *Xba*I, SP6: sense; Price et al., 1991), *Xenopus Xdll4* subclone (*Pst*I, T3: antisense; Papalopulu and Kintner, 1993), mouse *Emx-1* subclone (*Eco*RI, SP6: antisense; Simeone et al., 1992b), chick and turtle *Emx-1* subclones (*Hind*III, T7: antisense; *Eco*RI, SP6: sense; Patarnello et al., 1997), *Xenopus Emx-1* subclone (*Hind*III, T7: antisense; *Eco*RI, SP6: sense), mouse *Emx-2* subclone (*Hind*III, T7: antisense; Simeone et al., 1992b), chick *Emx-2* subclone (*Hind*III, T7: antisense; Patarnello et al., 1997). The mouse *Pax-6* subclone (*Pst*I, T3: antisense; *Hind*III, T7: sense; gift of Dr S. Saule) consisted of a 1.8 kb fragment including part of the paired box domain, and the whole homeobox domain, subcloned in pBluescript KS. The quail *Pax-6* subclone (*Eco*RI, T7: antisense; Turque et al., 1994). This probe cross-reacted with chick *Pax-6* RNA under the conditions used.

### Single-colour and double-colour *in situ* hybridization

Mouse, chick, turtle or frog embryos were fixed by immersion or perfused through the heart with 4% paraformaldehyde, and kept at 4°C until being dehydrated in methanol/PBT (PBT: PBS with 0.1% Tween-20). After rehydration, the telencephalon was dissected out and embedded in gelatin/albumin (30% albumin, 0.5% gelatin, in phosphate buffer 0.1 M pH 7.3 hardened by adding 1.25% glutaraldehyde). Sections, 200 µm thick, were cut in the desired orientation on a

vibrating slicer (Campden Instruments) and kept at -20°C overnight after dehydration in methanol/PBT.

Single *in situ* hybridization was performed as described for whole-mount preparations (Wilkinson, 1992). Probes were labeled with digoxigenin-UTP (Boehringer Mannheim) using the Riboprobe



**Fig. 2.** Fate map of the telencephalic vesicle at stage HH9. (A-C) Dissected brains of quail-to-chick chimeras which received homotopic transplants in the telencephalic vesicle (left panel) at the 8- to 9-somite stage and were fixed at E7 (A,C) or E5.5 (B). The grafts (in brown) are revealed with a quail-specific antibody. (A) Caudal transplants give rise to dorsocaudal regions of the telencephalic vesicle. (B) More anterior grafts produce an intermediate sector of the telencephalic vesicle. The basalmost region of the telencephalic vesicle is not derived from the graft. This transplant extended laterally beyond the telencephalic anlage and involved the eye primordium. Notice that the optic stalk contains quail cells. (C) The rostralmost tip of the neural dorsal hemitube gives rise to 'basal' structures of the telencephalic vesicle; the dotted line marks the limit of the chick striatum. (D,E) Coronal sections through the brains of chimeras with rostral (D) and caudal (E) grafts similar to those illustrated in C and A, respectively. The orientation of the sections is indicated by a dotted line in C and A. The arrow (compare with Fig. 3H, asterisk) and arrowhead in E point to the lateralmost position of two streams of labeled cells far from the grafted neuroepithelium. The levels of the sections shown in D and E, are indicated in C and A respectively. (F-I) Successive coronal sections through the brain of a chimera with a medial graft. The limits of the grafted neuroepithelium are marked with arrowheads. A population of neurons (its limit is indicated by arrows in H and I and F) is observed in the basal telencephalon far from the grafted neuroepithelium. (J) Schematic representation of the relative positions of the primordia of the telencephalic vesicle (in grey) and its major subdivisions (pallial, red; striatal, green) in the neural tube of 8-somite and E5 chick embryos. The intermediate domain is intercalated between the pallial and striatal domains. The bar represents 1500 µm in A,C; 1800 µm in B; 500 µm in D; 650 µm in E-G; 300 µm in H,I.

Gemini System II kit (Promega). They were added to the hybridization buffer at a concentration of 1-2 mg/ml; sections were hybridized at 70°C overnight in a water bath. An anti-digoxigenin alkaline-phosphatase-coupled antibody (Boehringer Mannheim) was used diluted 1/2000; alkaline phosphatase activity was revealed using NBT/BCIP as substratum.

For double in situ hybridization, the probes were synthesized either with digoxigenin-UTP or fluorescein-UTP; they were added together to the hybridization buffer and revealed sequentially as described in Bally-Cuif and Wassef (1994) except that the anti-fluorescein antibody was used at a concentration of 1/2000 and that between the revelation of the first and second probe the sections were incubated for 10 minutes in 0.1 M glycine-HCl pH 2.2 plus 0.1% Tween-20 in order to eliminate the first alkaline-phosphatase-coupled antibody (Hauptmann and Gerster, 1994).

## RESULTS

A schematic representation of the main neuroanatomical subdivisions of mouse, chick, turtle and frog rostral telencephalon is given in Fig. 1. The telencephalic subdivisions are illustrated on transverse sections of adult brains, an orientation adopted in most previous neuroanatomical comparative studies. In green, the territories considered as homologs of the mouse striatopallidum (Reiner et al., 1984; Medina and Reiner, 1995; Northcutt and Kaas, 1995) are designed thereafter as striatum. This term should not be confused with the so-called 'neostriatum' a component of the chick ADVR (see below). The ADVR, a thickening of the lateral telencephalic wall, was initially described on morphological grounds in reptiles. The ADVR is bounded by two sulci, which can be detected early in development. Many investigators (Karten, 1969; Ulinski, 1983) considered the ADVR to be homologous to avian neostriatum and ventral hyperstriatum, which are therefore collectively referred to as ADVR. In the following descriptions, unless specified, DVR refers to ADVR.

### Organization of the telencephalic subdivisions in the 8-somite chick embryo

Earlier fate maps in chick (Couly and Le Douarin, 1987) and *Xenopus* (Eagleson and Harris, 1990; Eagleson et al., 1995) suggested that the primordium of the striatum is more anterior in the neural plate, rather than more basal, than the primordium of the dorsal pallium. In order to better understand how the telencephalic subdivisions are organized in the closed neural tube before the bulging out of the telencephalic vesicle, we performed a fate-mapping study of the dorsal chick prosencephalon at the 8-somite stage. Quail neural tube fragments were transplanted homotopically and isochronically into the dorsal prosencephalon of 7- to 9-somite chick embryos (HH9). Unilateral transplants were taken from the dorsal aspect of the neural tube as illustrated in Fig. 2. Our aim was to identify the relative positions of the telencephalic primordia on a rough fate map. The embryos were fixed after 1, 3 or 5 days survival, the grafts were identified using a quail-specific monoclonal antibody (QCPN).

At the 8-somite stage, the primordium of the telencephalic vesicle was entirely located in the anterior portion of the dorsal prosencephalon. The presumptive striatum, DVR and dorsal pallium anlagen occupied successive transversal domains along the anteroposterior axis of the neural tube (Fig. 2), the

presumptive striatopallidal territory being the most anterior (Fig. 2C). The prospective limits of these three major telencephalic subdivisions were roughly transversal to the axis of the neural tube (Fig. 2J). It was not possible, however, based on these grafts to delineate precisely each territory. Between E4 and E6, the basal ganglia were displaced from their early anterior to their traditional 'basal' position.

As noted previously (Balaban et al., 1988), postmitotic cells arising from the grafted neuroepithelium migrated tangentially and spread over the host neuroepithelium. In a few instances, accumulations of quail cells were observed in regions that were distant from the grafted neuroepithelium. Grafts giving rise mostly to pallium also produced a stream of quail cells extending in the lateral telencephalic vesicle (Fig. 2E). Grafts giving rise mostly to rostral pallial + intermediate territory produced a row of cells in the basal telencephalic vesicle (Fig. 2F-I).

### Subdivisions defined in the vertebrate telencephalon by *Emx-1* and *Dlx-1* expressions

The *Emx* and *Dlx* genes code for homeodomain-containing transcription factors related to *Drosophila ems* and *dll*, which are also expressed in restricted head domains in *Drosophila* (Price et al., 1991; Simeone et al., 1992a, 1994; Bulfone et al., 1993; Porteus et al., 1991, 1994; Gulisano et al., 1996; Yoshida et al., 1997). In mouse embryos, *Emx-1* and *Dlx-1* are expressed in complementary domains in the proliferative layer of the cortical and striatal anlagen. In order to localize the origin of the DVR in relation to the expression of the *Emx-1* and *Dlx-1* orthologs, we examined, at successive developmental stages, the expression domains of these genes in the forebrain of mouse, chick, turtle and frog embryos. Thick sections or dissected brains were treated for the detection of either or both transcripts. Although this method does not allow for high resolution analysis, it is well suited for the examination of large brain subdivisions.

The first stages examined, mouse E11.5, chick HH28 and turtle stage 17 (Pieau and Dorizzi, 1981) corresponded to the production of the first postmitotic neurons in the dorsal pallium. At this stage in all three species, both *Emx-1* and *Dlx-1* are already expressed at high levels in complementary territories: *Emx-1* in a dorsal pallial region and *Dlx-1* ventromedially. In order to facilitate comparison with the other two species, the pattern of expression of *Emx-1* and *Dlx-1* in the mouse telencephalon will be first briefly summarized, even if more complete descriptions have already been published (Simeone et al., 1992a,b; Price et al., 1991; Bulfone et al., 1993).

### A transient gap separates the expression domains of *Emx-1* and *Dlx-1* in the telencephalon of mouse embryos

At E11.5, in the ventricular zone of the dorsal (pallial) part of the telencephalic vesicle, the *Emx-1* expression domain extends from the prospective hippocampal region, medially, to the paleocortical anlage, laterally (Fig. 4A, Simeone et al., 1992; Boncinelli et al., 1993; Yoshida et al., 1997). *Emx-1* is also expressed in early postmitotic neurons of the 'preplate' (Caviness, 1982) and in a stream of cells that have already migrated over the paleocortical anlage (Boncinelli et al., 1993; Yoshida et al., 1997). The palliobasal (or striatopallial) angle

is an anatomical sulcus that has been generally considered as separating the prospective territories of the cortex dorsally and the basal ganglia ventrally (Smart and McSherry, 1982). Interestingly, the limit of *Emx-1* expression in the ventricular zone (upper arrowhead in Fig. 3A) lies clearly dorsal to the palliobasal angle.

The *Dlx-1* territory includes the broad subventricular zone characteristic of the prospective basal ganglia and septum. *Dlx-1*-positive cells constitute young postmitotic neurons and mitotically active progenitors in the subventricular and ventricular zones (Bulfone et al., 1993). The *Dlx-1* expression domain do not reach the palliobasal angle (lower arrowhead in Fig. 3B). Ventricular cells expressing neither gene can be clearly distinguished around the palliobasal angle between the *Emx-1* and *Dlx-1* domains (between arrowheads in Fig. 3A,B).

At E13.5, the relative position of the *Emx-1*- and *Dlx-1*-positive ventricular zone with respect to the palliobasal angle (arrowheads in Fig. 3C, D) has not changed much, although, at this stage, the negative gap is less conspicuous than at E11.5.

At E15.5, the *Emx-1*- and *Dlx-1*-negative ventricular zone at the level of the palliobasal angle is no longer distinguishable (Fig. 3E,F).

### In chick, turtle and frog the *Emx-1* and *Dlx-1* telencephalic domains are separated laterally by a growing intermediate territory

#### Chick

Stage HH 28-29 (about E6, Fig. 3G) is broadly comparable to mouse E11.5. At this stage, *Emx-1* is expressed in ventricular and postmitotic cells in a dorsal, pallial territory, whose position is comparable to that of mouse *Emx-1* at E11.5. *Emx-1* is also expressed in the whole caudal pole of the telencephalic vesicle. Some *Emx-1*-expressing cells are found in a ventral territory, corresponding probably to the olfactory tubercle. *Dlx-1* is expressed in a basal telencephalic territory corresponding to the paleostriatum and septum anlagen (not shown). The paleostriatum is considered to be the equivalent in birds of the striatum of mammals (Reiner et al., 1984). Most *Dlx-1*-positive cells are, as in the mouse, located in the subventricular zone.

A small territory, in which the ventricular zone and the overlying postmitotic cells are negative for both markers, separates the *Emx-1* and *Dlx-1* domains at stage HH 28-29. Its position is equivalent to that of the mouse intermediate territory although there is no palliobasal angle in birds.

At stage HH35 (E9, Fig. 3H), *Emx-1* is still expressed in the neuroepithelium of the dorsal pallium and hyperstriatum as well as in postmitotic cells. In addition, *Emx-1* expression is maintained in a stream of superficial postmitotic cells (Fig. 3H, asterisk; compare with Fig. 2E, arrow) which were probably generated at early stages in the dorsal territory (Tsai et al., 1981a,b, Fig. 6E). The limit of the *Dlx-1* territory coincides with the *dorsal medullary lamina*, a sheet of axons first detected at this stage which marks the boundary between the paleostriatum and neostriatum. At this stage, the primordium of the neostriatum lies in the gap between the *Emx-1* and *Dlx-1* expression domains whereas the hyperstriatum ventrale is located in the *Emx-1* domain.

Beginning from stage HH 36-37 (E10-11, Fig. 3I), the main subdivisions of the adult telencephalic vesicle are marked by anatomical landmarks (Tsai et al., 1981b). The *Emx-1* territory is delimited by the axons of the *lamina hyperstriatica* which,

in adults, mark the limit between the neostriatum and hyperstriatum. *Emx-1* expression is maintained in the hyperstriatum but downregulated in the dorsal pallium. The parahippocampus and the hyperstriatum accessorium, which originated from the early *Emx-1* dorsal domain, no longer express this gene. *Dlx-1* expression is restricted to the subventricular layer of the paleostriatum and septum. As in the mouse (Bulfone et al., 1993; Porteus et al., 1994; Anderson et al., 1997), *Dlx-1*-expressing cells are also detected in the subventricular zone of the chick dorsal telencephalon.

Thus, the neostriatum and hyperstriatum ventrale, two classical subdivisions of the avian DVR, arise from distinct neuroepithelial domain: the *Emx-1*-positive and the intermediate domain, respectively. Because the DVR was initially identified in reptiles and the term was secondarily extended to specific regions of the brain in birds (Ulinski, 1983), we decided to examine the relationship of the developing DVR with the *Emx-1* and *Dlx-1* expression domains in turtle embryos.

#### Turtle

The development of the telencephalic vesicle is comparable in stage 16-17 (Pieau and Dorizzi, 1981) *Emys orbicularis* embryos to that of mouse E11.5 and chick HH 28-29 embryos with a row of postmitotic neurons detected in a subpial position in the pallium. The expression domains of *Emx-1*, in the pallium, and *Dlx-1*, in a basal territory, are also very similar (not illustrated). However, the sulci in the lateral telencephalic wall used in previous studies (Ulinski, 1983) to delimit the DVR anlage are not formed yet at this stage.

Between stages 19 and 21, the dorsal ventricular ridge begins to bulge between the dorsal and lateral sulci. *Emx-1* is confined to a dorsal pallial region (Fig. 4A) and to the caudal part of the telencephalic vesicle. Its expression domain in the ventricular layer extends beyond the dorsal sulcus. *Emx-1* is also expressed in postmitotic cells in the lateral pallium. *Dlx-1* is expressed in a basal telencephalic domain comparable to that observed in chick and mouse (Fig. 4B). Interestingly, in the absence of a clear subventricular zone in turtle embryos, *Dlx-1* is expressed in cells of the ventricular layer. The sharp limit of the *Dlx-1* expression domain lies slightly dorsal to the lateral sulcus (Fig. 4B). In the most prominent part of the DVR bulge, a negative ventricular zone is intercalated between the expression domains of *Emx-1*, dorsally, and *Dlx-1*, basally. This intermediate domain is narrow at stage 19 and widened by stage 21 (Fig. 4A,B). At stage 21, the *Emx-1*-positive domain in the caudal telencephalon can be identified as the basal dorsal ventricular ridge (BDVR). Some isolated cells in the cellular layers of the medial and dorsomedial cortices in the medial pallium are *Dlx-1* positive at this stage.

At stage 23, *Emx-1* expression is restricted to postmitotic cells of various pallial derivatives. *Dlx-1* expression has decreased. Their expression domains are now delimited by cytoarchitectonic discontinuities.

Thus, as in mouse and chick embryos, a narrow territory intervening between the *Emx-1* and *Dlx-1* domains is also detected in early turtle embryos. At later stages, although the width of the intermediate territory increases, it is not bounded by the ventricular sulci, which classically delimit the DVR but include most of the DVR bulge. These observations indicate that the major part of the DVR, as classically defined, does not belong to the *Emx-1* territory.

The presence of an intermediate telencephalic territory in the telencephalon of mouse chick and turtle embryos suggested that it could represent an archetypic brain subdivision already present in their common ancestor. To test this possibility, we examined the expression patterns of *Emx* and *Dlx* genes in *Xenopus* embryo, an amphibian species.

### Frog

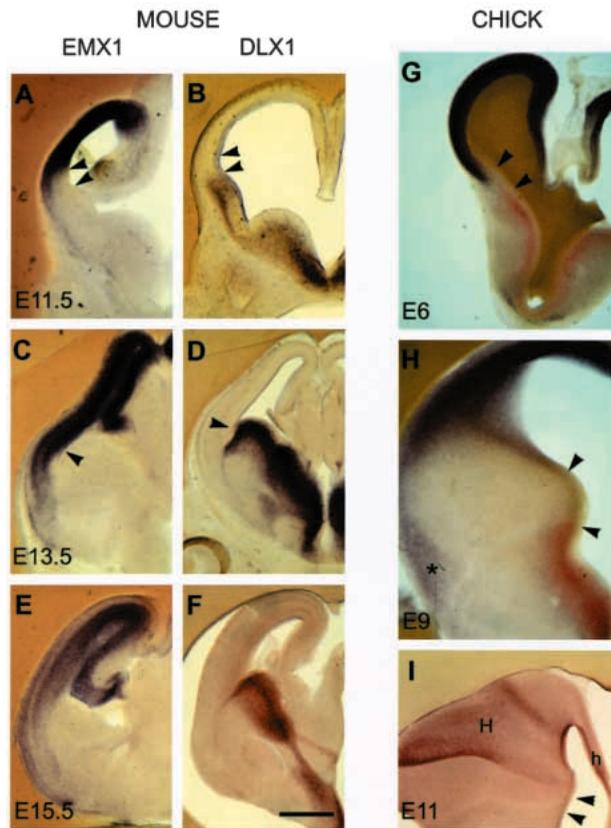
The expression patterns of *XEmx-1*, *Xdll-4* were compared in *Xenopus* embryos between stages 41 and 50 (Nieuwkoop and Faber, 1956).

A negative intermediate territory was observed between the

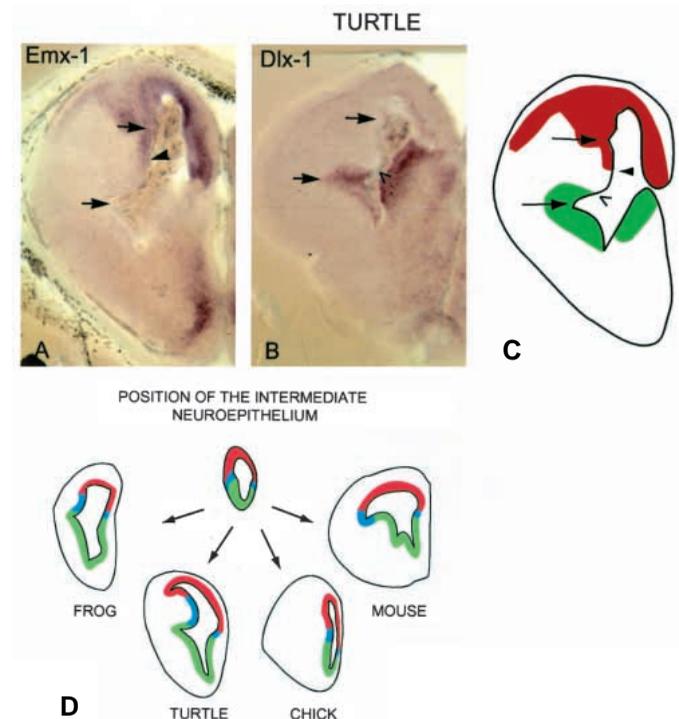
faintly positive caudal *XEmx-1* region and the rostral *Xdll-4* telencephalic domain on stage 41 embryos treated for whole-mount in situ hybridization (not shown). In stage 43 brains reacted in toto, the *XEmx-1* and *Xdll-4* expression domains were broadly complementary (see Papalopulu and Kintner, 1993). Later on, at stage 50, the intermediate telencephalic domain is composed of a large negative region in the lateral telencephalic wall and a smaller medial one. These domains are likely to give rise to the lateral pallium and to a septal domain, respectively. Thus, as in amniotes, an intermediate neuroepithelial domain, inserted between the *Emx-1* and *Dlx* telencephalic domains, is also observed in frog embryos (see Fig. 4C).

### *Pax-6* and *Emx-2* expression in the intermediate territory of mouse and chick embryos

Because a specific marker of the telencephalic intermediate territory was not available, we examined the expression patterns of the *Pax-6* and *Emx-2* genes, which are known to be expressed in the dorsal and lateral telencephalic vesicle (Walther and Gruss, 1991; Stoykova and Gruss, 1994;



**Fig. 3.** *Emx-1* and *Dlx-1* expression patterns in the telencephalic vesicle of mouse and chick embryos. Transverse vibratome sections through the forebrain region were treated for the detection of *Emx-1* (A,C,E,I), *Dlx-1* (B,D,F) or both (G,H). (A-F) Mouse embryos. At E11.5, the expression domains of *Emx-1* in the pallium and *Dlx-1* in the striatum are separated by a thin intermediate territory (between arrowheads) expressing neither gene. At E13.5, this intermediate territory is still visible (compare C and D) and surrounds the palliobasal angle (arrowheads). By E15.5, the domains of *Emx-1* and *Dlx-1* expressions are roughly complementary. (G-I) Chick embryos. At E6, there is a gap (arrowheads) between the limits of the *Emx-1* (purple) and *Dlx-1* (red) expression domains. At E9, a wide domain corresponding to the so-called neostriatum (between arrowheads) separates the *Emx-1* (purple) and *Dlx-1* (red) territories. The *Emx-1* expression domain extends superficially in the lateral part of the telencephalic vesicle (asterisk). At E11, the hyperstriatum (H) and hippocampus (h) express *Emx-1*, which has been downregulated in other pallial regions. The position of the axon tracts that delimit the neostriatum (not visible at this magnification) is marked by arrowheads. The scale bar represents 400  $\mu$ m in A,B; 550  $\mu$ m in C,D,H; 700  $\mu$ m in E,F,G; 1250  $\mu$ m in I.



**Fig. 4.** Expression of *Emx* and *Dlx* in turtle. (A,B) Transverse vibratome sections cut through the forebrain of a stage 21 turtle (*Emys orbicularis*) embryo and treated for the detection of *Emx-1* (A) and *Dlx-1* (B) transcripts. *Emx-1* is expressed in the pallial regions and *Dlx-1* in the basal telencephalon. In between, an intermediate negative domain (between arrowheads) comprises most of the bulging part of the DVR but is not coextensive with the DVR. (C) Diagram summarizing results in A and B; *Emx-1* (red) and *Dlx-1* (green) expression patterns in the neuroepithelium. (D) Schematic drawing summarizing the patterns of *Emx-1* (red) and *Dlx-1* (green) expression patterns in the neuroepithelium of the telencephalic vesicle in different species in relation to the various sulci which usually serve as landmarks. The intermediate neuroepithelium is labeled in blue. Scale bar 300  $\mu$ m.

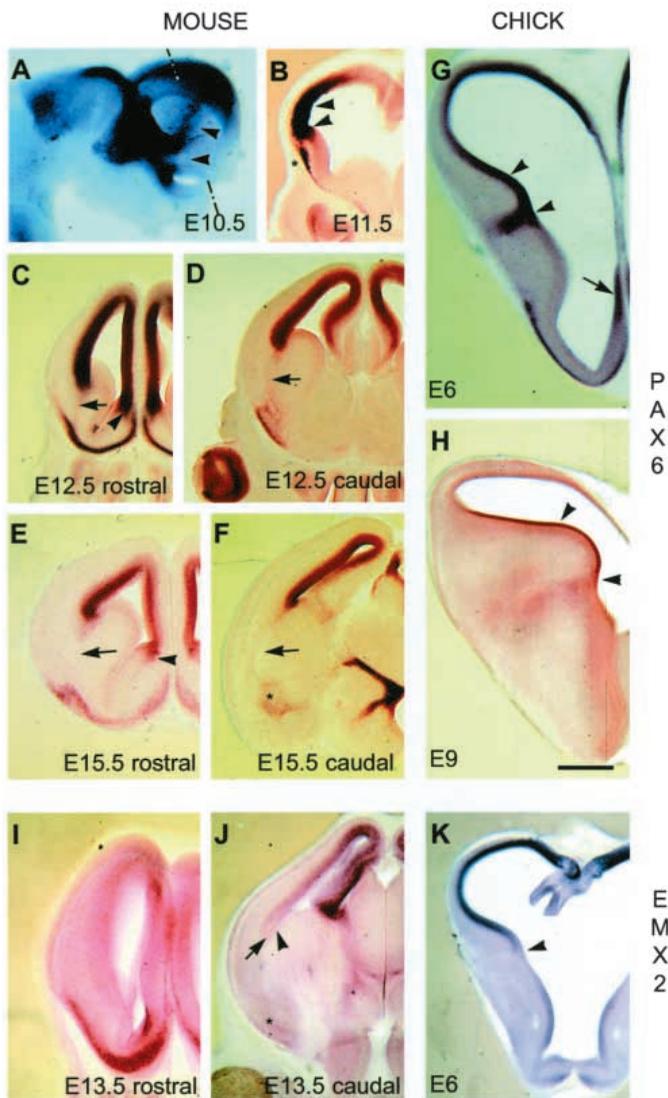
Stoykova et al., 1996; Simeone et al., 1992a,b; Gulisano et al., 1996; Yoshida et al., 1997). In addition, *Pax-6* has been shown to be important for the segregation of telencephalic domains in the mouse (Stoykova et al., 1996, 1997). We will concentrate on features of *Pax-6* and *Emx-2* expression in the telencephalic vesicle, which may be relevant for the present study.

In the E10.5-E11.5 mouse embryo, *Pax-6* is expressed in a dorsocaudal telencephalic territory (Fig. 5A,B), considered in previous studies as the cortical anlage and encompassing the *Emx-1* expression domain (compare Figs 5B and 3A). The *Pax-6* expression domain surrounds the palliobasal angle extending, beyond that of *Emx-1*, in the intermediate territory. At later developmental stages, both the *Pax-6* (Fig. 5A-F) and *Emx-2* (Fig. 5J) expression domains extend beyond the palliobasal angle. *Pax-6* is expressed at a high level in the ventricular zone of the intermediate domain (Fig. 5A, arrowheads, Fig. 5B, between arrowheads), both in the lateral telencephalic wall and in its thin medial extension located in the septum (Fig. 5C,E, arrowheads). At E11.5, a group of *Pax-6*-expressing postmitotic cells (Fig. 5B) lies close to the striatopallial angle between the lateral *Emx-1*-expressing neurons and the *Dlx-1* territory (compare Figs 3A,B, 5B). From E12.5 on, the *Pax-6*-

expressing nuclei are displaced superficially but maintain a topographical relationship with the striatopallial angle (Fig. 5C-F, arrows). These neurons can now be identified as belonging to several subnuclei, from medial to lateral, of the medial septum, the diagonal band of Broca, part of the amygdala and a lateral population which could be part of the paleocortex. Interestingly, these structures maintain their expression of *Pax-6* in adult mice (Stoykova and Gruss, 1994). *Emx-2*, but neither *Emx-1* nor *Dlx-1*, is expressed in a slightly different pattern in the same nuclei as *Pax-6* (Fig. 5I-J).

In chick embryos, *Pax-6* and *Emx-2* are expressed in the ventricular zone of the dorsal telencephalic vesicle. At HH28 (E6), the *Pax-6* and *Emx-2* expression domains include that of *Emx-1* but do not overlap with the *Dlx-1*-positive region. Medially, the dorsalmost part of the septum is *Pax-6* positive, whereas laterally *Pax-6* is expressed at high level in the neuroepithelium of the neostriatum, which belongs to the intermediate territory (see above). A group of postmitotic *Pax-6*-positive neurons surmounts the lateralmost *Pax-6*-positive neuroepithelium. A row of basally located nuclei contains both *Pax-6* and *Emx-2*-expressing neurons (not illustrated).

*Pax-6* is still strongly expressed in the ventricular zone of the neostriatum by E9 but its expression is progressively



**Fig. 5.** *Pax-6* and *Emx-2* expression in the telencephalic vesicle of mouse and chick embryos. (A) Whole mount and (B-K) vibratome sections through the telencephalic vesicle of mouse (A-F,I,J) and chick (G,H,K) embryos treated for the detection of *Pax-6* (A-H) or *Emx-2* (I-K) transcripts. (A) Whole mount of a E10.5 mouse embryo. The *Pax-6* domain in the caudal part of the telencephalic vesicle is delimited by a stripe of high intensity of expression (arrowheads); this band corresponds to the region delimited by arrowheads in B. (B) Transverse section through the telencephalon at E11.5 (orientation indicated in A). *Pax-6* is expressed in the pallial region and most intensely in the ventricular zone of the intermediate territory (between arrowheads; compare with Fig. 3A and B). A group of *Pax-6*-positive postmitotic cells is located more basally than the lateral stream of *Emx-1*-expressing neurons (Fig. 3A, asterisk). (C-F) Rostral (C,E) and caudal (D,F) transverse sections through the telencephalic vesicle of E12.5 (C,D) and E15.5 (E,F) mouse embryos. The neuroepithelium of the pallial and intermediate territories express *Pax-6*. The intermediate neuroepithelium is linked to the populations of *Pax-6*-positive neurons of the basal telencephalon through a thin stripe of *Pax-6*-positive cells, both laterally (arrows) and medially (arrowheads). (G) E6 chick: *Pax-6* is expressed in the ventricular zone of the dorsal pallium including the intermediate region where the labeling is more intense both laterally (delimited by arrowheads, compare with Fig. 3G) and medially (arrow). Two groups of *Pax-6*-positive postmitotic cells one lateral and one basal are located near the basal limit of the *Pax-6*-positive neuroepithelium. (H) E9 chick: *Pax-6* expression is still detectable in the ventricular zone of the region corresponding to the neostriatum (delimited by arrowheads) and the ventralmost part of the hyperstriatum. (I-K) Rostral (I) and caudal (J,K) transverse sections through the telencephalic vesicle of E13.5 mouse (I,J) and E6 chick (K) embryos treated for the detection of *Emx-2* transcripts. In the neuroepithelium, the domains of *Emx-2* and *Pax-6* expressions are very similar. In addition, in both mouse and chick, *Emx-2* labels populations of postmitotic neurons in the same regions as *Pax-6* (asterisks in F,J, arrows in D,F,J). Arrowheads in J,K point to the ventral limit of *Emx-2* expression in the neuroepithelium. The scale bar represents 500  $\mu$ m in A,B,G,J; 670  $\mu$ m in C,D,E,F,H,K,L; 400  $\mu$ m in I.

downregulated in the pallial region (Fig. 5H). Whereas a few scattered neurons expressing *Pax-6* are detectable in the paleostriatum (not shown), none were observed in the neostriatum.

The pattern of *Pax-6* expression in the ventricular zone is thus similar in both chick and mouse and provides a good marker of the intermediate telencephalic territory. On the contrary, similar early populations of postmitotic cells expressing *Pax-6* and *Emx-2* are observed close to the neuroepithelium of the intermediate territory. At later stages, however, the regulation of *Pax-6* expression in postmitotic neurons seems to diverge between species.

### Time of origin of the *Pax-6*-positive nuclei in the mouse telencephalon

In order to delineate the early generated basal telencephalic structures, we performed bromodeoxyuridine (BUdR) injections at different stages of development. All animals were killed at E18.5.

Most of the early postmitotic cells, born at E10.5, belonged to the *Pax-6*-positive structures of the basal telencephalon (Fig. 6A). BUdR injections at E11.5, labeled a large number of cells in these *Pax-6*-positive nuclei (Fig. 6B); in contrast, only a few scattered cells in the cortical and striatal territories become postmitotic at this stage. It is worth noting that this is the time when the gap between *Emx-1* and *Dlx-1* expression domains is most prominent.

BUdR injections at E14.5 resulted in the labeling of a high density of cortical and striatal cells whereas the *Pax-6*-positive nuclei appear as a BUdR-negative island containing few labeled nuclei (Fig. 6C). BUdR injected at E15.5 or later is not incorporated in the *Pax-6*-positive nuclei (Fig. 6D) indicating that these structures are produced before E15.5. The *Pax-6*-positive structures already display some of their adult morphological features by E15.5 and can be easily identified from this stage on. From E14.5 on, the *Emx-1* and *Dlx-1* expression domains seem to be contiguous.

These birthdating experiments indicate that most neurons in the *Pax-6*-positive nuclei are produced between E11.5 and E13.5, when the neuroepithelium of the intermediate territory can be detected. Previous neuronal birthdating experiments in chick embryos have shown that the neurons that transiently express *Pax-6* in the basal telencephalon are also among the earliest to become postmitotic (Tsai et al., 1981a,b, compare Figs 5G and 6E).

## DISCUSSION

In the present study, we compared the expression patterns of *Emx-1* and *Dlx-1/2* in chick, mouse, turtle and frog embryos. Our observations indicate that, at the onset of neurogenesis, the developing telencephalic vesicle can be subdivided into three conserved neuroepithelial domains: the dorsal pallium, the ventral striatopallidum and an intermediate territory. These territories, as deduced from our fate-mapping studies, corresponded to successive rostrocaudal domains of the dorsal neural tube. We propose that this initial partition in three territories should be considered as a developmental homology.

The fate of the intermediate territory differed between species. Whereas in mouse its neuroepithelium rapidly became

vestigial and could not be detected beyond E13.5, its width increased in the lateral wall of the telencephalic vesicle in chick and turtle overlapping with the neuroepithelium lining the growing DVR. In mouse and chick, we identified discrete neuronal populations that are located in similar positions, maintain a topographical relationship with the intermediate territory and express similar markers. We propose that these populations can also be considered as developmentally homologous. Conversely, we propose that, because of the protracted proliferation of the intermediate neuroepithelium in chick and turtle as compared to mouse, other neuronal populations belonging in particular to the chick neostriatum and to the turtle DVR have no developmental homolog in mouse.

### The telencephalic vesicle derives from the dorsal aspect of the prosencephalic vesicle

The position of the presumptive territory of the telencephalon in the early neural tube is still controversial. Most classical neuroanatomists implicitly consider that it derives from the rostralmost tip of the neural tube, being composed of a pallial part, dorsal in nature, and a basal part, issued from the ventral half of the tube (Altman and Bayer, 1995; Alvarez-Bolado et al., 1995). More recent descriptions of brain organization and in particular of telencephalic subdivisions were based on the prosomeric model (Puelles and Rubenstein, 1993; Rubenstein et al., 1994; Puelles, 1995). These authors assume that the telencephalic vesicle arises from the dorsal aspect of the tube (Bulfone et al., 1993, 1995). However, because most of their early schemes were based on gene expression patterns at E12.5 in the mouse, the striatal anlagen was depicted as more basal than the cortical anlagen. In a more recent study, based on gene expression patterns at earlier stages of mouse development, Shimamura et al. (1997) depict the presumptive cortex and striatum as aligned along the anteroposterior axis of the neural tube as we find in the chick fate map, instead of being successive dorsoventral structures. The transverse boundary that separates the *Emx-2/Pax-6* and *Dlx-2* domain receives the name of zona limitans intratelencephalica.

Our observations confirm earlier fate map studies performed at the neural plate stage in chick (Couly and Le Douarin, 1987) and *Xenopus* (Eagleson and Harris, 1990; Eagleson et al., 1995), which showed that the whole telencephalic vesicle is issued from the dorsal anterior part of the neural tube. In addition, in chick embryos, the primordium of the striatum ('floor of the telencephalon' in Couly and Le Douarin, 1987) is located more anteriorly than the pallial region ('roof of the telencephalon'). Similarly, in *Xenopus* (Eagleson et al., 1995), the striatal anlage is located more medially on the anterior neural ridge of the open neural plate (i.e. more anteriorly in the closed neural tube) than the primordium of the cortex. Our fate-mapping study indicates that the three major telencephalic subdivisions, delineated on the basis of differences in gene expression (see also Shimamura et al., 1997), originate from successive transversal stripes in the dorsal anterior prosencephalon which are arranged along the anteroposterior axis and correspond, from rostral to caudal, to the basal ganglia (septum/pallidum/striatum), the intermediate region (composed more prominently in the chick of the medial septum, a set of 'basal' nuclei and the neostriatum) and the

pallial region (including the hippocampus, hyperstriatum accessorium and hyperstriatum).

### ***Emx-1* and *Dlx-1* as markers of early telencephalic regionalization**

In the proliferative cell populations of the telencephalon, the *Dlx-1*, *Emx-1* and *Pax-6* transcripts were used as regional markers with the aim of identifying and following early telencephalic subdivisions in the absence of other landmarks. The expression of these transcripts, at later developmental stages, in several populations of postmitotic neurons were a priori considered as an independent issue needing complementary data to be interpreted.

The present study relies on two assumptions: (i) the early *Emx-1* and *Dlx-1* expression domains delineate homologous territories in different vertebrate species and (ii) the onset of neuronal production can be considered as a reliable reference for comparison of developmental stages between species.

There is a concern that gene duplications during evolution could have partially released the constraint on their spatial regulation resulting in a variability of their expression patterns in different species. As discussed below, it seems that, in the *Dlx* gene family, the variations in expression patterns between orthologs antedates the formation of amniotes (Morasso et al., 1995). Less is known about the expression patterns of genes of the *Emx* family. The available data in mouse, chick and zebrafish, however, indicate that the spatial regulation of *Emx-1* and *Emx-2* has probably been fixed early in evolution.

The *Dlx* gene family comprises at least 6 members (Stock et al., 1996) in the mouse. Except for *mDlx-3* (Robinson and Mahon, 1994) and *mDlx-7* (Shimamoto et al., 1997), which are not expressed in the central nervous system, all (*mDlx-1*, *mDlx-2*, *mDlx-5* and *mDlx-6*) are expressed in the subventricular zone of the medial and lateral ganglionic eminences and in the septum. At early developmental stages, their expression domains share a common limit at the border of the striatopallidal anlage (Porteus et al., 1991; Price et al., 1991; Simeone et al., 1994). This feature is likely to have been evolutionarily conserved between orthologs of these genes. In *Xenopus*, the *X-dll3* and *X-dll4* genes which belong to the *mDlx-5* and *mDlx-2* subfamilies, respectively, are expressed in the ventral forebrain (Papalopulu and Kintner, 1993; Dirksen et al., 1993), whereas *X-dll2* and *mDlx-3*, which belong to the same subfamily, are not expressed in the brain. Furthermore, frog regulatory sequences from the *X-dll2* promoter confer appropriate *Dlx-3* like expression on a *lacZ* reporter gene in transgenic mice (Morasso et al., 1995).

The vertebrate *ems* homologues that have been cloned in mouse (Simeone et al., 1992b), zebrafish (Morita et al., 1995), chick, frog and turtle (Patarnello et al., 1997) are all expressed in the forebrain. In mouse, chick and zebrafish embryos, *Emx-1* and *Emx-2* are similarly expressed in overlapping domains, *Emx-2* being expressed earlier and more broadly than *Emx-1*.

Thus, at least at early developmental stages, the expression domains of the *Emx-1* and *Dlx-1/2* genes in the telencephalic vesicle have been conserved during evolution

Our concern here was to use a way of comparing developmental stages which would be based on a parameter of differentiation and not on the pattern of gene expression. At the onset of neuronal production, the telencephalic vesicles of mouse chick and turtle are still very similar although they have

already acquired species-specific morphological traits. This stage also corresponds to the sharpening of the limits of *Emx-1* expression and is late enough to be close to the production of the neuronal structures that we wanted to compare. The fact that the patterns of *Emx-1*, *Dlx-1* and *Pax-6* expression are very similar at this stage in mouse chick and turtle and change progressively thereafter can be taken as a validation of this choice.

### **Derivatives of the transient intermediate territory in the mouse**

Structures derived from the mouse intermediate territory are expected to have two properties: first, they should be located laterobasally in the telencephalic vesicle on the basis of their site of origin and of the result of our grafting experiments in chick, and, second, because they are produced by a transient domain of the neuroepithelium, they are expected to become postmitotic at early developmental stages.

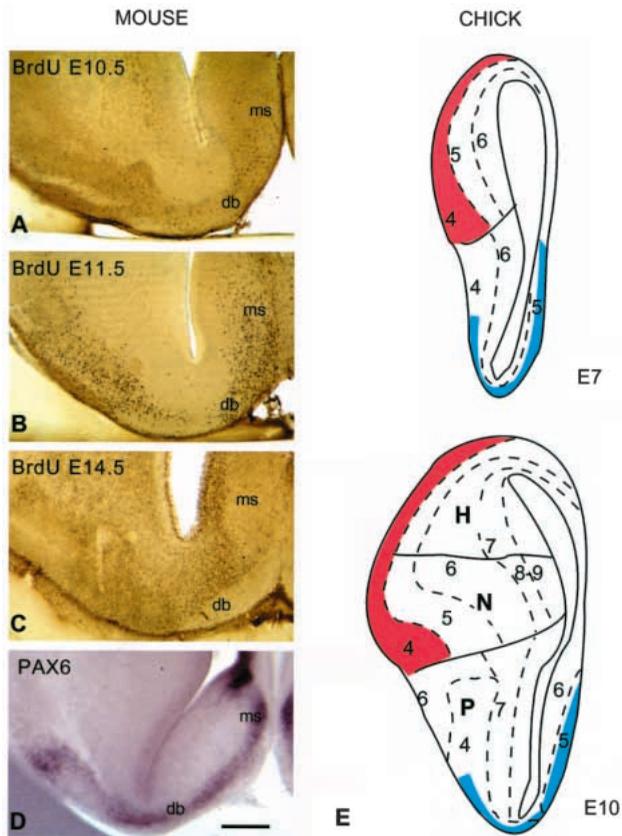
The radial glia provides a record of early topographical proximity between the ventricular and pial domains. Late in embryonic development, a subset of the glia fibers follows a curved trajectory at the border of the basal ganglia linking the neuroepithelium of the striatopallial angle to the ventrolateral part of the cerebral cortex; this glial palisade could serve as a substratum and a guidance cue for migratory cells (De Carlos et al., 1996 and references therein, Striedter and Beydler, 1997). It is unclear whether the *Pax 6* transcripts that line the striatum, linking the palliobasal angle with the *Pax-6*-positive amygdalar nuclei, are contained in radial glia or in migrating neurons. In any case, this pattern makes the laterobasal *Pax-6* structures a likely derivative of the transient intermediate neuroepithelium.

Besides their location and the fact that they maintain a connection with the striatopallial angle through a line of *Pax-6*-positive cells, the *Pax-6*-positive nuclei of the paleocortex, amygdala and diagonal band and medial septum are good candidates as derivatives of the intermediate telencephalic on the basis of their early generation and also because, in *Pax-6* (sey) mutants, both the intermediate domain and the ring of *Pax-6*-positive nuclei fail to form (Stoykova et al., 1996).

### **Derivatives of the intermediate territory in chick and turtle.**

According to Ulinski (1983), two ventricular sulci designed MVS and DVS mark the lateral wall of the telencephalic vesicle and demarcate the DVR. Their presence is a common feature in all reptiles and birds. It is interesting to note that the intermediate territory, defined as a gap between the *Emx-1* and *Dlx-1* expression domains, lies into the DVR neuroepithelium of chick and turtle embryos. It is, however, not bounded by the MVS and DVS sulci. In fact, sulci in the ventricular wall are not reliable markers of the limits of histogenetic subdivisions (Puelles and Rubenstein, 1993). These boundaries are in general marked by local histological discontinuities or coincide with limits of gene expression domains; they are also often marked by the course of major axonal tracts (Puelles and Rubenstein, 1993).

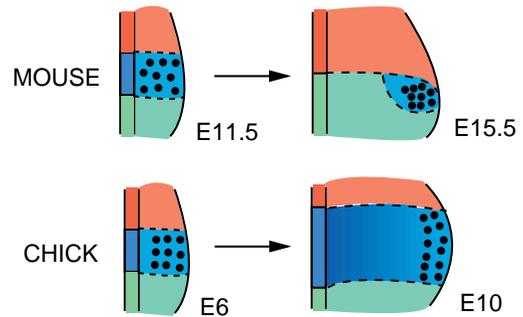
Such histological discontinuities are indeed observed in *Emys orbicularis* as step variations in both the thickness and the proliferative activity of the DVR neuroepithelium (Goffinet et al., 1986). These discontinuities do not coincide with the



**Fig. 6.** Birthdates of the *Pax-6* and *Emx-2* positive basal telencephalic nuclei. (A-D) Transverse vibratome sections showing the basal telencephalic region of E18.5 (A-C) and E15.5 (D) mouse embryos. (A-C) E18.5 embryos injected with BrdU at the stage indicated: the first neurons forming part of the medial septum (ms) and the diagonal band of Broca (db) are produced at E10.5. (A) Neurogenesis has not yet begun in the other telencephalic structures. (B) Most cells belonging to the *Pax-6*-positive nuclei are produced around E11.5. Neurogenesis is beginning in other telencephalic regions. (C) Neurogenesis is completed in the *Pax-6*-positive region whereas the production of neurons in the surrounding structures is now very active. (D) E15.5 embryo treated for the detection of *Pax-6*-positive neurons. (E) Schematic representation of coronal sections of E7 and E10 chick telencephalon illustrating variations in neuronal birthdates between territories (adapted from Tsai et al., 1981b). An early population, labeled in red, is related to the pallial region and expresses *Emx-1* during development (compare with Fig. 3H). The laterobasal part of this region is labeled with an asterisk in Fig. 3H. The basal telencephalon, in blue, contains early generated neuronal populations corresponding to the *Pax-6*- and *Emx-2*-positive domains. Compare this region to the one issued from the dorsomedially placed neuroepithelium from the graft showed in Fig. 2 F-I. The scale bar represents 300  $\mu$ m.

MVS and DVS sulci but define a subdomain in the middle of the classical DVR, which may correspond to our intermediate domain.

In E6 chick embryos (Tsai et al., 1981b), a distinct boundary is delineated by a difference in the distribution of cells labeled by [ $^3$ H]thymidine injections at E5. This limit prefigures the lamina medullaris dorsalis, which marks the basal limit of the DVR.



**Fig. 7.** Comparison of intermediate territory development in the telencephalon of mouse and chick embryos. Schematic representation of transverse sections through the lateral wall of the telencephalic vesicle at successive stages. The neuroepithelium (vertical stripe on the left) is subdivided into three territories: pallial (red), intermediate (blue) and striatal (green). During the phylotypic stage, which extends shortly after the onset of neurogenesis, the intermediate territory produces a population of neurons represented as black dots. At subsequent stages, the neuroepithelium of the intermediate territory disappears from the mouse telencephalon but is maintained in the chick. Both in mouse and chick, the early population of neurons produced by the intermediate territory is pushed superficially by later generated neurons (outside-in gradient of neuronal production). The gradient in the blue shading indicates the order of neuronal generations, which is likely to be accompanied by changes in neuronal identity.

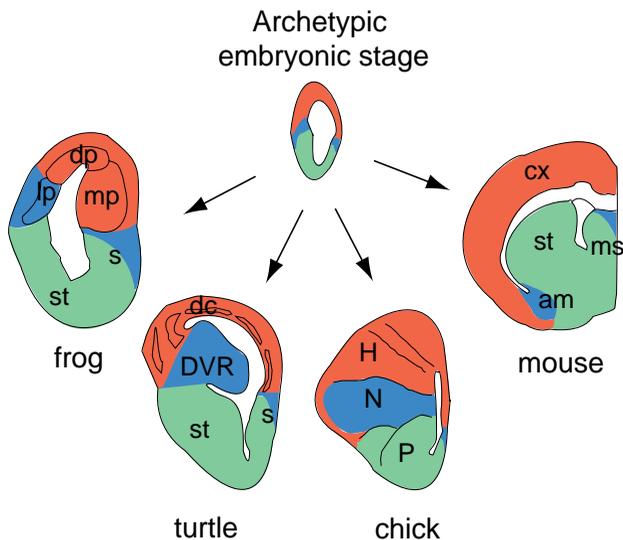
A great part of the chick and turtle DVR is thus likely to derive from the intermediate territory. Our results indicate, however, that the classical definition of the avian DVR (Ulinski, 1983), considered as an homolog of the reptilian DVR, is misleading. By analogy with reptiles, the bird DVR was delimited by two early sulci appearing in the lateral wall of the telencephalic vesicle. We propose that the structures that derive from the lateral portion of the intermediate telencephalic territory including most of the classical DVR of reptiles and exclusively the neostriatum in birds should be considered as DVR in other species; the hyperstriatum should thus be considered as a pallial derivative.

Besides the DVR, additional structures located in the basal telencephalon of adult animals could arise from the intermediate territory. In chick embryos, this sheet of basally located cells is not produced by the overlying neuroepithelium (Fig. 2F-I). It contains neurons generated early in development (E4, see Tsai et al., 1981 and Fig. 6E), which are transiently *Pax-6* and *Emx-2* positive.

In conclusion the intermediate telencephalic territory produces neuronal structures belonging to the classical DVR but does not coincide with it. In addition, some discrete neuronal populations of the basal and medial telencephalon, which express *Pax-6* and *Emx-2*, probably derive from the intermediate subdivision.

#### Are the structures that derive from the intermediate territory in different vertebrate species homologous?

During the early 'phylotypic' phase, the intermediate territory generates a population of neurons belonging to nuclei that, in chick and mouse, share their lateral and basal positions, their



**Fig. 8.** Interpretative scheme of the evolution of telencephalic regionalization. The adult structures produced by the three main subdivisions of the telencephalic vesicle neuroepithelium are depicted in different species based on the results of the present study (see text for details). The 'pallial' territory is shown in red, the 'intermediate' territory in blue and the 'basal' territory in green. Same abbreviations as in Fig. 1.

early birthdates and their expression of *Pax-6* and *Emx-2*. Each of these nuclei does not necessarily derive entirely from the intermediate telencephalon. Indeed, several rhombomeres contribute to the formation of single cranial nerve motor nuclei (Marin and Puelles, 1995). In the telencephalon, neurons produced in the *Dlx-1/2* domain have been shown to migrate dorsally and differentiate as GABAergic interneurons in the neocortex and olfactory bulb (Anderson, 1997a,b).

Our data, and those of others (Stoykova and Gruss, 1994; Gulisano et al., 1996), in mouse embryos, support the case for a dual origin of the nuclei constituting the mouse amygdalar complex: the corticomedial and central nuclei (both expressing *Emx-1*) are likely derivatives of the dorsal or 'pallial' territory, while those nuclei of the laterobasal complex (which express *Pax-6*) could derive from the intermediate territory. Taking into account our data in the other species studied, the search for an homolog of the reptilian DVR and the avian neostriatum in mammals should be centered in the laterobasal part of the amygdala, while traditional homologs of the amygdala in birds (the archistriatum) and reptiles (the BDVR) should now be tested as homologs of the corticomedial and central part of the amygdala (all these structures express *Emx-1*). Bruce and Neary (1995) reached a similar conclusion based on a detailed analysis of the connections of amygdalar subnuclei in different species.

We argue that the early generated populations of neurons can be considered as homologous in chick and mouse and probably in other vertebrate species and that, on the contrary, later generated structures, although produced by homologous developmental brain subdivisions, should not be considered properly as homologs (illustrated in Fig. 7). An argument in favor of this interpretation comes from the analysis of mouse mutants in which defined populations of early produced

neurons can be detected at late embryonic stages although their neuromere of origin is 'deleted' or failed to develop. The *Kreisler* and *Krox-20* genes, for example, are necessary for rhombomere development in mouse and zebrafish. In mutants, the affected rhombomeres express early position-specific markers but fail to develop further and either disappear in the case of *Krox-20* homozygous mutants (Schneider-Maunoury et al., 1993, 1997) or fail to acquire fully differentiated traits as in *Kreisler* mutants (Cordes and Barsh, 1994; McKay et al., 1994; Moens et al., 1996). In both *Krox-20* and *Kreisler* mutants, it has been noted that a normal contingent of early generated neurons is produced before the mutant phenotype is detected (Schneider-Maunoury et al., 1997; Moens et al., 1996). Similarly, *BF-1* function is required for normal development of the telencephalon and the size of the telencephalic vesicles is severely reduced in *BF-1* null mutants. Nevertheless, in the vestigial cerebral cortex of *BF-1* mutants, a neuronal population is detected that expresses markers of the preplate neurons, the earliest to be born in the wild-type cerebral cortex (Xuan et al., 1995). In *Dlx-1/Dlx-2* double mutants, the early population of striatal neurons is formed whereas late born striatal neurons fail to differentiate (Anderson et al., 1997a,b).

Alternatively, the whole set of intermediate telencephalon derivatives comprising the diagonal band of Broca, the DVR in chick and turtle and the mouse septal and amygdalar nuclei could be considered as developmental homologs (Fig. 8). We are less in favour of this interpretation because it underestimates the constraint on timing in the generation of neuronal structures. Two observations indicate that brain developmental time is constrained at a local as well as at a more global level. In the mammalian cerebral cortex, despite large variations in the duration of corticogenesis, the same proportion of the total time is devoted to the production of defined populations of neurons (Caviness et al., 1995). Furthermore, in mammalian species, the order of neurogenesis in various brain divisions is conserved across a wide range of brain sizes resulting in a strong constraint on the relative growth of each structure. Only late-generated structures show disproportional enlargement across mammalian species (Finlay and Darlington, 1995). Similar studies across different vertebrate lineages are hampered by their disparate brain organization, which precludes the comparison of brain divisions between species belonging to different lineages. It may however be supposed that the constraint on the relative growth of brain subdivisions is conserved in reptiles, birds and other non-mammalian lineages but not across lineages. Because there are no fossil records of brain structures and the duration of neurogenesis in a given brain subdivision may successively expand and shorten during evolution, it seems that only the structures that have been conserved across species can be considered as homologous and that the early generated structures are the most likely to have homologs in distantly related species.

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