A new approach to the development of the cerebellum provided by the quail–chick marker system

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Summary

We have used the quail–chick chimera system to reveal the cell migrations and settling pattern involved in the construction of the cerebellum. Three types of orthotopic transplantations were carried out, between quail and chick embryos, at the 12-somite stage: exchanges of (i) the whole metencephalic vesicle, (ii) the lateral half of this vesicle and (iii) the diencephalic plus the mesencephalic vesicles.

Histological study of chimeric embryos and young chicks provided the following results: longitudinal morphogenetic movements distort the embryonic neural tube as early as the fifth embryonic day, so that the dorsal limit of the mes-, met- and myelencephalic vesicles are displaced caudad and their ventral limits rostrad. This leads to a participation of mesencephalic vesicular material in the construction of the cerebellum. Cells originating in the mesencephalic vesicle are found in a rostromedial V-shaped region, in all the cerebellar cellular layers, except the external granular layer, the presumptive territory of which is entirely located in the metencephalic vesicle.

The chimerism of the rostromedial part of the cerebellum allows the analysis of the origin of the various cerebellar cell types. We find (i) that the Purkinje cells always have the same cellular marker as the ventricular epithelium radially beneath them. This strongly suggests that these cells reach their final localization following strictly radial migrations. (ii) Most of the small cells surrounding the Purkinje neurons and most of the neurons and glial cells of the molecular layer are also of the same type as the ventricular epithelium they surmount, i.e. different from the type of the external granular layer cells. Therefore, they are not derived from the external granular layer and are not of the same origin as the granule cells as previously believed.

Unilateral substitutions of the metencephalic vesicle revealed that transverse cell migrations occur across the sagittal plane. They have been observed mainly in the inner and external granular layers, but also, though to a lesser extent, in the molecular layer and in the cell layer located at the level of the Purkinje neurons.

These observations show that the position of cerebellar cells is determined by both morphogenetic movements and cell type-specific active radial and tangential migrations. The quail–chick chimera system is thus able to provide new information both on the origin of cerebellar cells and how each cell type assumes its final position.

Key words: brain development, quail-chick chimeras, cell migration, cerebellum.

Introduction

The cerebellum is an excellent system for studying cell migrations during brain ontogeny and the events leading to the formation of distinct cell populations in a structured organ. This part of the brain is built up of cellular layers organized into a number of folia. The cellular layers appear progressively during ontogenesis. By the end of development, when proceeding radially outward from the epithelium lining the fourth ventricle, they are: the cerebellar deep nuclei in the white matter, the inner granular layer, the Purkinje cell layer, the molecular layer and the transient external granular layer. The mechanisms governing the positioning of these distinct neuronal cells in each of these layers have been extensively studied in birds and mammals (see Cajal, 1911 and Jacobson, 1978, for reviews). The current view is that variate cellular migrations occur within the cerebellar anlage during ontogenesis. Classically, it has been thought that the external granular layer cells are generated in the rhombic lip, localized laterodorsally in the embryonic rhombencephalon, and then spread tangentially over the cerebellar anlage (Larsell, 1948; Saetersdal, 1956; Woodward, 1960; Hanaway, 1967; Altman, 1966, 1972, 1982; Altman and Bayer, 1985; Hansmann et al. 1987). They give rise to granule cells (Uzman, 1960; Miale and Sidman, 1961; Fujita et al. 1966; Fujita, 1967) which migrate centripetally along radial glial processes (Altman, 1972; Altman and Bayer, 1985; Rakic, 1971, 1972, 1981, 1985, 1988;
Rakic and Sidman, 1973; Rakic et al. 1974; Trenkner et al. 1984; Hatten et al. 1986; Linder et al. 1986; Chen Ming Chuong et al. 1987). In contrast, Purkinje cells are

These cellular migrations have been deduced from both classical histological staining and tritiated thymidine labelling of dividing cells (Uzman, 1960; Angevine and Sidman, 1961; Miale and Sidman, 1961; Wetts and Herrup, 1982; Altman and Bayer, 1985; Bertossi et al. 1986).

The quail–chick chimera system (Le Douarin, 1969) allows cells to be labelled in discrete areas of the embryonic tissues and their fate to be followed during ontogeny without any dilution of the label. The methodology entails exchanging homologous embryonic territories between chick and quail and subsequently identifying the species identity of cell bodies by staining the DNA using the Feulgen and Rossenbeck reaction (1924): quail heterochromatin is massed in the nucleolus while the chick heterochromatin is more uniformly dispersed.

Quail–chick chimeras have already been used to study the embryonic development of many organs and tissues in which cell migrations play a major role (see Le Douarin, 1982 and Le Douarin and McLaren, 1984). The ontogeny of the central nervous system has also been studied by means of this technique (Alvarado-Mallart and Sotelo, 1984; Kinutani and Le Douarin, 1985; Kinutani et al. 1989; Couly and Le Douarin, 1985, 1987, 1988; Balaban et al. 1988).

In the present work, we have applied the quail–chick chimera system to a study of the cerebellar ontogeny and the fate of the embryonic territories neighbouring the metencephalic vesicle at the 12-somite stage. This was done by exchanging regions of the embryonic neural tube between quail and chick embryos and studying the chimeras during embryonic life and after hatching.

Materials and methods

Embryos

Fertilized chick eggs (Gallus gallus domesticus, JA-57, commercial source) and quail eggs (Coturnix coturnix japonica, commercial source) were incubated horizontally for 40 to 44 h and for 36 to 40 h, respectively, at 37.5°C (at 70% humidity), until the embryos reached the 11- to 13-somite stage.

Surgery

After the removal of about 0.5 ml of albumin, a window was opened in the shell and surgery was performed immediately as described below. The window was then closed with adhesive tape and the operated eggs were reincubated at 37.5°C at 70% humidity in a forced-draft incubator.

At the 12-somite stage, the cephalic neural tube consists of successive vesicles separated by constrictions perpendicular to the anteroposterior embryonic axis. The transverse constrictions, which limit the metencephalic vesicle from the myelencephalic vesicle caudally and from the mesencephalic vesicle rostrally, were reference points used to define the limits of the grafts.

Three different types of microsurgical isotopic transplantation were performed, from quail to chick and vice versa, following a procedure already described (Le Douarin, 1982). The three regions involved in the operations were: (1) the entire metencephalic vesicle (MT), (2) the lateral half of the metencephalic vesicle (LMT), (3) both the mes- and diencephalic vesicles (DMS) (Fig. 1). For the grafts involving the lateral half of the metencephalic vesicle, the medioventral border followed the notochord and the mediadorsal border followed the dorsal suture of the neural tube.

The neural epithelium was not isolated by proteolytic digestion from the surrounding tissues, as in our classical technique, and some of the adjacent ectoderm and mesenchyme covering the neural tube were included in the grafted tissue.

Histological procedure

The operated birds were killed for histological studies at ages ranging from embryonic day 5 (E5) to 11 days after hatching (P11) (Table 1).

Embryos (from E5 to E16) were removed from the egg. Their heads were fixed in Zenker or Carnoy solutions. After hatching, chimeras P0 to P11 were anesthetized with Pentobarbital, then perfused transcardially by Zenker or Carnoy solution. Heads or dissected brains were embedded in paraffin. Any animal showing brain malformations was eliminated. Serial, transverse or longitudinal (sagittal and parasagittal) 5 μm sections were stained with cresyl violet (Merck) or according to the Feulgen and Rossenbeck (1924) method for DNA staining, which reveals the quail nuclear marker (Le Douarin, 1969).

Immunocytological procedure

Immunocytological staining was performed as previously described (Lance-Jones and Langenaur, 1987) with the polyclonal antibody raised against quail antigens, kindly provided by Dr Cynthia Lance-Jones.

Results

Irrespective of whether they were made from quail to chick (Q/C) or conversely (C/Q), the three types of transplantation (MT, LMT, DMS, see Table 1) gave similar results with no significant variation from embryo to embryo. At all the stages studied, the border between host and implanted tissues remained distinct at the level of the ventricular epithelium with no cell mixing.

(A) Transplantations of metencephalic (MT) and mesencephalic (DMS) vesicles

Evolution of the grafted territories at the level of the ventricular epithelium

At the time of operation, the boundary between host and grafted tissues followed the constrictions of the neural tube separating the metencephalon from the adjacent myel- and mesencephalon (Fig. 1).

By E5, E6 (MT-311, MT-312), the isthmus is well defined and its roof is made up medially of cells of the mesencephalic species type. The anterior limit of the graft is located at a significant distance from the isthmus...
Table 1. Table of the different experiments performed and age of killing

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MT, graft of the total metencephalic vesicle; LMT, graft of the right lateral half of the metencephalic vesicle; DMS, graft of di- and mesencephalic vesicles; O/C, graft performed with quail tissues transplanted in a chick embryo; C/Q, graft performed with chick tissues transplanted in a quail embryo, the numbers refer to individual chimeras.

By E8 (MT-115), large scale cellular movements have pushed the ventral metencephalic material rostrally and the dorsomedial mesencephalic material caudally (Fig. 3), resulting in a lateral distortion of the territories derived from the metencephalic vesicle in this area. These relative movements become more pronounced at E10 (MT-220) and E12 (MT-253) and reach their maximum by E15 (MT-195). From this stage onward, and particularly in hatched chimeras (see Fig. 4), the ventricular epithelium corresponding to the ventral metencephalic vesicle lies in the ventral part of the fourth ventricle from the posterior level of the isthmus area backwards. In contrast, the ventricular epithelium of mesencephalic origin lines dorsally the rostromedial part of this ventricle up to about half of the length of the cerebellum.

At all stages observed, the posterior limit of the metencephalic vesicle falls within the anterior part of the choroid plexus to which it makes a small contribution (see Fig. 3D).

\[ \text{Formation of the cellular layers of the cerebellar cortex} \]

Marking the metencephalic vesicle differently from the mesencephalic vesicle leads to the formation of a chimeric cerebellum.

By E5 (MT-311, MT-312), the cerebellar anlage is made up of an epithelium about 15 cell bodies thick. This neuroepithelium is of metencephalic origin, except for a V-shaped rostromedial region of mesencephalic...
Fig. 2. Sagittal section of the chimera MT-311, C/Q, E5, showing the penetration of mesencephalic material into the metencephalic vesicle. (A) Cresyl violet staining of the entire embryo. Is, isthmus; III, third ventricle; IV, fourth ventricle; Mes, mesencephalon; Met, metencephalon. The framed area is enlarged on an adjacent section in B. Scale bar: 1.5 mm. (B) Dorsal metencephalo-mesencephalic limit. The framed area is enlarged in C. Scale bar: 350 μm. (C) Boundary between quail (Q) and chick (C) tissues within the cerebellar anlage. Scale bar: 40 μm. Arrowheads indicate the limits of the grafted tissues. (B) and (C), Feulgen-Rossenbeck staining.

origin, as already described (see Fig. 2). By E8 (MT-115), the cerebellar anlage, of mixed origin, is covered by the external granular layer which derives entirely from the metencephalic vesicle (see Fig. 3). At E10 (CE-220), the external granular layer is fully developed, with a thickness of about 10 cell bodies (data not shown).

Purkinje cells can be observed from E8 (MT-115) onward due to their large size and their peripheral localization underneath the external granular layer. At all stages, Purkinje cells are of the same species type as the ventricular epithelium they surmount (Fig. 4), i.e. they are of metencephalic origin laterally and caudally and of mesencephalic origin in a V-shaped, rostromedial part of the cerebellar anlage.

In the hatched chimeras (MT-230, MT-216, DMS-688), small cells of morphology akin to that of the granule cells surround the Purkinje cells and generally carry the same species marker as the latter (Fig. 5). However, at the border between the mesencephalic and metencephalic parts of the cerebellum, there is mixing of quail and chick cells among this cellular population. As a consequence, chick or quail Purkinje cells can be surrounded either by a pure population of cells of the same origin or by a mixed population of small quail and chick cells (Fig. 6).

The inner granular layer is clearly discernible by E12 and thereafter its thickness progressively increases. Like the cells that form the external granular layer, the inner granule cells are generated in the metencephalic vesicle, confirming that they settle following a centripetal migration from the external granular layer. However, rostrally, among the metencephalic granule cells, a few scattered cells are derived from the primary mesencephalic region (Fig. 5). Some of these are two to three times bigger than the granule cells and scattered...
Fig. 4. Parasagittal median (A) and sagittal (B) sections of the cerebellum of the chimera MT-230, Q/C, P8.
(A) Distribution of the quail (red triangles) and chick (green triangles) Purkinje cells correlated with the quail and chick ventricular epithelium cells (respectively, red and green spots). The Purkinje cells overlie a ventricular epithelium of the same species type. Cresyl violet staining. Scale bar: 1 cm. (B) Staining of the chimeric cerebellum with the chick anti-quail immune serum and peroxidase. (The quail tissues appear dark, while the chick tissues are lightly stained). III, IV, respectively, third and fourth ventricle; Is, isthmus. Scale bar: 1 cm.
Fig. 3. Sagittal section of the chimera MT-115, Q/C, E8, showing the participation of primitive mesencephalic material in the cerebellar anlage. (A) Low magnification of the cerebellar anlage. Arrows indicate the relative movements of the mes- and metencephalic material. (B) Anterior limit of the external granular layer (EGL), made up of metencephalic quail cells. ChP/I, choroid plexus. (C) Rostrodorsal limit between the grafted metencephalic quail and host chick cells within the ventricular epithelium (VE), in the cerebellar anlage. No cell mixing occurs in this cell layer, whereas cell mixing can be observed beyond it. (D) Posterior dorsal limit between the grafted metencephalon and the host myelencephalon. The choroid plexus is principally composed of host chick cells. III, third ventricle; IV, fourth ventricle. Arrowheads indicate the limits of the grafted tissues. Feulgen-Rossenbeck staining. Scale bars: (A) 350 μm, (B and C) 30 μm, (D) 40 μm.
in the granular cell layer. They are most probably Golgi cells. Others have a size comparable to that of the inner granule cells.

The molecular layer was studied only in the oldest chimeras, when the inward migration of the granule cells from the external granular layer to the inner granular layer is nearly complete. Histological analysis reveals that, at the anteromedial level of the cerebellum (MT-230, MT-254, DMS-524, DMS-688), most cells of the molecular layer are of mesencephalic origin (Fig. 5) whereas, in the lateral and posterior levels, they are of metencephalic origin. Graded mixing of quail and chick cells has been observed between these two levels.

The deep nuclei neurons can be identified by their large size and their position in the cerebellum from E10 (MT-220) onward (see Table 1). Both cellular markers are present in these chimeras (Fig. 7). The neurons of the rostral levels are of mesencephalic origin while
those of the caudal parts of the nuclei are metencephalic-derived. Some mixing exists between the anterior and the posterior levels.

Much mixing is also observed in the white matter from E8 onward.

Fig. 6. Sagittal section of the chimera DMS-688, Q/C, P7. Gradual anteroposterior cellular mixing occurs within the population of small cells surrounding the Purkinje cells. (A) Folium I: quail Purkinje cell surrounded by quail cells. (B) Folium VIII: chick Purkinje cell surrounded by quail and chick cells. (C) Folium IXc: chick Purkinje cell surrounded by chick cells. Feulgen-Rossenbeck staining. Scale bar: 15 μm.

Fig. 7. Parasagittal section of the chimera DMS-688, Q/C, P7. The presence of quail mesencephalic (arrows) and chick metencephalic deep nuclei neurons indicates that these cells are generated in a region lying in both the mes- and metencephalic vesicles. Feulgen-Rossenbeck staining. Scale bar: 35 μm.

(B) Transplantations of the lateral half of the metencephalic vesicles (LMT)

In transplantations involving only one lateral half of the metencephalic vesicle, the anterior and the posterior borders of the graft were the same as for whole metencephalic vesicle transplantations. Nine chimeras were observed, from E5 to P8 (see Table 1), and the behaviour of the cells along the longitudinal axis was the same as previously described for the two former types of transplants. In addition, the unilateral transplantations revealed transverse cellular migrations across the sagittal plane: from E12 (LMT-148, LMT-149) onward, cells of the contralateral side were found within the inner and external granular layers and among the cell population of the white matter.

In the external granular layer such mixing mainly occurred in the deeper zone (Fig. 8). Quail cells were observed up to 1.2 mm from the sagittal plane at E15 (LMT-202). After hatching (LMT-216), the external granular layer becomes very thin and does not exceed 3 cell bodies in thickness. At that time, no significant cellular mixing can be detected within it.
Fig. 8. Transverse section of the folium IV of the cerebellum of the chimera LMT-192, Q/C, E16 through the external granular layer. The external granular layer is cut tangentially, thus revealing that the cell migrations between the grafted right side and the host left side occur mainly in the deeper zone of the layer (appearing in the center of the section) and in both directions. (A) Low magnification of the section, scale bar: 100 μm. (B) Higher magnification of the chick left side. (C) Same magnification of the quail right grafted side. Scale bar for B and C: 35 μm. Feulgen–Rossenbeck staining.
Development of chick cerebellum

Fig. 9. Parasagittal sections of the cerebellum of the chimera LMT-216, Q/C, P8, at the posterior level of the cerebellum (folium X). Cell mixing occurs within the inner granular layer (IGL) across the sagittal plane, in both directions. (A) Parasagittal section through the left host (chick) side. (B) Parasagittal section through the right grafted (quail) side. The slices are separated by about 30 μm. VE, ventricular epithelium; IGL, inner granular layer; ML, molecular layer; PC, Purkinje cell layer. Scale bar for A and B: 35 μm. These pictures also reveal that Purkinje cells overlie a ventricular epithelium of the same species type (see Fig. 5).

Discussion

This study is a part of a more comprehensive investigation in our laboratory of the fate map of the avian cephalic vesicles at the 12-somite stage using the classical quail–chick marker system. Here, we have focused on the metencephalon and its contribution to the construction of the cerebellum by marking differently defined regions of the chick metencephalic vesicles or of the neighbouring myel- and mesencephalic vesicle by interspecific grafts. We also followed cell movements and migrations at the borders of the implanted tissues.

Since the quail and the chick have different sizes and differ in the length of their incubation period, it is extremely important to carry out the grafts in both directions. As previously observed in experiments dealing with the ontogeny of the neural crest (see Le Douarin, 1982, for a review), the cellular behaviour and morphogenetic processes were found to be fundamentally similar from embryo to embryo and in both directions. Furthermore, hatched chimeras did not exhibit obvious behavioural abnormalities.

Topographical origin of the cerebellum in the 12-somite encephalic vesicles

The most original finding resulting from these experiments is that a large part of the cerebellum, rostromedially, has a dual origin, both met- and mesencephalic origin. A longitudinal morphogenetic distortion affects the neural tube from E5 onward and this leads to the displacement of its ventral aspect rostrally and its dorsal aspect caudally, at the level of the encephalic vesicles we studied. As a result, mesencephalic material is carried backwards in a V-shaped area inserted into the metencephalic roof. In this area, all the cerebellar cortical layers, except the inner and external granular layers, are composed of cells of mesencephalic origin.

Consequently, the embryonic neural tube constrictions are dynamic structures which do not necessarily correspond to definitive adult anatomical brain regions. We demonstrate here that the cerebellum arises from a territory that transgresses the primitive mes-metencephalic boundary (Figs. 3,4 and 10). These distortions probably result from mechanisms similar to those that have been recognized in the modelling of the neural plate, earlier in development, such as changes in cellular morphology and local cellular proliferation (Jacobson and Gordon, 1976; Jacobson, 1984).

Different cerebellar cell types have different, specific patterns of migration

Chimerism was established within the cerebellum using different experimental designs. In one series of experiments, it was created by the intrusion of dorsal mesencephalic material into the rostromedial part of the cerebellum. The others involved transverse cell migration revealed by grafts of quail or chick hemimetencephalic vesicle. The chimerism observed in these various cases differentially affected each of the cellular layers. This is particularly informative for deducing the
different specific patterns of migration and settlement of the various cell types.

In these experiments, the Purkinje cells appear to migrate radially from the ventricular epithelium to their definitive location, since they invariably have the same nuclear marker as the ventricular epithelium that they surmount, i.e. quail Purkinje cells cover a quail ventricular epithelium and chick Purkinje cells a chick ventricular epithelium (Figs. 4 and 9). Moreover, no significant mixing (defined as penetration exceeding 5 cells width) occur between quail and chick Purkinje cells in any direction, at any stage observed. Thus, we confirm that Purkinje cells reach their final destination following an outward radial migration already documented in other systems (Miale and Sidman, 1961; Altman, 1982; Altman and Bayer, 1985).

The cerebellar deep nuclei neurons are the first cerebellar neurons to appear during ontogenesis in rodents. From early development onward, they remain close to the ventricular epithelium (Altman and Bayer, 1985). Quail/chick cell mixing is observed in these nuclei (Fig. 7) showing that they develop from an area extending across the mes–metencephalic boundary.

As expected (Cajal, 1911; Miale and Sidman, 1961; Altman and Bayer, 1985), the presumed Golgi cells located in the inner granular layer were found to be of the same type as the corresponding Purkinje and ventricular epithelium cells (Fig. 5).

Concerning the smaller, scattered mesencephalic cells in the inner granular layer, and the cells mixed in the white matter, no precise conclusion can be drawn yet concerning their phenotype in the absence of information obtained by using specific cell markers.

The external granular layer cells are of the same species as the metencephalic vesicle regardless of the experimental designs used. This infers a metencephalic origin, at the 12-somite stage, for this layer as proposed by many authors, the territory concerned being referred to as the rhombic lip (Cajal, 1911; Larsell, 1948; Altman, 1982; Altman and Bayer, 1985). The precise limits of the rhombic lip were not defined in these experiments.

In grafts of total mes- or metencephalon, the ventricular epithelium (and thus also the Purkinje cells), of a V-shaped rostral cerebellar area, is derived from the mesencephalic vesicle, while the inner granular layer is almost entirely composed of cells of metencephalic species type, as the external granular layer. Our results are thus in accord with the idea that the positioning of the granule cells in the inner granular layer results from a centripetal migration from the external granular
layer. This confirms that the inner granule cells are derived from cells of caudal and superficial origin (Altman, 1972; Altman and Bayer, 1985; Hansmann et al. 1987; Pehlemann et al. 1987; Rickmann, 1987).

However, the inward migration of the cells deriving from the external granular layer is far from being strictly radial. In fact, the unilateral metencephalic vesicle transplants reveal that significant cell mixing occurs across the sagittal plane in the external granular layer (mainly in the deeper zone) and particularly in the inner granular layer. Some isolated cells could even be seen reaching the extreme lateral aspect of the cerebellum in this latter cell layer. We did not demonstrate that all the cells that had crossed the sagittal plane were granule cells. However, according to our results, granule cells are involved in such movements (see Figs. 8 and 9).

Radial migrations, whether directed inward or outward, have been extensively documented in several systems and primarily in the establishment of the cerebral cortical layers of mammals. They have been interpreted as using transitory radial glial cells as substrate (Uzman, 1960; Angevine and Sidman, 1961; Miale and Sidman, 1961; Altman, 1966, 1972, 1982; Fujita et al. 1966; Fujita, 1967; Rakic and Sidman, 1973; Rakic, 1971, 1972, 1981, 1985, 1988; Rakic et al. 1974; Chen Ming Chuong et al. 1987; Linder et al. 1987). Non radial migrations have been more difficult to perceive, although they are thought to exist (for examples see Harkmark, 1954; Clarke, 1982; Zagon et al. 1985; Puelles and Martinez-de-la-Torre, 1987; Rickmann, 1987; Bourrat and Sotelo, 1988). They have recently been demonstrated as contributing extensively to the positioning of cells in the cerebral hemispheres of birds (Balaban et al. 1988).

The possible cues followed by laterally migrating cells have not been documented. Cajal (1911) described changes in the morphology of inwardly migrating granule cells in the external granular layer. These cells acquire a bipolar shape in the deeper zone of the external granular layer, with two cellular processes running transversely. Later on, they extend a third cytoplasmic process which leads them radially downward (Rakic, 1971). It is then possible that they follow a cytoplasmic process transversally and/or they could migrate transversally within the inner granular layer itself.

Tangential migrations cannot be revealed by tritiated thymidine labelling, since this method only shows up cellular migrations from a labelled to an unlabelled, i.e. non proliferative, region of the neural epithelium. It thus identifies cellular mixing between layers generated at different times in development but not within a particular, homogeneous cell layer.

Molecular layer cells, such as the basket and stellate cells, have been studied in hatched chimeras with Feulgen and Rossenbeck and cresyl violet stainings. At this time, the inward granule cell migration is nearly completed and the relative proportion of molecular layer specific cells has thus increased compared to earlier stages.

In the rostromedial mesencephalic part of the cerebellum, the species type of the cells of the molecular layer is found to be the same as that of the mesencephalic vesicle, i.e. the opposite type to the external granular layer (Fig. 5). Moreover, their position with respect to the ventricular epithelium of either quail or chick reveals that their migration is mainly radial but also involves a non-negligible tangential component. Our results challenge the classical conclusion that the cells of the molecular layer arise from the external granular layer. This notion stemmed from tritiated thymidine labelling experiments, performed in rodents (Miale and Sidman, 1961; Uzman, 1961; Altman and Bayer, 1985), which showed that these cells are generated late in development, at a time when the ventricular epithelium is no longer in a proliferative state whereas the external granular layer still is. This led to the conclusion that cells appearing at this age, close to the external granular layer, are generated in this structure. Our technique shows that the cells of the molecular layer and those of the external granular layer are two independent populations of cells of distinct origin, but does not provide any information concerning the time at which these cells are generated.

Our experiments also reveal that a population of small cells, surrounding the Purkinje cells and located at the border between the molecular layer and the inner granular layer, is of the same type as the Purkinje and the ventricular epithelium cells. Consequently, they do not belong to the inner granular layer sensu stricto, i.e. to the progeny of the external granular layer. To our knowledge, this has never been noted before.

In conclusion, it has to be underlined that, in three different types of grafts we performed from quail to chick as well as from chick to quail, we always obtained similar results. Furthermore, hatched chimeras did not show obvious behavioural abnormalities which could have been generated by the graft. Our data confirm the well documented active cell migration such as the inward movement of granule cells or the outward migration of Purkinje cells that occurs during cerebellar ontogenesis. Consequently, we believe that the hitherto undescribed cellular behaviour revealed by this method corresponds to phenomena occurring in normal development as well. These are: the morphogenetic movements that massively displace the neuroepithelial cells, the active tangential migrations affecting cells of the granular layers, the specificity of the cell bodies surrounding the Purkinje cells and the origin of the cells of the molecular layer, which do not derive from the external granular layer as previously believed.

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