A subset of clones in the chick telencephalon arranged in rostrocaudal arrays

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Background: Different areas of the vertebrate central nervous system appear to follow different rules during development for determining the position of sibling cells. For example, in the chick hindbrain, clones are frequently confined to a single functional unit that derives from a single rhombomere. In contrast, clones in the mammalian cerebral cortex often cross functional boundaries because of the extensive migration of sibling cells in orthogonal directions. We have investigated whether the pattern of clonal distribution in the chick telencephalon is similar to that of the hindbrain or to the more functionally analogous mammalian cerebral cortex. Progenitor cells in the chick telencephalon were marked using a retroviral library encoding alkaline phosphatase and over 10^5 distinct molecular tags. Patterns of dispersion were detected using alkaline phosphatase histochemistry, followed by the recovery and sequencing of the molecular tag. We also analyzed the phenotypes of cells that occurred within the clones.

Results: A subset of progenitors gave rise to clones that were found in rostrocaudal arrays resembling tubes. Arrays were restricted in the mediolateral and dorsoventral planes but could span up to 4 mm in the rostrocaudal direction. They were found throughout the telencephalon and a single clone often spanned more than one telencephalic nucleus. Rostrocaudal clones comprised 60 % of clones containing five or more cells and contained many different types of neurons, astrocytes, oligodendrocytes, or various combinations of these cell types.

Conclusions: Telencephalic progenitors are multipotent, producing progeny that become distinct cell types. Clonally related cells can migrate rostrocaudally within domains that are restrained in the mediolateral and dorsoventral directions. A subset of rostrocaudal clones resemble those seen in the mammalian cerebral cortex, with respect to the crossing of functional boundaries, but all rostrocaudal clones differ from the cerebral cortical clones in the pattern of spread of sibling cells, with the rostrocaudal clones being more constrained in the mediolateral and dorsoventral directions. A role for lineage in the patterning of the chick forebrain is supported by these observations. In addition, these data suggest a role for cues within the telencephalic marginal zone that serve to guide clones in their rostrocaudal migration.

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Background

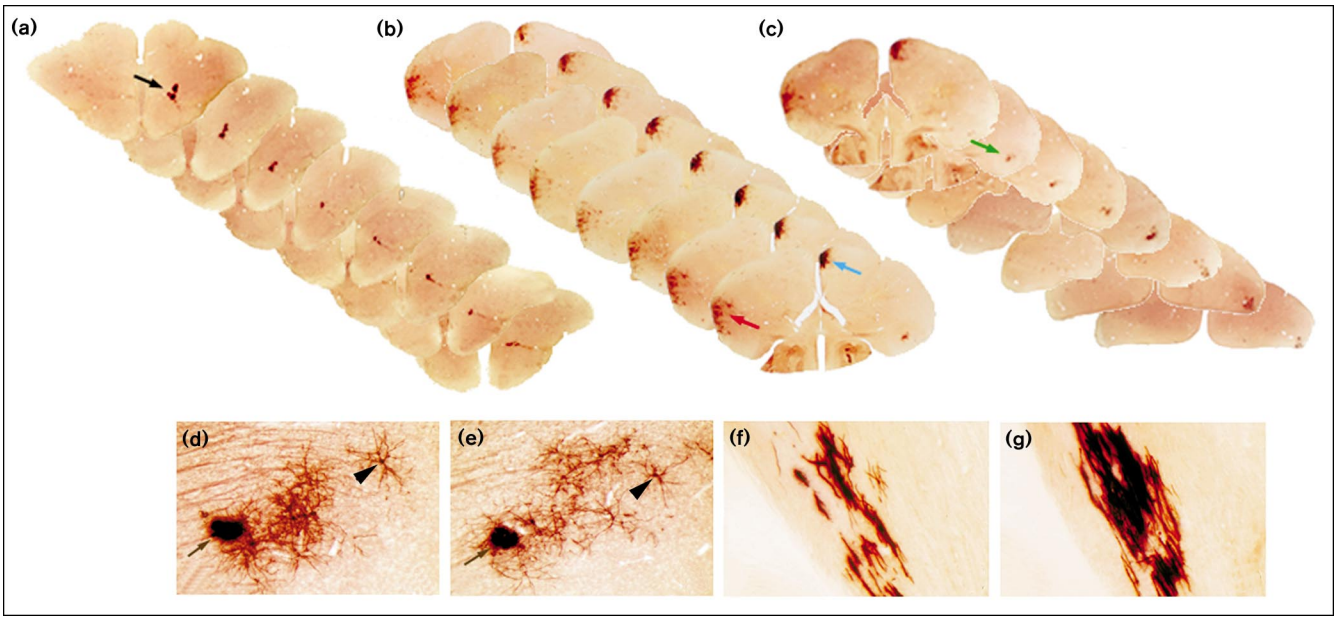
Mature neurons of the vertebrate central nervous system (CNS) are typically located some distance from their site of genesis. They are usually born adjacent to a ventricle — in the ventricular or subventricular zone (VZ and SVZ, respectively [1,2]) — from where they migrate to form the various laminated and nuclear structures of the CNS. The paths taken by newborn neurons have not been completely described, although it is known that most newborn neurons begin their migration from the ventricular surface by moving along radial glial guides [3,4]. There are, however, exceptions to this mechanism — for example, some cells can migrate orthogonally to the radial glial fibers [5–7], causing them to end up in different functional domains [5,8–10], and cells in particular parts of the brain, such as the olfactory bulb and cerebellum, use rather extended and circuitous routes that appear uniquely suited to certain aspects of their structure [11,12].

The chick telencephalon is similar to the mammalian cerebral cortex in function, but has a nuclear rather than laminar structure [13]. The current study was undertaken to define the migration routes, final locations, and phenotypes of clonally related cells in the chick telencephalon, and to compare these features with those of the mammalian cerebral cortex.

Results

The CHAPOL retroviral library encodes human placental alkaline phosphatase and a highly complex pool of

Figure 1



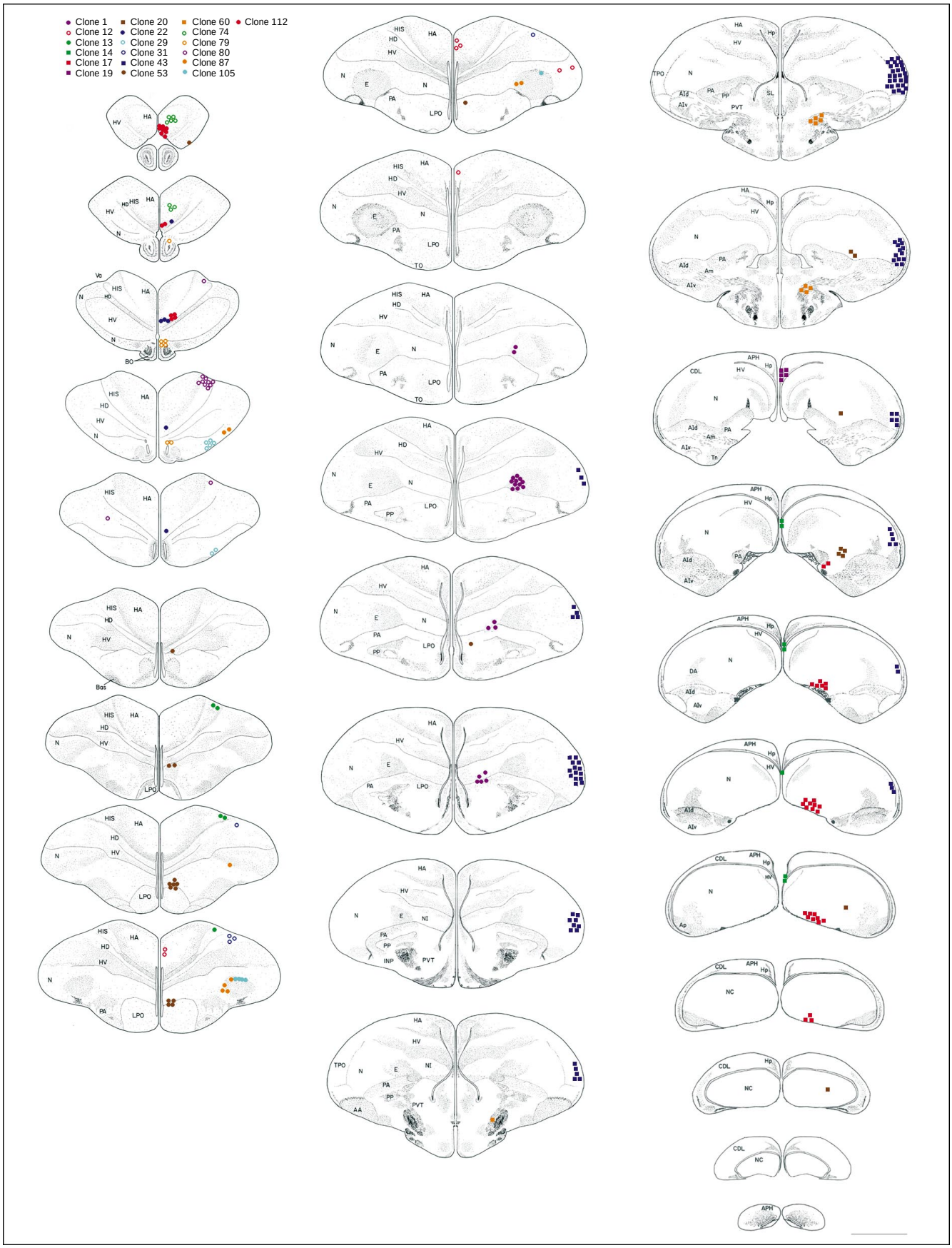
(a–c) Transverse sections of the chick forebrain showing four rostrocaudal clones. The 60 μm sections in (a,b) are contiguous (480 μm total) and show only a portion of the clones, which continue in both the rostral and caudal directions. In (c), which includes almost the entire clone, every sixth section (2880 μm total) is shown. Sections in (a,c) extend in a rostral to caudal direction; sections in (b) extend in a caudal to rostral direction. In (a), the clone (arrow) was composed of astrocytes found in the neostriatum close to the lobus parolfactorius. Note that within each consecutive tissue section, members of this clone were found in the same dorsoventral and mediolateral position. Portions of two clones are shown in (b); the one on the left (red arrow) was in the neostriatum and was composed only of neurons. The neostriatal clone is also shown in Figure 2 (clone 43). The clone on the right (blue arrow) was in the hyperstriatum accessorium of the visual wulst, and was composed of both astrocytes and neurons. Note the high density of cells found in the wulst clone in (b). The entire wulst clone is shown in light blue in Figure 3. Both clones maintain their dorsoventral and mediolateral positions as they extend along the rostrocaudal axis. The sections in (c) show almost the entire extent of a clone found in the region of the archistriatum. A few cells in this clone were found more caudally. **(d,e)** High-magnification views (120 μm apart) of the clone depicted in green in (c). This clone was composed primarily of neurons (black arrowheads) but also contained a few astrocytes (black arrows). **(f,g)** Oligodendrocytes in a rostrocaudal clone found in the lateral forebrain bundle; sections are 120 μm apart.

degenerate oligonucleotide sequences [14]. CHAPOL was injected into the neural tube of chick embryos at the beginning of neurogenesis in the telencephalon at embryonic day 2.5 (E2.5). Brain sections from post-hatch day 14 to 21 (P14–21) chicks were stained for alkaline phosphatase (AP) activity and positive cells were picked, amplified using the polymerase chain reaction (PCR) and sequenced [14]. Clones were defined as cells that contained the same oligonucleotide sequence. The location of each cell was recorded and the positions of the clones were reconstructed in two and three dimensions. A total of 2 082 samples (referred to as picks) from three brains were successfully amplified and sequenced, leading to the identification of 192 clones. Similar clonal patterns were found in all eleven brains analyzed.

Clones in the chick telencephalon ranged in size from 1 cell to more than 100 cells, and several different types of clones

Figure 2 (facing page)

Localization of 19 rostrocaudal clones in the chick telencephalon. Each transverse section represents a tissue width of 400 μm. Clones were found in both the left and right hemispheres of the brain, but are shown only on the right for clarity. Each coloured dot, circle or square denotes one pick (picks most often contained one cell). These clones were from three brains. Note the extensive rostrocaudal spread of individual clones and the lack of dispersion in other directions. For example, clone 53 spanned 5580 μm rostrocaudally and clone 1 spanned 1980 μm. Note that clone 80, which spanned 600 μm rostrocaudally, was primarily located in the hyperstriatum intercalatus but also has a contralaterally located cell. Clone 60 was composed entirely of oligodendrocytes (also shown in Fig. 1f,g). Clones 14 and 17 are also depicted in Figure 3 (green and purple clones, respectively). Sections were modified from the atlas of Kuenzel and Masson [29]. AA, archistriatum anterior; AId, archistriatum intermedium, pars dorsalis, Alv: archistriatum intermedium, pars ventrale; Am, archistriatum mediale; Ap, archistriatum posterior; APH, area parahippocampalis; Bas, nucleus basalis; CDL, area corticoidea dorsolateralis; DA, tractus dorsoarchistriaticus; E, ectostriatum; HA, hyperstriatum accessorium; HD, hyperstriatum dorsale; HIS, hyperstriatum intercalatum supremum; Hp, hippocampus; HV, hyperstriatum ventrale; INP, nucleus intrapeduncularis; LPO, lobus parolfactorius; N, neostriatum; NC, neostriatum caudale; NI, neostriatum intermedium; PA, paleostriatum augmentatum; PP, paleostriatum primitivum; PVT, paleostriatum ventrale; SL, nucleus septalis; Tn, nucleus taeniae; TO, tuberculum olfactorium; TPO, area temporo-parieto-occipitalis. Scale bar = 3 mm.


Clone 1
Clone 12
Clone 13
Clone 14
Clone 17
Clone 19
Clone 20
Clone 22
Clone 29
Clone 31
Clone 43
Clone 53
Clone 60
Clone 74
Clone 79
Clone 80
Clone 87
Clone 105
Clone 112
HA
HV
HIS
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Hp
APH
CDL
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PP
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AId
AIv
Am
DA
Ap
NC
NI
INP
AA
Tn

Figure 3

(a) Three-dimensional reconstruction of the entire chick forebrain showing seven rostrocaudal clones from two brains. Each cell in a given tubular array was picked, amplified and sequenced, and was always found to contain the same insert. Clones are depicted on the surface of the tissue, but, as seen in Figures 1 and 2, they were often located in more interior areas. Individual colors represent separate clones. Each dot denotes a single cell, whereas streaks of color denote groups of cells. Sections from the atlas of Kuenzel and Masson [29] were scanned into a computer and reconstructed using Spyglass (Visualogic). Each of the 55 sections represents a width of 200 μm. The clone depicted in red spanned 1080 μm, and was composed of two rostrocaudal tubes found in the hyperstriatum; one sibling cell was located outside the rostrocaudal arrays. The majority of rostrocaudal clones were composed of one array only. The clone depicted in yellow was found very close to the olfactory bulb in the ventral neostriatum, and contained 33 cells that spanned 1200 μm; the yellow streak represents 24 cells clustered together. This clone also contained four cells that are out of view on the contralateral side. The clone depicted in brown was one of the largest clones; it spanned 4200 μm and was composed primarily of astrocytes. The distribution of this clone was reconstructed from 135 sequenced picks, each of which contained 1–6 astrocytes. Interestingly, a single neuron was found within this clone. The brown clone was found at the lateral edge of the most anterior one third of the neostriatum, but gradually extended to the ventricle, strongly suggesting rostral migration. Note that five cells were found outside the rostrocaudal tube. The clone depicted in light blue was found in the wulst; it spanned 3400 μm and contained large numbers of astrocytes and neurons. The very dense part of the clone is shown in eight consecutive 60 μm sections in Figure 1b (blue arrow). Note that four out of 32 picks were outside of the rostrocaudal tube, and three additional cells were located in the contralateral hemisphere. The small clone depicted in dark blue spanned 540 μm and was found in the hippocampus; it was composed of a dense cluster of astrocytes and neurons. The clone depicted in green spanned 2600 μm rostrocaudally and was found in the corticoseptalis white-matter tract. The area in between positive picks was often occupied by similar small cuboidal AP-positive cells, from which no viral genome was recovered. Some other clones that were restricted to distinct rostrocaudal tubes contained cells separated by long stretches of unlabelled tissue. The clone depicted in purple spanned 2100 μm and was found in the ventral neostriatum; it contained neurons with fine processes, and very small cell bodies that were impossible to pick individually and were therefore picked in 28 clusters.

were found. The members of some of the clones were clustered together, whereas cells from other clones appeared to be dispersed in a random fashion. However, a subset of clones showed an interesting non-random pattern of distribution. As seen in Figures 1–3, 30 clones containing five or more cells were found in arrays that extended in the rostrocaudal direction (rostrocaudal clones: approximately 15 % of all clones, and approximately 60 % of all clones that contained five or more cells). These clones were quite restricted in the dorsoventral and mediolateral planes. Most often they contained cells in contiguous sections and seemed to form tubular arrays (Fig. 1a–c). Some rostrocaudal clones had fewer cells at their rostral or caudal ends than in the middle (for example, the clone indicated by the green arrow in Fig. 1c). Some rostrocaudal clones were discontinuous, in that siblings were separated along the rostrocaudal axis by areas containing no sibling cells (for example, the clone depicted in green in Fig. 3). In transverse section, the shape of the tubes ranged from round (Fig. 2, clone 74) to amorphous (Fig. 2, clone 43). Some rostrocaudal clones had a few members outside of the tubular array that were randomly dispersed (Fig. 2, clones 12 and 80; Fig. 3, clones shown in red, brown and light-blue) and a few contained two or more tubular arrays within one transverse section (Fig. 3, clone depicted in red).

Rostrocaudal clones were found in most of the major nuclei of the chick forebrain (Figs 2,3), including the hippocampus, corticoid area, wulst and ectostriatum (visual processing areas), neostriatum, lobus parolfactorius (olfactory processing) and paleostriatum (analogous to the mammalian basal ganglia). A single rostrocaudal clone often spanned several functional and/or anatomical domains (Fig. 2, clone 87). The majority of rostrocaudal clones were parallel to the pial surface or to the ventricles, and many were close to these surfaces (Fig. 2; clones 13, 31, 53, 79, 80 and 112); some were found in the middle of the tissue (Fig. 2; clones 1, 74, 87 and 105). A few clones were oriented at oblique angles (Fig. 2, clone 1) and could be followed to their origin on the ventricular surface (Fig. 3, clone depicted in brown). Those that could be assigned an origin appeared to contain cells that migrated rostrally. The majority of rostrocaudal clones were found in the gray matter; however, of the two that were located in the white matter, one was a large clone of oligodendrocytes in the lateral forebrain bundle (Fig. 1f,g), and the other contained small cuboidal cells in the tractus corticoseptalis (Fig. 3, green clone).

The formazan precipitate from the AP histochemical reaction clearly labeled the cell bodies and processes of many different types of neurons, as well as astrocytes and oligodendrocytes (Fig. 1d–g). A single clone frequently contained both neurons and glial cells (Fig. 1d,e). There was no correlation between the size of a rostrocaudal clone and its cellular composition. The number of cells in each rostrocaudal clone ranged from five (an arbitrary minimum)

to more than 100. Clone size was underestimated because amplification was not successful for all picks (61 ± 3 % were successfully amplified). Thus, it is probable that each rostrocaudal clone has more members than we identified, and that, as a group, rostrocaudal clones are likely to be more frequent than reported here.

In contrast to the cells of the mammalian cerebral cortex, cells born at early developmental stages in the chick telencephalon are found close to the pial surface, whereas cells born at later stages remain close to the ventricles [15]. It was therefore possible that there was a correlation between the time of infection and the distance of a rostrocaudal clone from the ventricle along the mediolateral axis. However, we infected embryos between stages 12 and 19 and saw no correlation between the time of injection and either the lateral distance of clones from the ventricles or the frequency of rostrocaudal clones. This suggests that progenitors that give rise to rostrocaudal clones are mitotically active within the VZ throughout these stages, and that the mediolateral localization of rostrocaudal clones is independent of their time of birth. Furthermore, there was no correlation between the size of the clones and their mediolateral localization.

Discussion

Rostrocaudal clones exhibit a novel pattern of distribution along the rostrocaudal axis. The mechanism that restricts rostrocaudal clones to this pattern of dispersion is not known. To date, patterns of anatomical or biochemical substrates that could account for these patterns of clonal distribution have not been found. It is possible that the cells of rostrocaudal clones migrate along fiber tracts in the intermediate zone of the developing telencephalon. Anterior–posterior migration along axons has been proposed for oligodendrocytes in the white matter of the developing chick spinal cord [16], and GnRH neuroblasts migrate along axon tracts to populate the hypothalamus [17,18]. Alternatively, cells in the chick forebrain may recognize an extracellular matrix molecule or an adhesion molecule arranged so as to promote rostrocaudal migration. In the mouse, expression of PSA–NCAM (polysialic acid-modified neural cell-adhesion molecule) is essential for neuroblasts to migrate from the SVZ of the anterior lateral ventricle through a glial meshwork to the olfactory bulb, in what is known as the rostral migratory stream (RMS) [19–21]. This type of directed rostral migration appears to have the purpose of populating the olfactory bulb with late-born granule neurons; these neurons must derive from an adjacent VZ because the VZ of the olfactory bulb is depleted by the postnatal period [11,22,23].

Whereas the RMS is an example of actual migration in a proliferative zone, it is unclear to what extent migration in proliferative zones contributes to histogenesis in other areas of the brain. In previous work from our laboratory, we found that a small number of clones were dispersed in the VZ and/or SVZ of the mammalian cortex [7]. Tangential migration in the SVZ of the developing striatum appeared more frequently, occurring in approximately 40 % of clones, primarily in the dorsoventral plane [24]. In a study using live imaging techniques, seemingly random movements within the plane of the proliferative zones were seen within the murine telencephalon [25]. Recently Reid *et al.* [26] used a retroviral library to examine clonal patterns in the rat cerebral cortex and, on the basis of their observations and previous data [10], proposed a model in which migration in the proliferative zones accounts for systematic clonal organization [26]. They found that clones formed subunits that were distributed in a periodic fashion along the rostrocaudal axis, although clones were also dispersed in the mediolateral and dorsoventral planes. The clones reported here do not have a periodic distribution along the rostrocaudal axis, and do not show dispersion in the mediolateral and dorsoventral planes. Nonetheless, *a priori*, it is possible that extensive rostrocaudal migration in the VZ, followed by migration along radial glia, could result in the presence of rostrocaudal clones in the chick telencephalon. However, this seems unlikely because preliminary observations of labelled cells within embryos harvested several days postinfection suggest that there is little to no dispersion of sibling cells in the proliferative zones of the chick telencephalon. In contrast, radial migration along radial glia within the VZ, followed by orthogonal migration away from radial glia as cells exit the VZ, is a common finding (F.G.S. and C.L.C., unpublished observations).

The observation that descendants of a particular progenitor appear to be restricted in their movement in the mediolateral and dorsoventral planes, and that the location of the rostrocaudal migration varies from clone to clone, suggests that there may be a system of positional cues recognized by members of a rostrocaudal clone. If so, the recognition of a cue by almost all members of a particular clone suggests that the recognition is derived from information transmitted from the progenitor — that is, lineage appears to play a role in the patterning of the chick telencephalon. What the underlying pattern represents with respect to the organization of the telencephalon is an intriguing question. The pattern suggested by these clones is not one that would be predicted by the known nuclear boundaries or physiological connections within the chick forebrain [13]. Patterns of gene expression have not been investigated within the chick telencephalon, and it is therefore possible that gene expression patterns would correlate with the distribution of rostrocaudal clones. The rostrocaudal clones may reflect or comprise a novel type of compartment within the brain. Alternatively, clonally related cells may not recognize cues within the tissue, but might follow each other, recognizing some surface property or attractant produced by their siblings. Clones might migrate in rostrocaudal chains that are located randomly,

as dictated by the migratory routes of the first-born members of a clone. Although they might use a different mechanism, this would be reminiscent of the migration of chains of neuroblasts in the RMS, and could be similar to the behavior of growth cones following a pioneer axon. An understanding of the mechanisms underlying the formation of rostrocaudal clones during development of the chick forebrain will rely upon identification and perturbation of the molecules involved in these processes.

Conclusions

We describe a novel pattern of distribution of sibling cells in the chick telencephalon. Siblings within rostrocaudal clones in the chick telencephalon are distributed in restricted domains along the rostrocaudal axis, indicating directed, rather than random, migration. Rostrocaudal clones can contain neurons, glia, or neurons and glia; unlike the majority of clones within the chick hindbrain, they can cross functional and anatomical boundaries, as seen for some clones within the chick diencephalon. In the functionally similar mammalian cerebral cortex, clonally related cells were found to be separated rather extensively along the rostrocaudal axis and, in many cases, the distances appeared to be of a discrete and periodic nature, unlike the continuous distribution reported here. Rostrocaudal clones and clones of the mammalian cerebral cortex also differ in that rostrocaudal clones are constrained in their dispersion in the mediolateral and dorsoventral planes. Rostrocaudal clones thus appear to have a unique pattern of dispersion that may reflect or contribute to a previously unappreciated aspect of patterning within this area of the brain.

Materials and methods

Infections and histology

Chick embryos were infected with the CHAPOL retroviral library encoding human placental AP [14] at Hamburger and Hamilton stages 12–19 (HH12–19) [27]; neurogenesis in the chick telencephalon starts at HH24 (E4) and ends at E10 [15]. A total of 11 chicks were sacrificed between P14 and P21. Animals were perfused transcardially with 4 % paraformaldehyde, and brains cryoprotected in 30 % sucrose. Sections (60 μm) were cut on a cryostat in the transverse plane. AP activity was detected according to Golden *et al.* [14].

PCR and sequencing

Telencephalic cells with AP activity were analyzed by sequencing the viral oligonucleotide tag following PCR amplification of a small area of the tissue (a pick) [14]. Some clones contained such a high density of cells (see for example, Fig. 1b, blue arrow) that it was impossible to pick single AP-positive cells. In these cases, several cells were included in each pick. The majority of these 'multiple picks' contained only one sequence. The area was not assigned a clonal identity if it contained more than one sequence. To date, the same viral tag has not been recovered from more than one independent infection using the original preparation of viral stock [14] that was used in the study by Golden and Cepko [28]. To date, 682 different inserts have been recovered from this and previous studies [14,28], yielding an even distribution (as each was recovered once) and a complexity of more than 10^5 members [14].

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