Formation of the final pattern of muscles is not a single event. It depends on a series of patterning processes that take place at different times during development. These integrate both the segment-specific signals provided by homeotic genes, and signals that are common to all segments—provided directly or indirectly by segmentation and dorso/ventral patterning genes. We show that an early cue to define this pattern is provided autonomously by homeotic gene expression in the mesoderm. Recent work (K. Vijayraghaven, J. Fernandes, S. E. Celniker and E. B. Lewis, manuscript submitted) shows that later steps in the development of the thoracic muscle precursor cells depend on both inductive signals from the epidermis and further signals provided autonomously by homeotic genes expressed in the mesoderm. By allowing the selective and inducible mis-expression of genes, the GAL4 system[11] provides a powerful tool to dissect such complex patterning processes. □

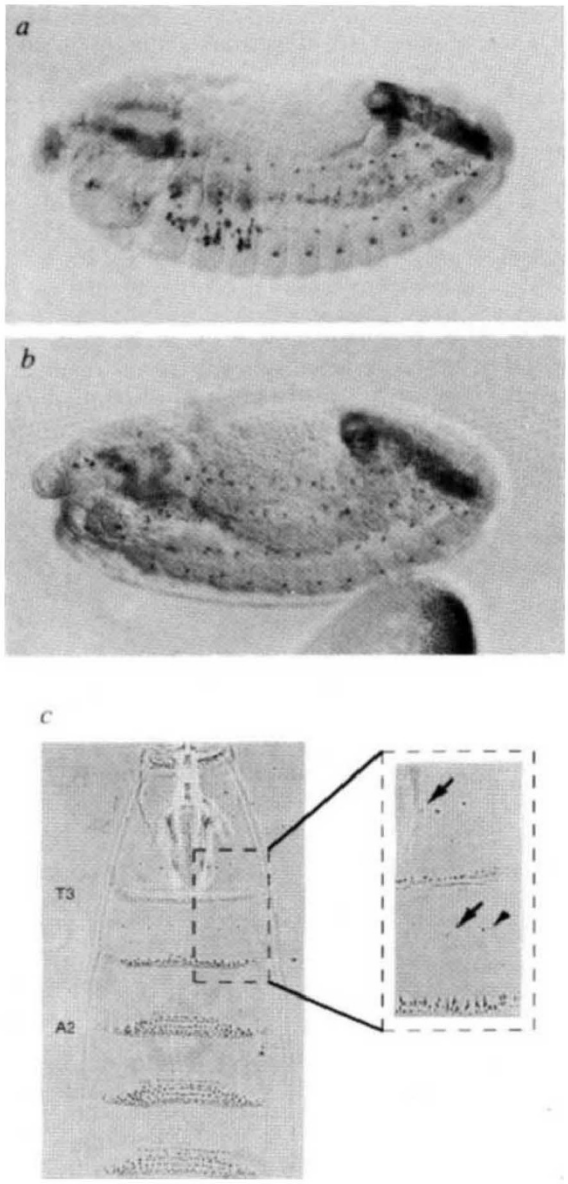

FIG. 3 *a* and *b*, Pattern of adult muscle precursor cells in germ band retracted embryos (late stage 14) that are wild-type (*a*) or express *abd-A* protein throughout the mesoderm (*b*). Of 47 late stage-14 and stage-15 embryos from the cross shown in Fig. 1, 26 showed the wild-type pattern seen in *a*. The remaining embryos all had a pattern very similar to that shown in *b*. The most striking features of this transformed pattern are that the groups of anti-Twist-staining cells found in the wild-type, thoracic segments are missing and the staining pattern in the thoracic segments now strongly resembles that seen in the wild-type abdominal segments A1 to A7. Embryos were stained for *twist* protein as described in Fig. 2. *c*, Cuticle preparation showing the thoracic segments from an animal similar to *b*. The thoracic segments are indistinguishable from wild type. Arrow, Keilins organs; arrowhead, ventral pit; T3, denticle belt of thoracic segment 3; A2, denticle belt of abdominal segment 2.

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1. Haget, A. *Bull. Biol. Fr. Belg.* **87,** 123–217 (1953).
2. Bock, E. *Roux Arch. dev. Biol.* **141,** 159–247 (1942).
3. Williams, G. J. A. & Caveney, S. *J. Emb. exp. Morph* **58,** 13–33 (1980).
4. Sahota, T. S. & Beckel, W. E. *Can. J. Zool.* **45,** 421–434 (1967).
5. Beer, J., Technau, G. M. & Campos-Ortega, J. A. *Devl Biol.* **196,** 222–230 (1987).
6. Bate, M. in *The Development of* Drosophila melanogaster (eds Bate, M. & Martinez-Arias, A.) (Cold Spring Harbor Laboratory, New York, 1993).
7. Lawrence, P. A. & Johnston, P. *Cell* **45,** 505–513 (1986).
8. Akam, M. & Martinez-Arias, A. *EMBO J.* **4,** 1689–1700 (1985).
9. Hooper, J. E. *EMBO J.* **5,** 2321–2329 (1986).
10. Lawrence, P. A. in *Genetics: New Frontiers, Proceedings of the XV International Congress of Genetics* (Oxford and IBH, New Delhi, 1983).
11. Brand, A. & Perrimon, N. *Development* (in the press).
12. Fischer, J. A., Giniger, E., Maniatis, T. & Ptashne, M. *Nature* **332,** 853–856 (1988).
13. Ornitz, D. M., Moreadith, R. W. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **88,** 698–702 (1991).
14. Jiang, J., Kosman, D., Ip, Y. T. & Levine, M. *Genes Dev.* **5,** 1881–1891 (1991).
15. Thisse, C., Perrin-Schmitt, F., Stoetzel, C. & Thisse, B. *Cell* **65,** 1191–1201 (1991).
16. Karch, F., Bender, W. & Weiffenbach, B. *Genes Dev.* **4,** 1573–1588 (1990).
17. Macias, A., Casanova, J. & Morata, G. *Development* **110,** 1197–1207 (1990).
18. Campos-Ortega, J. A. & Hartenstein, V. *The Embryonic Development of* Drosophila melanogaster (Springer, Berlin, 1985).
19. Leptin, M. *Genes Dev.* **5,** 1568–1576 (1991).
20. Bate, M. A., Rushton, E. & Currie, D. A. *Development* **113,** 7–89 (1991).
21. Jiang, J., Hoey, T. & Levine, M. *Genes Dev.* **5,** 265–277 (1991).
22. Pirrotta, V. in *A Survey of Molecular Cloning Vetcors and their Uses* (eds Rodriguez, R. I. & Denhardt, D. T.) 437–456 (Butterworth, Boston, 1988).
23. Roth, S., Stein, D. & Nusslein-Volhard, C. *Cell* **59,** 1189–1202 (1989).

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Clonal dispersion in proliferative layers of developing cerebral cortex

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In the adult cerebral cortex, many retrovirally labelled clones are widely dispersed[1], though the mechanisms of this dispersion are not well understood. Here we investigate the temporal sequence of clonal dispersion after labelling progenitors of rat cortical cells with replication-incompetent retroviruses at early stages of cortical neurogenesis, 14–15 days after conception (E14/15). The location of labelled daughter cells was determined 3, 6 or 10 days later. Labelled sibling cells were radially arrayed three days after infection (E18). In contrast, by six days after infection (E20/21), 43% of cortical clones were dispersed non-radially by at least 500 μm. Four of these widespread clones were dispersed longitudinally by ≥2 mm, implying sustained rates of dispersion of >15 μm per hour. Dispersed sibling cells occurred within proliferative zones of the forebrain in 35% of widely dispersed clones, suggesting that some dispersion reflects movement of dividing cells. Some clones dispersed beyond the neocortex into the olfactory bulb. Progenitor cell dispersion represents a previously unrecognized mode of migration by which sibling cells become widely dispersed in the developing forebrain.

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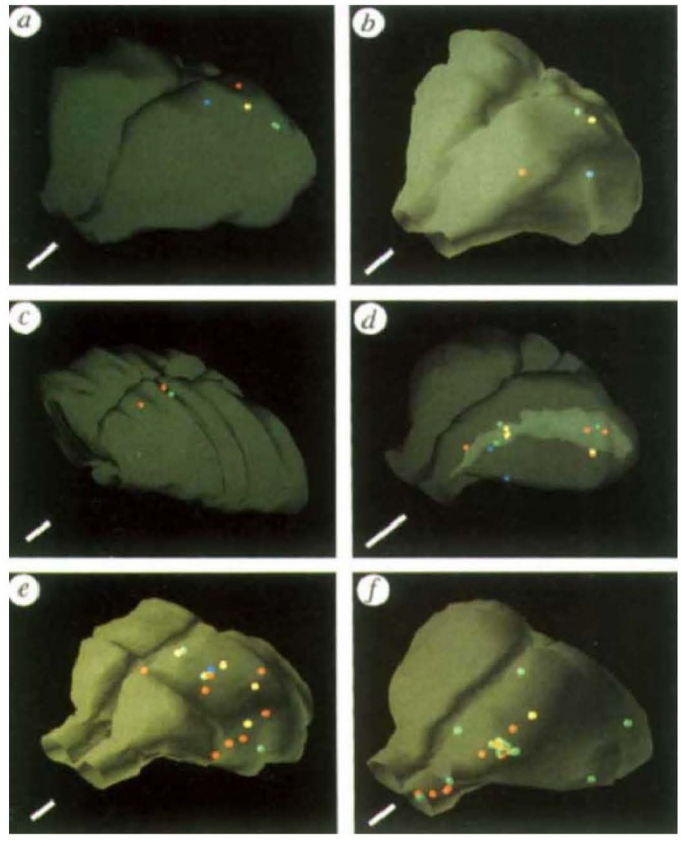

FIG. 1 Dispersion of cerebral cortical clones. Three-dimensional computer reconstructions are illustrated from six experiments following injection of the BAG retroviral library at E15. Brains were processed for X-gal histochemistry and PCR analysis of clonal relationships at E18 (*a*, *b*), E21 (*c*, *d*) or P3 (*e*, *f*). Each brain is oriented so that the rostral olfactory bulbs are in the lower-left corner of the picture. Distinct clones are indicated by up to five different colours: red-orange, yellow, green, dark blue and light blue. Each symbol represents a single cell or a tight cluster of cells sharing the same viral tag and are enlarged for clarity (symbol diameter, 120 μm in *a* and *b*, 150 μm in *c* and *d*, 200 μm in *e* and *f*). For each experiment, all histochemically labelled cells in the entire cortex were analysed, but only cells whose DNA tag was successfully amplified are illustrated. Clones consisted of single cells or tight cell clusters at E18. At E21, some clonally related cells were dispersed from one another (such as the red clone in *c*, or red, yellow and green clones in *d*), often parallel to the lateral ventricle (indicated by lighter shading in *d*). At P3, a substantial proportion of clones covered nearly the medial–lateral extent (green clone in *e*), rostral–caudal extent (red clone in *e*), or both dimensions of the cortex (green clone in *f*). Histochemical staining for β-galactosidase showed that widespread clones comprised several cell types, defined using criteria described before[1,15]. For example, in *f* the green clone included several widely scattered neurons posteriorly, a dense concentration of glial cells in the frontal cortex, and several cells in the subcortical zones and olfactory bulb. Scale bars, 1 mm. METHODS. Histochemical techniques were as described[1], except that in order to increase staining sensitivity, some brains processed for X-gal histochemistry at E18 were fixed and sectioned at 60 μm. They were rinsed and reacted for 4 h in 1 mg ml^{-1} X-gal, 0.5 mg ml^{-1} nitroblue tetrazolium, in phosphate-buffered saline (pH 7.3) with 2.5 mM $MgCl_2$. Sections were then rinsed in PBS and coverslipped in Gelvatol. Histochemically labelled cells were plotted using a computer reconstruction package, CARP[8]. One clone in the brain shown in *c*, containing a single cortical cell, was not illustrated.

Retrovirus-mediated transfer of a histochemical reporter gene allows labelling of mitotic cortical progenitor cells and their progeny. Individual clones can be distinguished by using a library of vectors that carry distinct DNA inserts as tags, and by using the polymerase chain reaction (PCR) to analyse the tags[1]. The most accurate determination of clonal patterns with this technique requires experiments with few virally labelled clones[1-3]. The analysis was thus limited to experiments with ≤5 PCR-defined clones per cortical hemisphere. In the rat, cortical neurons are generated between days 14 and 21 after conception[4] (E14–E21, the day of conception being E0), in two proliferative zones: the ventricular zone (VZ) and the subventricular zone (SVZ)[5]. During the period of neurogenesis and the ensuing 7–10 days after birth, post-mitotic neurons migrate through the intermediate zone (IZ) to the cortical plate (CP), the precursor of the cerebral cortex[6]. Therefore labelling cortical progenitors at E14 or 15 should enable most cortical neurons to be analysed.

Cortical clones showed little dispersion when analysed three days after infection at E18 (Fig. 1*a*, *b*). Clones consisted of single cells (4 clones) or clusters of ≤5 cells in the VZ, SVZ and IZ (8 clones). Most of these cells were so closely spaced that multiple cells were processed for PCR in single tissue fragments, making quantitative analysis of clone size difficult (Table 1). As previously noted in studies using a single retrovirus in rat[7] and mouse[8,9], cortical clusters were often oriented radially (Fig. 2*a*–*c*) as arrays up to 200 μm tall and ≤120 μm (usually 30–50) in the medial–lateral or rostral–caudal dimension. In one of 11 clusters analysed at E18, PCR analysis showed that nearby cells contained different viral tags, and thus constituted two adjacent clones. All other clusters contained cells with only one viral tag, however, and no widespread dispersion of cells with the same viral tag was seen after analysis of 12 clones at E18 (Table 1).

In contrast to E18 brains, brains analysed at E20/21 (six days after infection) showed very different patterns. Although there were equivalent numbers of viral tags per cortical hemisphere

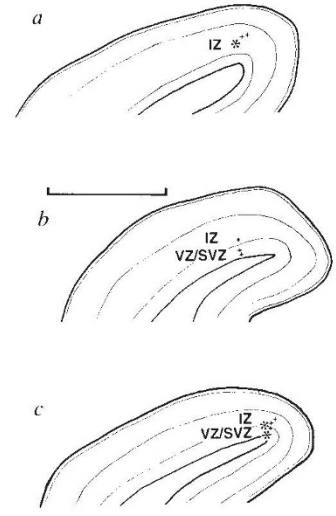

FIG. 2 Laminar locations of retrovirally labelled cells, analysed at E18. *a*, *b* and *c*, Camera lucida drawings of coronal sections from brains injected with the BAG library at E15 and analysed at E18. In each drawing, dorsal tip is up, with the brain outline shown as a heavy trace. Layers of the developing cortex are indicated with finer lines, and layers containing labelled cells are indicated. Histochemically labelled cells were tightly clustered in the VZ, SVZ and IZ, so that each cluster was included in 1–2 coronal sections taken at 60 μm. PCR analysis showed that cells in 11/12 of these clusters contained one viral tag. Asterisks indicate single cells for which PCR was used to amplify a single viral tag. Dots indicate cells for which PCR amplification was unsuccessful. Such PCR-negative cells are not illustrated in Fig. 1. As labelled cells were dissected for PCR analysis in tissue chunks ~300 μm square, two or more tightly clustered cells were sometimes contained in one tissue fragment. Crosses incicate 2–3 cells contained in a single tissue fragment from which a single PCR product was amplified (Table 1). Tight clusters like these are indicated by single dots in Fig. 1*a* and *b*. Scale bar, 1 mm.

TABLE 1 Summary of clonal patterns in the developing cortex

Cortical hemisphere	Clone	Tissue fragments (cells)					Dispersion (mm)	
		Total	VZ/SVZ	IZ/WM	CP	Non-cortex	Longitudinal	Transverse
E18-K, left	A	1 (4)	1 (4)					
	B	2 (3)		2 (3)			0.0	0.04
E18-U, left	A	1			1			
E18-U, right	A	3 (5)	2 (4)	1			0.06	0.05
E18-J, left	A	1 (3)	1 (2)	(1)				
	B	1	1					
	C	2	1	1			0.06	0.1
	D	1 (2)	1 (2)					
E18-J, right	A	1 (3)	1	(2)				
	B	1	1					
	C	1 (2)		1 (2)				
	D	1	1					
E18 total		16 (28)	10 (17)	5 (10)	1			
Average		1.3 (2.3)	63%	31%	6%		0.04	0.06
E21-A, left	A	1			1			
	B	2 (4)			2 (4)		0.0	0.3
	C	1			1			
	D	2	1		1		1.3	~1.4
E21-A, right	A	5 (10)	2	1 (3)	2 (5)		0.2	0.7
E21-H, left	A	3 (5)			3 (5)		0.1	0.3
E21-H, right	A	4	1	1	2		3.1	~0.8
	B	3	2			1 {s/ic}	2.3	~0.3
	C	2		1	1		0.0	0.6
	D	3	1	2			2.3	~0.7
E21-I, left	A	1			1			
	B	5 (7)	4 (6)		1		0.4	0.3
	C	2			2		0.2	0.2
	D	2			2		0.8	~0.4
E21-I, right	A	2	1	1			0.5	0.5
	B	2		2			0.1	0.08
E21-C, left	A*	3	2			1 {s/ic}	2.9	~0.7
E21-C, right	A	1			1			
	B	2			2		1.1	~0.9
	C*	7			1	6 {s/ic}	2.2	~0.8
E20-B, right	A	3 (4)	3 (4)	1			0.1	0.1
	B	1						
E20/21 total		57 (6.9)	17 (20)	9 (11)	23 (30)			
Average		2.7 (3.3)	35%	18%	47%		1.0	0.5
P3-L, left	A	2			2		0.1	3.2
	B	5	1		4		1.0	2.6
	C	1			1			
	D	8		1	7		3.2	3.2
	E	1	1					
P3-M, right	A	1 (2)		1 (2)				
	B	7 (24)	1 (2)			6 (22) {ob}	1.9	0.2
	C	15 (22)	15 (22)				3.1	1.1
P3-S, left	A	21 (26)	2	4 (5)	9 (13)	6 {ob}	3.6	1.3
	B	20 (27)		4 (5)	14 (20)	2 {ob}	6.4	2.6
	C	1			1			
P3-N, right	A	1 (2)		1 (2)				
P3 total		83 (121)	20 (28)	11 (15)	38 (48)			
Average		6.9 (10.1)	29%	16%	54%		2.8	2.0

Results are shown from 18 experiments (defined as a hemisphere of an infected brain) after injection of the BAG retrovirus library at E15 and analysis 3 days later (E18), injection at E14 or E15 and analysis 6 days later (E20/21), or injection at E15 and analysis 10 days later (P3). One clone (asterisk) dispersed across the midline into both hemispheres. Each clone is listed as a row of the table and indicated by A, B and so on. The total number of tissue fragments that contained the same viral tag is indicated for each clone. In case where tissue fragments contained >1 histochemically labelled cell, the total number of labelled cells in all tissue fragments is indicated in parentheses. For each clone, the location of PCR-positive cells in the ventricular or subventricular zones (VZ/SVZ), intermediate zone or subcortical white matter (IZ/WM) or cortical plate (CP) is listed; again, the number of histochemically labelled cells in each fragment is given in parentheses. Non-cortical cells were found that had the same viral tag as cortical cells. They were located in the striatum/internal capsule and in the olfactory bulb (indicated as {s/ic} or {ob}); they were included in calculations of clonal dispersion. Between E18–E21, the cortex increases in length from 3.3 to 5 mm while the average longitudinal dispersion increases more than 20-fold. Between E21 and P3, the cortex grows from 5 to 7.5 mm in length, and longitudinal dispersion increases more than 2.5-fold. With increasing age, clones increase in size, and cells migrate from the VZ/SVZ to the CP. Construction, characterization, and injection of the retroviral library are described elsewhere[1]. The library consists of a mixture of retroviral vectors that encode lacZ. Each member of the library also contains a DNA segment of unique size and/or pattern of restriction enzyme digestion. Viral inoculations were made such that ≤5 clones were recovered from each hemisphere. After histochemical processing of tissue sections, labelled cells were removed in small tissue fragments (containing up to ~10,000 unlabelled cells) and digested in a proteinase K solution; viral tags were amplified with PCR. Standard dideoxy sequencing methods were used to determine whether assignment of clonal relationships based upon restriction enzyme digestion patterns was accurate. Sequencing was attempted on 23 amplified tags and was successful on 21 (the other two sequencing reactions yielded no product); DNA sequence analysis showed that the 21 tags had been assigned correctly to 9 clones by the restriction enzyme digestion patterns (data not shown). In 291 clones analysed so far, 85 members of the library have been recovered, suggesting that some members of the library may be absent or present in relatively low amounts[2]. To confirm that differences in clonal patterns were not due to coincidental infections of two or more clones with one viral tag[3], the Wilcoxan rank sum test was applied to compare frequencies of dispersed clones at E18 versus E21 and P3. Even though the number of clones per experiment was similar at all ages, the difference in frequency of dispersed clones was significant ($P < 0.0002$). The probability of coincidental double infections can also be calculated directly using statistical models[2,3] available over e-mail (at church@rascal.med.harvard.edu). When ≤5 clones are labelled in an experiment, the probability that two clones coincidentally carry the same tag is <0.10–0.15, whereas the probability that three or more clones carry the same tag is <0.005; when ≤3 clones are labelled in an experiment, the probability that two clones carry the same tag is <0.05. The longitudinal dispersion separating the most widespread members of each clone was measured by multiplying the number of coronal sections separating the cells by the section thickness. Transverse dispersion was measured by superimposing tracings of cell position when clonally related cells were ≤0.5 mm apart longitudinally. When clonally related cells were dispersed longitudinally >0.5 mm, transverse dispersion was estimated by comparing the medial–lateral position of labelled sibling cells relative to the midpoint of the lateral ventricle. Data from one of these 18 experiments were also included in a previous analysis[1].

(average, 2.4 tags per hemisphere at E18; 2.3 tags per hemisphere at E20/21), cells bearing the same tag were frequently widely dispersed when analysed at E20/21. Forty-three per cent of the labelled clones (9 of 21) were dispersed over >500 μm (Table 1), and four of these clones were dispersed longitudinally over >2 mm, a distance equivalent to about half the length of the forebrain at this age (Fig. 1*c*, *d*). Dispersion continued postnatally: in clones analysed three days after birth (P3, counting the day of birth as P0), more than half of the labelled clones were widely dispersed (Table 1) and dispersion ranged up to 6 mm (Fig. 1*e*, *f*).

Further examination of labelled clones gave an indication of the routes taken by cells during rapid dispersion. Six clones (4 clones analysed at P3, two at E21) contained cells at multiple sites within the telencephalic VZ or SVZ, separated by up to 3.6 mm (average, 2.2 mm). Three of these clones contained cells in the cerebral cortex (Fig. 3*a*; Table 1); three others showed cells in proliferative regions of the cortex and olfactory bulb, where neurogenesis continues postnatally (Fig. 1*f*)[10]. These patterns suggest that substantial dispersion can occur before cortical cells exit the proliferative layers, as has been clearly illustrated *in vitro*[11]. Other dispersed clones contained cells in one site of the VZ or SVZ, with other cells in the IZ or CP (five clones; see Fig. 3*b*); five other clones were restricted to the CP or IZ (Fig. 3*b*).

Clonal dispersion longitudinally was on average twice as great as transverse dispersion at E21, which largely reflects 4 clones (19%) that were extremely widely dispersed longitudinally. Clones with large longitudinal dispersion were often oriented strikingly parallel to the lateral ventricle (Fig. 1*d*), perhaps reflecting rapid longitudinal dispersion in proliferative zones (Fig. 3*a*). Other longitudinal routes of migration, such as longitudinal migration along subcortical fibre tracts, may also occur, however.

Direct observation of migrating cells in ferret cortical slices[12] shows that some (~10%) cells migrate transversely within the IZ (see also refs 4, 13, 14). In our material, clones dispersed in the transverse (medial-lateral) plane as well as longitudinally. Although transverse dispersion was seen prenatally, it was more extensive postnatally (Fig. 1*e*, *f*), when many clones were dispersed in both transverse and longitudinal directions. Some of the transverse dispersion seemed to occur after cells left the proliferative zones and moved through the IZ, because it was more extensive after cortical neurogenesis was complete and it involved many cells in the CP (Fig. 3*c*; Table 1).

Transverse and longitudinal clonal dispersion can also be seen in animals analysed as adults, when most retrovirally labelled clones contain one or more neurons[1,15]. The decrease in frequency of widespread clones in adults (58% of all clones at P3; 24% in adults) may reflect lower efficiency of PCR analysis (inactivation of the histochemical marker, DNA degradation, poorer tissue lysis). Moreover cell death in the cerebral cortex, which occurs postnatally in rodents[16], may cause this decrease. Cell death, either random or selective for cells in widespread clones, would leave single cells or single clusters remaining from initially widespread clones.

The migration patterns shown by murine cortical cells 2–6 days after retroviral infection has been studied using a single lacZ-encoding retrovirus[8]. Cells were grouped according to geometric criteria, and statistical analysis indicated that most groups were probably clones. To compare patterns in rat and mouse, and to re-evaluate the criteria used to group murine cells, the same grouping criteria were applied to the present results using rat brains. At six days post-infection (E20/21), both the lumping of unrelated cells into groups and the splitting of sibling cells into different groups were very frequent. But dispersion patterns appear to be similar in the two species at shorter survival times, because the same geometric criteria accurately defined clones in rat brains analysed at E18.

As the retroviral library method detects the relative dispersion of sibling cells, not the movement of a single labelled cell, it cannot determine the precise age at which non-radial migrations begin. Cell dispersion may occur throughout neurogenesis. Widespread clones may only be detectable with the retroviral technique after several cell divisions of a moving progenitor cell have produced sibling cells at multiple locations. In this case, maximal rates of dispersion would be ~15 μm per hour (if cells dispersed ≥2 mm in 6 days). An alternative possibility is that cortical progenitor cells change their behaviour during development, so that most non-radial dispersion occurs in the later stages of neurogenesis at correspondingly faster rates (maximal rate ≥30 μm per hour). Although the roles and mechanisms of clonal dispersion are still being established, our results indicate that several cell types and stages of cortical development involve non-radial movements. □

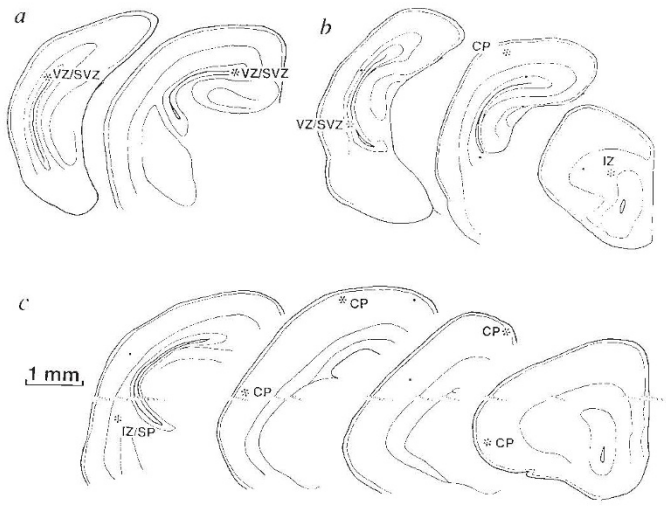

FIG. 3 Laminar locations of retrovirally labelled clones of cells analysed at E21 (*a*, *b*) or P3 (*c*). Asterisks indicate cells sharing the same viral tag. PCR analysis was successful in 40–70% of histochemically labelled cells; cells for which PCR was unsuccessful, or which were part of other clones, are indicated by dots. In *a*, two VZ/SVZ cells were separated longitudinally by 1.5 mm. In *b*, labelled cells were dispersed longitudinally by 3.1 mm. Cells of this clone (shown in red in Fig. 1*d*) occupied the CP and VZ/SVZ, and one cell was in either the IZ or the cortical subplate (SP)[17]. Note that it is difficult to calculate precisely the degree to which cells in this clone were dispersed transversely. At P3 (*c*), cells in the indicated clone (shown in red in Fig. 1*e*) were located in the CP, except one cell that was either in the IZ or SP, as indicated. Cells in this clone were also dispersed both transversely and longitudinally. Only some of the cells in each clone are illustrated owing to space constraints.

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1. Walsh, C. & Cepko, C. L. *Science* **255,** 434–440 (1992).
2. Kirkwood, T. B. L., Price, J. & Grove, E. A. *Science* **258,** 317 (1992).
3. Walsh, C. *et al. Science* **258,** 317–320 (1992).
4. Altman, J. & Bayer, S. A. *Neocort. Dev.* (Raven, New York, 1991).
5. The Boulder Committee *Anat. Rec.* **1666,** 257–262 (1970).
6. Rakic, P. *Science* **241,** 170–176 (1988).
7. Walsh, C. & Cepko, C. L. *Science* **241,** 1342–1345 (1988).
8. Austin, C. & Cepko, C. L. *Development* **110,** 713–732 (1990).
9. Luskin, M. B., Pearlman, A. & Sanes, J. R. *Neuron* **1,** 635–647 (1988).
10. Bayer, S. A. *Expl Brain. Res.* **50,** 329–340 (1983).
11. Fishell, G., Mason, C. A. & Hatten, M. E. *Nature* **362,** 636–638 (1993).
12. O'Rourke, N. *et al. Science* **258,** 299–302 (1992).
13. Rakic, P. *Trends Neurosci.* **4,** 184–187 (1981).
14. Misson, J.-P. *et al. Cerebr. Cort.* **1,** 221–231 (1991).
15. Grove, E. A., Kirkwood, T. B. L. & Price, J. *Neuron* **8,** 217–229 (1992).
16. Finlay, B. L. & Slattery, M. *Science* **219,** 1349–1351 (1983).
17. McConnell, S. K., Ghosh, A. & Shatz, C. J. *Science* **245,** 978–982 (1989).

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