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Dorsal Spinal Cord Neuroepithelium Generates Astrocytes but Not Oligodendrocytes

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used monoclonal antibody O4, which recognizes sulfatide and other antigens on oligodendrocyte precursors (Sommer and Schachner, 1981; Bansal et al., 1989), to

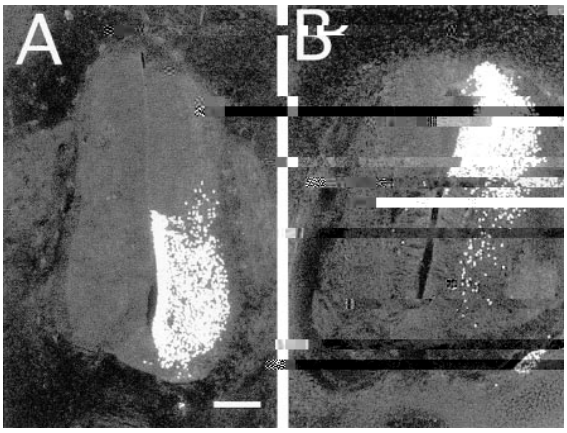

Figure 1. Chick–Quail Chimeras Fixed and Labeled with Antibody QCPN at E5 to Visualize Quail Cells

Quail tissue grafted on E1.5 into the ventral (A) or dorsal (B) chick neural tube incorporated seamlessly into the chimeric cord, and by E5 gross morphology was normal. There was little mixing of graft and host cells at this age, although there appeared to be directed migration of cells from the dorsal graft into ventral territory (B). Scale bar = 200 μm.

neural tube cells either alone or in coculture with quail dorsal or ventral cells. Oligodendrocytes always developed from ventral, but not dorsal, neural tube cells. Our data therefore support the bulk of other evidence for a localized ventral source of oligodendrocytes.

Our grafting experiments also provide evidence that dorsal neuroepithelium gives rise to astrocytes in the dorsal white matter but to no cells, glial or otherwise, in ventral white matter. Astrocytes in ventral white matter must therefore be derived from ventral neuroepithelium. In support of this, astrocytes developed in cultures of both dorsal and ventral neural tube cells. Therefore, there are at least two (and possibly many) sites in the spinal cord neuroepithelium that generate astrocytes, but there is only one source of oligodendrocytes.

integrated well and the chimeric cords were morphologically normal. The boundary between graft and host was usually close to the dorsoventral midpoint at E5. There was always a sharp boundary between graft and host, including the VZ, and little or no contribution to the contralateral side of the cord (Figure 1A). The length of the grafts ranged from 0.9 mm to 1.5 mm in the rostrocaudal direction. Four of the five grafts that we examined occupied half or less of the dorsoventral axis of the cord all along their lengths, while the fifth graft extended into dorsal territory for a short distance at its anterior end.

Oligodendrocytes Develop from Ventral Grafts

We allowed some chimeras to develop until E15–E18 in order to investigate oligodendrocyte development. We cut serial transverse sections through the spinal cord from the rostral toward the caudal end, labeling every tenth section with monoclonal QCPN to locate the graft. Only chimeras with a normal spinal cord morphology were analyzed further. An example of a chimera labeled on E18 is shown in Figure 2A. At this age, QCPN-labeled quail cells were present in all parts of the operated half of the cord, including the dorsal-most regions. The majority of quail cells could be classified as either cells with small nuclei, which were concentrated in white matter, or cells with larger nuclei, which were mainly restricted to ventral gray matter (Figure 2A). We presume that the former are glial cells and the latter, ventral neurons. A small number of quail cells had crossed into the contralateral side in the vicinity of the commissural tract beneath the central canal and more into the contralateral dorsal funiculus. It is clear that the QCPN-positive cells in the dorsal parts of the cord must have migrated there from the ventral graft through the host tissue.

In addition, all the ependymal cells lining the lumen of the cord on the operated side were QCPN positive. There were many chick (i.e., QCPN negative) neurons in the dorsal gray matter of the chimeric cord that must have developed earlier from chick precursors in the VZ

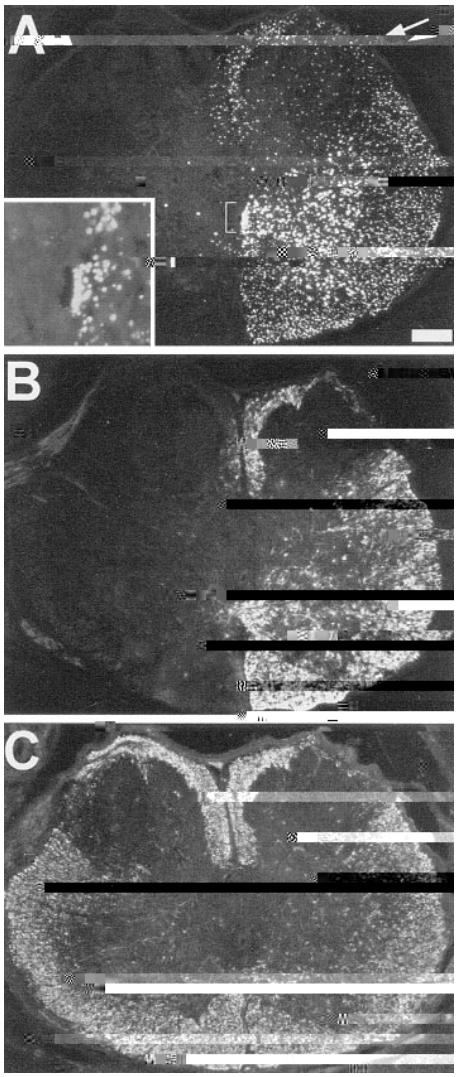

Figure 2. A Quail Ventral Graft Analyzed on E18

Three consecutive sections through the chimeric region of the cord

oligodendrocytes develop from ventral neural tube, in agreement with previous evidence (see Introduction) and consistent with the experiments of Cameron-Curry and Le Douarin (1995). The patterns of SMP and MBP labeling on the operated side were very similar even in the dorsal-most white matter, indicating that ventrally derived quail oligodendrocytes have no preference for specific axon tracts. Their presence in the dorsal funiculus (Figures 2B and 3E–3H) is a clear demonstration that oligodendrocytes or, more likely, their progenitors can migrate from ventral to dorsal.

Long-Range Longitudinal Migration of Oligodendrocyte Progenitors

To assess the ability of oligodendrocyte lineage cells to migrate in the longitudinal direction, we examined sections outside of the body of the ventral quail grafts. We defined the ends of the graft as the last sections to contain QCPN-labeled neurons and ependymal cells, which did not seem to spread significantly into host tissue as they were lost abruptly over a distance of about 150 μm at both the rostral and caudal ends of the graft (Figures 3A and 3B). However, quail oligodendrocytes were present in the white matter more than 2 mm beyond the ends of the graft (Figures 3D and 3H). This shows that oligodendrocyte lineage cells (presumably progenitors) can migrate long distances along axon tracts during normal development. The furthest-migrating cells were preferentially located in the dorsal and lateral fiber tracts (Figures 3D and 3H).

Dorsal Neural Tube Grafts

We also grafted segments of dorsal E1.5 quail neural tube into equivalent regions of E1.5 chick embryos (dorsal grafts) below the ninth somite. We fixed and examined some of the grafts at E5–E7.5 (chick stages 27–32). We cut serial 15 μm transverse sections through the chimeric spinal cords and labeled every tenth section with QCPN to reveal quail cells. Only grafts that had integrated well into the host, giving rise to a morphologi-

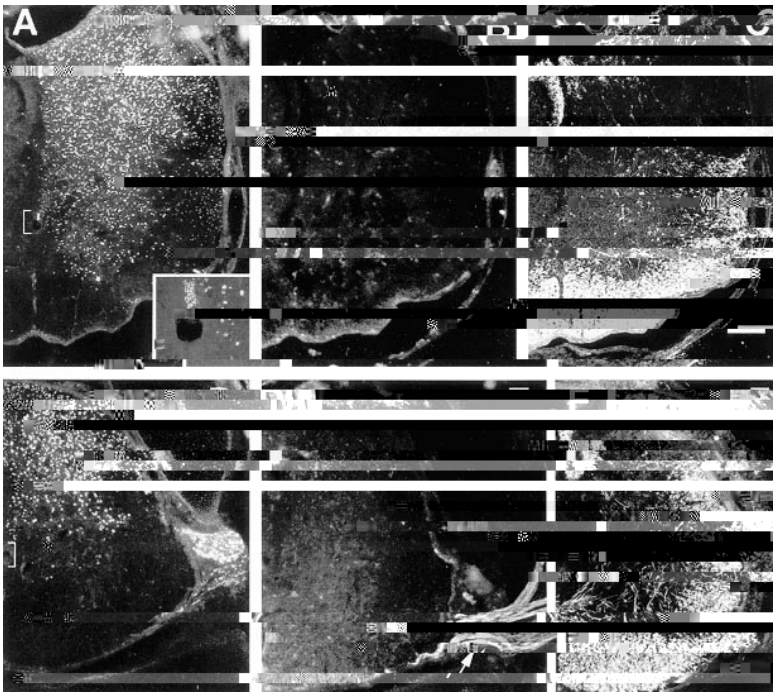

Figure 4. Dorsal Quail Grafts Analyzed on E18

Two different chimeric spinal cords are shown ([A–C] and [D–F], respectively). Consecutive or nearby sections (not more than 100 μm apart) were labeled with antibody QCPN (A and D), anti-SMP (B and E), or anti-MBP (C and F). Both dorsal grafts gave rise to many quail neurons in the dorsal region of the cord (A and D). The position of the spinal cord lumen is indicated by a square bracket in (A) and (D). The graft shown in (A), which descended further ventral than that in (D), gave rise to a small number of neuroepithelial precursors at the midline above the lumen ([A], inset), unlike the graft of (D), which did not generate any midline cells. Neither graft contributed to ependymal cells around the open lumen. Unlike ventral grafts, no SMP-positive (quail) oligodendrocytes were generated by dorsal grafts (B and E). Note that neural crest–derived peripheral quail myelin labeled with anti-SMP, providing a control for antibody labeling ([E], arrow). Anti-MBP labeled chick myelin in all parts of the white matter (C and F). We conclude that oligodendrocytes do not arise from dorsal neuroepithelium. Scale bars = 100 μm.

Cameron-Curry and Le Douarin (1995): the criterion they used to define a "dorsal" quail graft—i.e., the presence of quail ependymal cells around the dorsal part of the lumen at E15—indicates that the dorsal grafts they de-

earlier ages when precursor cells were being specified (see Discussion).

Further compelling evidence that the ependymal lining of the mature spinal cord lumen is derived from only the

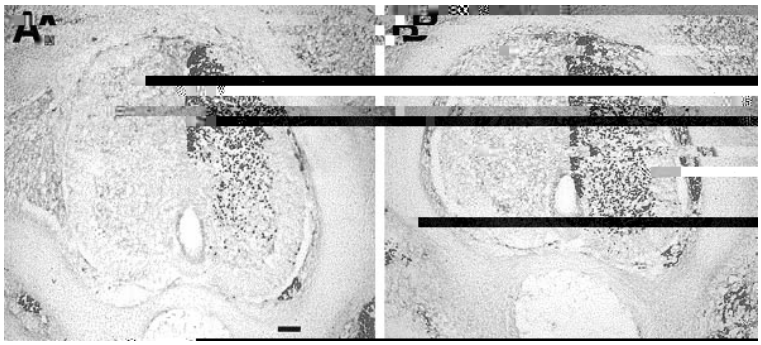

Figure 6. Dorsal Quail Graft Analyzed on E9

(A and B) illustrate different parts of the same dorsal quail graft. The sections are immunolabeled with antibody QCPN to visualize quail cells. This was a wedge-shaped graft that descended deep into ventral territory at one point along its length (B). Note the densely labeling VZ cells at the midline (diaminobenzidine reaction product). In (A) the graft covers around half of the VZ but does not come close to the open lumen; in (B) the graft covers about nine-tenths of the VZ, including the part that surrounds the dorsal half of the open lumen. By reference to the open lumen, therefore, the graft in (B) could perhaps be described as "dorsal" (Cameron-Curry and Le Douarin, 1995), although in our opinion only grafts such as that in (A) are truly dorsal. This distinction probably underlies the different conclusions drawn by ourselves (this paper) and Cameron-Curry and Le Douarin (1995) regarding

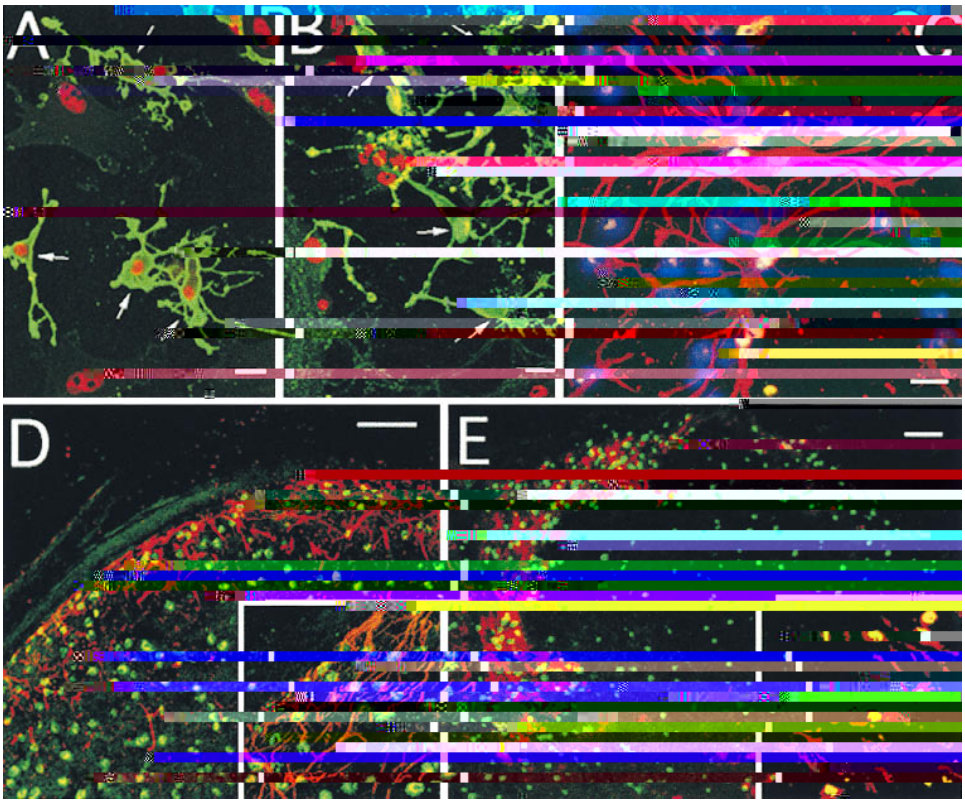

Figure 7. Quail Glial Cells in Culture and in Chimeric Spinal Cords In Vivo

Table 1. Cocultures of Chick and Quail Spinal Cord Cells

Experiment	Culture Type	Total Cells Counted	Quail Cells	Chick OLs	Quail OLs	Ventral OLs % of Total
1	CV + QD QV +	720	328 (45%)	223	1	>99%

sometimes called the dorsal glial septum (Böhme, 1988). Later, they disappear altogether, possibly by programmed cell death (Isomura et al., 1986). In any case, the central canal and its associated VZ at ages after E12 are only ventral remnants of what was present earlier, before E8.

Cameron-Curry and Le Douarin (1995) defined a dorsites within the VZ remains to be seen. Miller and Szigeti (1991) have described several different morphological varieties of astrocytes in spinal cord cultures, but it is not known whether these represent different lineages, possibly specified in different parts of the VZ, or whether they have distinct functions or locations in the intact

quail. For immunolabeling, it was diluted 1000-fold in PBS containing

O1, O4 and R-mAb used in the analysis of oligodendrocyte develop-

of notochord and floor plate grafts, and of sonic hedgehog. Mech. Dev. *60*, 13–32.

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