Identification of an adult-specific glial progenitor cell

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Summary

We have found that glial progenitor cells isolated from the optic nerves of adult rats are fundamentally different from their counterparts in perinatal animals. In our studies on bipotential oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells, we have seen that O-2A² adult progenitor cells can be distinguished from O-2A I perinatal progenitors by their morphology and antigenic phenotype, their much longer cell cycle time (65 h versus 18 h), slower rate of migration rate (4 μm h⁻¹ versus 21 μm h⁻¹), and their time course of differentiation into oligodendrocytes or type-2 astrocytes in vitro (≤3 days versus >5 days). At least some of the differences between O-2A² adult and O-2A I perinatal progenitor cells appear to be clearly related to the differing cellular requirements of the adult and perinatal central nervous system (CNS).

The properties of the O-2A² adult progenitor cells may make these cells ideally suited for the needs of the adult CNS, where rapid exponential increases in the number of oligodendrocytes and O-2A progenitor cells would be inappropriate. However, the properties of the O-2A² adult progenitor cells are such that they may not be able to replace oligodendrocytes in sufficient numbers to repair extensive or recurrent damage in the adult brain, such as in patients suffering from the human demyelinating disease multiple sclerosis. Moreover, available information about other tissues suggests that the transition from perinatal to adult progenitor cell types may represent a developmental mechanism of general importance.

Key words: adult, astrocyte, cell cycle, central nervous system, development, differentiation, glia, multiple sclerosis, oligodendrocyte, optic nerve, progenitor, remyelination.

Introduction

The development of a multicellular organism is in many ways dependent upon the rapid division of tissue-specific precursor cells which, through differentiation and sometimes through further cell division, generate the specialized cell types that make up the various tissues of the body. In contrast, in most tissues of adult animals, generation of new differentiated cells from precursor cells only occurs as a part of maintaining homeostasis, e.g. to compensate for naturally occurring cell death, or in response to injury. The mechanisms that control proliferation of precursor cells in developing and adult animals may therefore be very different. Moreover, although similar cell types may be produced in adult animals and during embryogenesis, fetal and adult precursor cells and their differentiated progeny may differ in at least some properties. For example, the generation of fetal versus adult forms of haemoglobin by erythrocytes appears to be determined at the level of the haematopoietic stem cells (Wood et al. 1985, 1988), suggesting that fetal and adult haematopoietic stem cells differ from each other and are specialized in ways that reflect the differing physiological requirements of fetal and adult animals.

Despite the obvious importance of understanding the properties of the precursor cells that function during development and in the adult animal, relatively little is known about these cells or about the mechanisms that control their division and differentiation. Attempts to understand the biological properties of precursor cells in general, and of adult-specific precursor cells in particular, have been greatly hampered by the inability to identify unambiguously most precursor cells or to grow these cells in conditions that would allow a detailed analysis of their properties. Thus, it has not yet even been possible to distinguish directly and unambiguously adult from perinatal precursor cells either during development or in vitro.

One of the few cell types in which the properties of fetal and adult cells with precursor function can be directly compared is the oligodendrocyte-type-2 astrocyte (O-2A) progenitor cell of the rat optic nerve (Raff et al. 1985b). These bipotential progenitor cells give rise to two of the major cell types of the optic nerve: oligodendrocytes, the myelin-forming cells of the central nervous system (CNS) and type-2 astrocytes, which enwrap bare axons in astrocytic processes at the nodal gaps between adjacent myelin sheaths (ffrench-Constat & Raff, 1986b). O-2A progenitor cells can be
readily identified and grown in tissue culture, where they can be induced to divide and differentiate in response to either specific cell–cell interactions or defined mitogens or differentiation-inducing factors (Raff et al. 1983b; Noble & Murray, 1984; Hughes & Raff, 1987; Noble et al. 1988; Raff et al. 1988; Richardson et al. 1988; Hughes et al. 1988; Lillien et al. 1988). Although it is the O-2A progenitor cells from the optic nerves of perinatal rats which have been the most extensively examined, studies in our laboratory (G. Wolswijk, unpublished observations), and independently by french-Constant & Raff (1986a), have demonstrated that O-2A progenitor cells can be isolated from the optic nerves of adult rats.

In the course of our studies on O-2A progenitor cells of the optic nerve of adult rats, it has become clear that these cells are fundamentally different from O-2A progenitor cells isolated from optic nerves of perinatal animals. In this paper, we present evidence that O-2A progenitors isolated from the optic nerves of adult rats (O-2A<sup>adult</sup>) differ from their perinatal counterparts (O-2A<sup>perinata</sup>) in antigenic phenotype, morphology, rate of migration, cell cycle time and time course of differentiation into either oligodendrocytes or type-2 astrocytes in vitro. The properties of the O-2A<sup>adult</sup> progenitor cells may make these cells ideally suited for the needs of the adult CNS.

Materials and methods

Preparation of monolayers of purified type-1 astrocytes

Monolayers of purified type-1 astrocytes were prepared by modifications of previously described methods (Noble et al. 1984; Noble & Murray, 1984). Dissociated cells from the cerebral cortex of newborn or 1-day-old Sprague Dawley rats were plated at a density of 2 brains per poly-l-lysine-coated (PLL; Sigma: 175 000 M<sub>r</sub>, 20 μg ml<sup>−1</sup>) NUNC tissue culture flask (85 cm<sup>2</sup> surface area) and grown in Dulbecco’s modified Eagle’s medium containing 4.5 g l<sup>−1</sup> glucose (DMEM; Imperial Laboratories) and supplemented with 10% heat-inactivated fetal calf serum (FCS; Imperial Laboratories), 2 mm-glutamine (Sigma) and 25 μg ml<sup>−1</sup> gentamicin (Flow Laboratories) (DMEM + 10% FCS). Once a confluent monolayer of flat cells had been formed (usually after 7–10 days), the layer of process-bearing cells growing on top of the flat cells was removed by shaking the flasks overnight on a rotary platform (100 revs min<sup>−1</sup>) at 37°C. Cultures were allowed to recover for 20 min. The suspension was centrifuged (5 min, 500 g), the supernatant removed and the tissue was then resuspended with an equal volume of 30 000 i.u. ml<sup>−1</sup> trypsin in DMEM-CMF was added and incubation at 37°C was continued for another 20 min. The suspension was centrifuged (5 min, 100 g), the supernatant removed and the tissue was then resuspended and further incubated in a solution of 15 000 i.u. ml<sup>−1</sup> trypsin and 0.27 mM-EDTA in DMEM-CMF for 20 min. The digestion with trypsin was terminated by addition of an equal volume of SBTI-DNAs, followed by an incubation at 37°C for 10–20 min and a short centrifugation (500 g, 5 min). The supernatant was replaced with the medium appropriate for any given experiment, followed by trituration of the tissue through a 5 ml blow-out pipette and 25 G and 27 G hypodermic needles. 100 μl of the resulting cell suspension was plated onto either PLL-coated coverslips or coverslips coated with monolayers of type-1 astrocytes (as in Noble & Murray, 1984). The cells derived from 1 pair of adult optic nerves were usually plated onto 8–15 coverslips, while optic nerve cells derived from newborn to 7-day-old animals were plated at a density of 3000–4000 cells per coverslip. When the adult optic nerve cells were plated onto PLL-coated coverslips, cells were kept in DMEM + 10% FCS for 3–4 h, rinsed with L-15 medium [to remove debris and myelin] and placed in Falcon 6-well trays (3–4 coverslips per well). Cells were either fed with defined medium [DMEM containing 4.5 g l<sup>−1</sup> glucose and supplemented with 25 μg ml<sup>−1</sup> gentamicin, 2 mm-glutamine, 0.234 i.u. ml<sup>−1</sup> bovine pancreas insulin (Sigma), 100 μg ml<sup>−1</sup> human transferrin (Sigma), 0.0286% (v/v) BSA pathocyte (Miles Laboratories, Inc), 0.2 μM-progesterone (Sigma), 0.1 μM-putrescine (Sigma), 0.45 μM-l-thyroxine (Sigma), 0.224 μM-selenium (Sigma) and 0.49 μM 3,3',5-
Characterization of O-2A<sub>adult</sub> progenitor cells

triiodo-l-thyronine (Sigma); modified from Bottenstein & Satо, 1979] (DMEM-BS), or fed with 50% fresh and 50% type-1 astrocyte-conditioned DMEM-BS supplemented with 0.5% FCS (DMEM-BS/0.5%FCS) or fed with DMEM + 10% FCS. Optic nerve cells that were plated directly onto type-1 astrocyte monolayers were kept overnight on raised platforms before being rinsed with L-15 medium and placed in 50% conditioned and 50% fresh DMEM-BS/0.5% FCS. In some experiments, adult optic nerve cells were plated into 85 cm<sup>2</sup> Nunc tissue culture flasks that had been coated previously with 1 x 10<sup>6</sup> purified type-1 astrocytes and fed with 50% fresh and 50% type-1 astrocyte-conditioned DMEM-BS/0.5% FCS. Two thirds of the culture medium was changed 2–3 times a week. Live cells were photographed through a Leitz inverted microscope using Kodak Tri-X-pan 125 ASA films.

**Indirect immunofluorescence**

To identify the cell types of interest, cultures were immunolabelled with various antibodies. Cells were incubated at room temperature in 40–50 μl of each antibody solution for 20–30 min, followed by an incubation in the appropriate rhodamine- or fluorescein-conjugated second antibody solution. Antibodies were diluted in Hank’s balanced salt solution (Imperial Laboratories) containing 5% heat-inactivated bovine donor calf serum (Imperial Laboratories) and 0.02 mM Hepes (Sigma) (HBSS-5% DCS). After every incubation, cells were washed several times with HBSS-5% DCS. Surface antigens (A2B5, O4, NSP-4 and GalC) were visualized on living cells, intracellular antigens [i.e. fibronectin and all classes of intermediate filaments including glial fibrillary acidic protein (GFAP) and vimentin] after fixation in methanol at −20°C for 10 min. Cultures were immunolabelled with mouse IgM monoclonal antibody A2B5 (ascites, 1:1000), mouse IgM monoclonal O1 and O4 (concentrated hybridoma supernatants, 1:100; Sommer & Schachner, 1981), mouse IgM monoclonal antibody NSP-4 (hybridoma supernatant, 1:1; Rougon et al. 1983), mouse IgG3 anti-GalC monoclonal antibody (hybridoma supernatant, 1:10), rabbit anti-fibronectin antisera (1:1000; Price & Hynes, 1985), rabbit anti-GFAP antiserum (1:1000; Pruss, 1979), mouse IgG1 anti-vimentin intermediate filament monoclonal antibody (used at a concentration of 4 μg ml<sup>−1</sup>, Boehringer Mannheim), and the mouse IgG1 monoclonal antibody called intermediate filament antigen (IFA; fresh undiluted hybridoma supernatant; Pruss et al. 1981). All rhodamine- and fluorescein-conjugated class-specific anti-mouse immunoglobulin (Ig) second antibodies were purchased from Southern Biotechnology Associates, Inc., USA and diluted 1:100 prior to use. The binding of A2B5, NSP-4, O1 and O4 antibodies was detected with either fluorescein- or rhodamine-conjugated goat anti-mouse IgM (anti-IgM-F/Fl/Rd), anti-GalC antibody with goat anti-mouse IgG3-Fl or goat anti-mouse IgG3-Rd, anti-vimentin and IFA monoclonal antibodies with goat anti-mouse IgG1-Fl and the binding of the rabbit anti-GFAP and anti-fibronectin antibodies with sheep anti-rabbit Ig-Fl (Wellcome, 1:100). In stainings involving both A2B5 and O4 (both mouse IgM monoclonals), cells were sequentially labelled with A2B5 and anti-IgM-Rd, followed by O4 and anti-IgM-Fl or vice versa. The first staining method revealed cells that expressed O4 and not A2B5 [i.e. mature oligodendrocytes (Sommer et al. in preparation)], the latter method, cells that were A2B5*O4−. In some experiments, cultures growing on PLL-coated coverslips were incubated simultaneously with the A2B5 and the anti-GalC antibodies, followed by anti-IgM-Rd and anti-IgG3-Fl. Cells were then fixed and labelled sequentially with anti-GFAP antibodies and anti-rabbit Ig-Fl. In such experiments, cells that were surface fluorescein<sup>+</sup> (i.e. were GalC<sup>−</sup>) were easily distinguishable from cells that were internally labelled by the fluorescein conjugate (i.e. were GFAP<sup>+</sup>). After the immunolabelling, coverslips were washed, mounted in a drop of glycerol containing 22 mM-1,4-diazobicyclo[2,2,2] octane (Sigma) to prevent fading (Johnson et al. 1982; Davidson & Goodwin, 1983), sealed with transparent nail varnish and examined in a Zeiss Universal microscope equipped with phase contrast, epifluorescence, and rhodamine and fluorescein optics and equipped for photography. All immunolabelled cells in cultures of adult optic nerve cells were counted; in cultures of newborn optic nerves cells, at least 200 immunolabelled cells were scored per coverslip in each experiment. Immunolabelled cells were photographed using Kodak-Tri-X-pan 400 ASA films, while their corresponding phase-contrast image was recorded using Ilford XP1 400 films.

**Autoradiography**

For in vitro autoradiography experiments, dissociated optic nerve cells were plated onto monolayers of type-1 astrocytes, grown for 2 days in DMEM-BS/0.5% FCS and exposed to 2 μCi ml<sup>−1</sup> [3H]thymidine (Amersham, 2 μCi mmol<sup>−1</sup>) for 20h. After the radiolabelling period, cells were immunolabelled, fixed, dehydrated in ethanol, air-dried, and mounted face up on microscope slides (with Gurr fluoromount mountant, BDH Chemicals Ltd). Slides were then coated with Ilford L4 autoradiographic emulsion, which had been diluted with an equal volume of distilled water and kept at 45°C, and exposed for 2 days in a light-tight box at 4°C. After development with Ilford contrast FF and fixation, cultures were covered with a coverslip and sealed with nail varnish. Cells with more than 5 silver grains above their nucleus were scored as cells that had taken up [3H]thymidine during the pulse.

**Complement-mediated cytolysis of adult optic nerve cells**

After 1 day in culture, coverslips with adult optic nerve cells growing on monolayers of type-1 astrocytes were placed onto raised platforms and incubated in 100 μl culture medium containing agarose-absorbed rabbit complement plus either A2B5, O4 and anti-GalC monoclonal or O4 and anti-GalC antibodies. After 30 min at 37°C, cultures were washed with L-15 and placed back into the original culture medium. Effectiveness of the complement kill was checked by immunolabelling with appropriate antibodies directly following the incubation with complement and antibodies.

**Analysis of the expansion of O-2A progenitor cell colonies in adult optic nerve cultures**

Dissociated cells derived from one pair of adult optic nerves were plated into 85 cm<sup>2</sup> Nunc tissue culture flask coated with 1 x 10<sup>6</sup> purified type-1 astrocytes and were grown in DMEM-BS/0.5% FCS. From day 2 onwards, cultures were screened for well-isolated colonies of O-2A progenitor-like cells. When colonies were found, their location was marked and their expansion was then followed daily. As the flask contained fewer than 20 colonies of O-2A progenitor-like cells, it is likely that each colony was derived from a single cell. The appearance of multipolar oligodendrocyte-like cells in these colonies confirmed that the colonies that were followed contained O-2A progenitor cells.

**Time-lapse microcinematography**

For migration studies, dissociated optic nerve cells of adult rats were plated in the centre of PLL-coated 6 cm Nunc Petri
The oligodendrocyte-type-2 astrocyte lineage

In vitro studies have shown that the optic nerve of perinatal rats contain two glial lineages: one lineage gives rise to both oligodendrocytes and type-2 astrocytes, the other to type-1 astrocytes. The various perinatal optic-nerve-derived glial cells can be identified unambiguously by their morphology and antigenic phenotype (see text for specific details).

Results

Identification of glial cells derived from the optic nerves of perinatal rats

Cultures prepared from embryonic and postnatal rat optic nerve contain members from the oligodendrocyte-type-2 astrocyte (O-2A) lineage and the type-1 astrocyte lineage, the two glial lineages of the rat optic nerve (see Fig. 1 for summary).

O-2A progenitor cells, the precursors to both oligodendrocytes and type-2 astrocytes, have a characteristic bipolar morphology (Temple & Raff, 1986; Small et al. 1987) and have been identified antigenically as cells that are labelled with the monoclonal antibodies A2B5 and NSP-4, but that express neither galactocerebroside [GalC: a specific marker for oligodendrocytes (Raff et al. 1978)] nor glial fibrillary acidic protein [GFAP: a protein specifically expressed by astrocytes (Bignami et al. 1972)] (Raff et al. 1983a; Raff et al. 1985; ffrench-Constant & Raff, 1986a). In addition, O-2A progenitors derived from the optic nerves of perinatal rats contain vimentin intermediate filaments (IFs) (Raff et al. 1984a). O-2A progenitor cells also start expressing an antigen recognized by a monoclonal antibody, called O4 (Sommer & Schachner, 1981), shortly before birth; 7 days after birth more than 95% of the O-2A progenitor cells are O4+ in vivo (Sommer et al. in preparation).

When O-2A progenitors differentiate into oligodendrocytes, they acquire a multipolar morphology, express GalC and gradually lose vimentin filaments and the ability to bind the monoclonal antibody A2B5. In contrast, O-2A progenitor cells differentiating into type-2 astrocytes remain A2B5+NSP-4+ and acquire GFAP filaments. Type-2 astrocytes, like oligodendrocytes, also have a multipolar morphology.

Type-1 astrocytes have a fibroblast-like morphology and label with the Ran-2 monoclonal antibody (Bartlett et al. 1981), express GFAP and do not label with the A2B5 and NSP-4 antibodies. Their precursor cells, which are also flat cells, are Ran-2+GFAP-
Fig. 2. O-2A progenitor cells isolated from the optic nerves of adult rats are A2B5+ and O4+ and do not express vimentin or any other intermediate filament proteins. Optic nerve cells from adult rats were plated onto PLL-coated coverslips, grown overnight in 50% fresh and 50% type-1 astrocyte-conditioned DMEM-B5/0.5% FCS and then immunolabelled with O4 (C), A2B5 (F, I), anti-GalC (B), anti-vimentin (E) or monoclonal antibodies against the IFA antigen (which is shared by all classes of intermediate filaments (Pruss et al. 1981)) (H). Most of the O-2A progenitor cells (indicated with arrows) derived from the optic nerves of adult animals are A2B5+O4+GalC− and lack intermediate filaments, including vimentin, A, D, G, phase-contrast; B, E, H, fluorescein; C, F, I, rhodamine optics. Bar in A, 100 μm.


O-2A progenitor cells from the optic nerves of adult and perinatal rats can be distinguished antigenically from each other

The first question we addressed was whether we could use antibodies to distinguish adult O-2A progenitor cells from their counterparts in perinatal optic nerve cultures. Although these two populations express many similar antigens, we found differences in the expression of intermediate filament proteins and in the expression of a cell surface antigen recognized by the O4 monoclonal antibody (Sommer & Schachner, 1981).

O-2A progenitor cells isolated from the optic nerves of adult rats, like their perinatal counterparts, are A2B5+ and do not express GalC or GFAP unless induced to differentiate into oligodendrocytes or type-2 astrocytes, respectively (ffrench-Constant & Raff, 1986a; G. Wolswijk, unpublished observations). Per pair of adult nerves, we obtained between 300 and 700 cells (average: 505 ± 117) that were labelled with the A2B5 monoclonal antibody, although not so heavily as O-2A progenitor cells derived from perinatal optic nerves, but which were not labelled with anti-GalC and anti-GFAP antibodies. Immunolabelling of such cells with the NSP-4 antibody revealed that these cells, like their counterparts in perinatal optic nerve cultures (ffrench-Constant & Raff, 1986b), were also NSP-4+ (not shown). Approximately 11% of the O-2A lineage cells isolated from the optic nerves of adult rats expressed GalC and none were A2B5−GFAP− type-2 astrocytes after 1 day of in vitro growth.

When cultures of adult optic nerve cells growing on type-1 astrocyte monolayers were immunolabelled with the O4 monoclonal antibody and with anti-GalC antibodies, the number of O4+GalC−GFAP− cells was very similar to that of the number of A2B5+GalC−GFAP− cells after 1 day in culture, suggesting that many O-2A progenitor cells were also O4+. Direct double-labelling analysis confirmed that >97% of the O-2A progenitors found after 1 day of in vitro growth were O4+ (Table 1; Fig. 2).

In contrast to the virtually ubiquitous expression of vimentin IFs in both O4− and O4+ O-2A progenitor cells isolated from perinatal rats (Wolswijk & Noble, in preparation), none of the O4+ cells in cultures of adult optic nerve cells, growing on glass coverslips and immunolabelled 1 day after plating, contained vimentin IFs (Fig. 2; Table 1). Again unlike perinatal O-2A progenitor cells, the few A2B5+O4− cells derived from adult optic nerve were vimentin+. In addition, the adult optic nerve-derived O-2A progenitor cells could not be labelled with the anti-intermediate filament antigen (IFA) monoclonal antibody that recognizes a determinant shared by all classes of intermediate filaments
Table 1. Antigenic phenotype of O-2A progenitor cells in cultures of newborn and adult optic nerve cells after 1 day of in vitro growth

<table>
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<tr>
<th>Antigenic phenotype of O-2A progenitor cell (A2B5+GalC-GFAP+)</th>
<th>% total O-2A progenitor population in each phenotype category</th>
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<td></td>
<td>Newborn optic nerves</td>
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<td>O4+ vimentin+</td>
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Optic nerve cells derived from the optic nerves of either newborn or adult animals were plated onto coverslips, grown for 1 day in type-1 astrocyte-conditioned DMEM-BS/0.5%FCS and then immunolabelled with various antibodies as described in Materials and Methods. After one day in vitro, virtually none of the A2B5+ optic nerve cells expressed either GalC or GFAP; thus, these cells appeared to be O-2A progenitors. The values are the mean ± S.E.M. of three separate isolations. The adult optic nerve cultures also contained some GalC+ oligodendrocytes, a few GFAP+A2B5- type-1 astrocyte-like cells and variable numbers of fibronectin+, vimentin+, fibroblast-like cells.

(Pruss et al. 1981), suggesting that the adult O-2A progenitor cells express no intermediate filament proteins (Fig. 2). In contrast, virtually all O-2A progenitors from newborn to postnatal day 7 (P7) optic nerves were IFA+ in vitro (Fig. 3).

O-2A progenitor cells from the optic nerves of adult rats differ morphologically from those isolated from perinatal animals

In addition to revealing antigenic differences between adult and perinatal O-2A progenitors, the immunolabelling experiments also indicated that these cells differed morphologically from each other.

As reported previously, O-2A progenitor cells derived from the optic nerves of perinatal rats have a characteristic bipolar morphology when grown in vitro (Temple & Raff, 1986; Small et al. 1987; see also Fig. 3 and Fig. 4). Because of their morphology, they are easily distinguishable from oligodendrocytes and type-2 astrocytes, which both have a multipolar morphology, and from type-1 astrocytes, which have a fibroblast-like morphology (Raff et al. 1983a,b). Virtually all of the bipolar cells found in cultures of newborn and P7 optic nerve cells growing on type-1 astrocytes or in type-1 astrocyte-conditioned medium (Astro-CM) appear to be O-2A progenitor cells (Temple & Raff, 1986; Small et al. 1987). Most of these bipolar cells are O4- and all are A2B5+ in vitro (Sommer et al. in preparation). The perinatal O-2A progenitors that are O4+ when grown in similar conditions are multipolar with several thin processes arranged in a radially symmetric manner (Sommer et al. in preparation).

In contrast to the bipolar morphology of A2B5+O4- O-2A progenitor cells found in cultures of perinatal optic nerve cells, over 65% of the O-2A progenitor cells isolated from the optic nerves of adult animals had one major branched process plus several smaller thinner processes when grown on type-1 astrocytes or in Astro-CM for 3 days and could be described as unipolar cells (Fig. 4). Those adult-derived O-2A progenitor cells that were not unipolar were generally multipolar and resembled the multipolar O4+ O-2A progenitor cells found in cultures of perinatal rat optic nerve cells.

O-2A progenitor cells derived from the optic nerves of perinatal rats have a cell cycle of <24 h, while O-2A progenitor cells derived from adult optic nerves have a cell cycle of about 65 h

In previous studies, the ability to identify unambiguously O-2A progenitor cells derived from perinatal animals allowed us to determine that proliferation of these cells is stimulated in vitro by factor(s) secreted by type-1 astrocytes (Noble & Murray, 1984; Raff et al. 1985). When O-2A progenitor cells derived from peri-

Fig. 3. O-2A progenitor cells from the optic nerves of perinatal rats are IFA+ in vitro. Dissociated cells derived from the optic nerves of 1-day-old rats were plated onto PLL-coated coverslips and grown overnight in the presence of DMEM-BS/0.5%FCS previously conditioned by type-1 astrocytes. Cultures were then double immunolabelled with the mouse monoclonal antibodies A2B5 (A) and the IFA antibody (B). First-layer antibodies were visualized by labelling with class-specific fluorescein- and rhodamine-coupled secondary antibodies. The figure illustrates that O-2A progenitor cells isolated from the optic nerves of 1-day-old rats are IFA+. Similar staining was observed in O-2A progenitor cells derived from newborn to P7 optic nerves. Two type-1 astrocyte-like, IFA+A2B5- cells are indicated with an arrow. Cultures were examined using rhodamine (A), fluorescein (B) and phase-contrast (C) optics. Bar in C, 50 μm.
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Fig. 4. O-2A progenitor cells derived from newborn optic nerves are mostly bipolar, whereas those isolated from adult nerves are mostly unipolar. Optic nerve cells were grown for 3 days in type-I astrocyte-conditioned DMEM-BS/0.5%FCS and photographed, when still alive, through an inverted microscope. Immunolabelling afterwards confirmed that the newborn optic nerve cells (A,B) and the adult optic nerve cells (C,D) were A2B5+ GalC− O-2A progenitor cells. Bar in D, 50 μm.

Natal optic nerves were grown on monolayers of type-I astrocytes or in the presence of Astro-CM, their numbers doubled approximately every day, corresponding with an average cell cycle time of <24 h (Noble & Murray, 1984; Temple & Raff, 1986; Noble et al. 1988).

When adult optic nerve cells were grown on type-I astrocytes in a chemically defined medium supplemented with 0.5% fetal calf serum (DMEM-BS/0.5%FCS), the number of O-2A lineage cells increased much more slowly than has been seen for populations of perinatal O-2A lineage cells. In a representative experiment with adult optic-nerve-derived cells shown in Fig. 5, the number of O-2A lineage cells (mostly O-2A progenitor cells and oligodendrocytes) increased just over fourfold between day 1 and day 7, corresponding to a doubling time for the total O-2A lineage population of 70 h (average for 3 separate experiments: 64±5 h). In addition, only about 40% of adult O-2A progenitor cells took up radiolabelled thymidine in a 20 h pulse (applied after 2 days in vitro) as compared with 75% of perinatal O-2A progenitors in newborn optic nerve cultures (Table 2).

Two lines of evidence indicated that the lower labelling index of adult optic-nerve-derived O-2A progenitors was due to a long cycle time. (1) The vast majority of the O-2A progenitor cells proliferated when grown on monolayers of type-I astrocytes. When we determined the proportion of the adult O-2A progenitor cells present on day 1 that had formed colonies of 2 or more cells after 14 days, we found that 65–85% of the O-2A progenitors had proliferated and produced colonies ranging in size from 2 to 36 cells. (2) When the expansion of individual colonies of O-2A progenitor-like cells was followed on a daily basis, the colonies initially expanded with an average doubling time of 62±10 h (n=14) (Fig. 6). With the appearance of multipolar cells (after 7–10 days in vitro), which most likely were oligodendrocytes, the doubling time of a colony decreased gradually. Time-lapse microcinematography experiments involving one of these colonies (colony N in Fig. 6) showed that cells with the unipolar morphology of adult optic-nerve-derived O-2A progenitor cells divided every 65±18 h (n=24) when followed from day 11 to day 21. In contrast, bipolar O-2A progenitor cells present in cultures of perinatal optic nerves divided every 18±4 h when grown under similar conditions (Noble et al. 1988).

Adult O-2A progenitor cells that proliferate in vitro are O4+

A further distinction between perinatal and O-2A progenitor cells is in the antigens expressed by dividing O-2A progenitors. While the majority of the proliferating perinatal O-2A progenitor cells were A2B5+O4− in vitro, the majority of the adult O-2A progenitors expressed O4 labelling. In the autoradiography experiments described in an earlier section, we found that about 92% of the O-2A progenitor cells that took up [3H]thymidine in optic nerve cell cultures of newborn rats were A2B5+O4−, while about 97% of the proliferating O-2A progenitor cells in cultures of adult optic nerve were O4+ (Table 2). Furthermore, in contrast to the widespread uptake of [3H]thymidine by O4+ O-2A progenitor cells derived from adult optic nerves, less than 10% of the O4+ O-2A progenitors had done so in cultures derived from the optic nerves of newborn rats.

As reported previously in studies on perinatal optic nerve cells (Noble & Murray, 1984), GalC+ oligodendrocytes present in both the newborn and the adult
optic nerve cell cultures did not synthesize DNA in response to factor(s) produced by type-1 astrocytes (Table 2).

O-2A progenitor cells from the optic nerves of adult rats, like those isolated from perinatal rats, are migratory cells, but migrate more slowly

In our previous studies, we found that perinatal O-2A progenitor cells were very motile cells, capable of migrating in vitro at speeds of up to 100 μm h⁻¹. The motile behaviour of the perinatal O-2A progenitors seems to be associated with a requirement for migration of this lineage into the optic nerve during development (Small et al. 1987). However, such rapid migration might not be necessary in adult tissues, and we were therefore interested whether adult and perinatal O-2A progenitors also differed in their capacity for migration.

Time-lapse microcinematography studies on adult O-2A progenitor cells demonstrated that cells with the morphology of adult O-2A progenitors were motile cells which migrated with an average speed of 4.3 ± 0.7 μm h⁻¹ (n = 21) when grown on PLL-coated coverslips in Astro-CM, less than 20% of the 21.4 ± 1.6 μm h⁻¹ speed of perinatal O-2A progenitor cells observed under similar conditions. When adult O-2A progenitor cells were grown on type-1 astrocyte monolayers, these cells migrated at a speed of only 2.3 ± 1.0 μm h⁻¹ (n = 61) The unipolar adult O-2A progenitor cells migrated in the direction of their large process and, following a division, the progeny cells moved away in opposite directions. Adoption of a multipolar oligodendrocyte-like morphology by such cells coincided with the loss of their migratory behaviour, as has been found in previous studies on O-2A progenitor cells derived from the optic nerves of perinatal rats (Small et al. 1987).

The time-course of differentiation of O-2A progenitor cells derived from the optic nerves of adult rats is almost three times as long as that of O-2A progenitor cells isolated from the optic nerves of perinatal animals

Over 95% of the bipotential progenitor cells of P7 optic nerves differentiate into oligodendrocytes or type-2 astrocytes within 3 days of plating in either chemically defined medium (DMEM-BS) or medium containing 10% FCS (DMEM + 10% FCS), respectively (Raff et al. 1983a). In contrast, in cultures of adult optic nerve cells plated onto PLL-coated coverslips and kept in either DMEM-BS (to allow differentiation along the oligodendrocyte pathway of development) or DMEM+10%FCS (to induce differentiation along the type-2 astrocyte pathway), more than 65% of the O-2A progenitor cells were still GalC⁻ and GFAP⁻ after 3 days (Fig. 7). With increasing length of culture time in
Characterization of \(O-2A\) progenitor cells

Table 2. When grown on type-1 astrocytes, fewer \(O-2A\) progenitor cells isolated from the optic nerves of adult animals take up radiolabelled thymidine in a 20 h pulse than \(O-2A\) progenitor cells from newborn nerves

<table>
<thead>
<tr>
<th>Age of animals</th>
<th>Newborn</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of all (O-2A) progenitor cells radiolabelled</td>
<td>77 ± 1</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>% of (O^4-) (O-2A) progenitor cells radiolabelled</td>
<td>86 ± 4</td>
<td>ND*</td>
</tr>
<tr>
<td>% of (O^4-) (O-2A) progenitor cells radiolabelled</td>
<td>7 ± 2</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>% radiolabelled (O-2A) lineage cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) (A2B5^+O4^-)</td>
<td>92 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>(ii) (O4^+)</td>
<td>8 ± 1</td>
<td>97 ± 1</td>
</tr>
<tr>
<td>(iii) (GalC^+)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

*Not determined; as the cultures of adult optic nerve cells contained very few \(A2B5^+O4^-\) cells, an accurate assessment of the proportion of these cells that were radiolabelled could not be made.

Optic nerve cells from newborn and adult rats were grown on type-1 astrocyte monolayers in DMEM-BS/0.5%FCS for 2 days and then exposed to \(2\muCi\cdot ml^{-1}\) \([^{3}H]\)thymidine for 20h. After the labelling period, cultures were immunolabelled with anti-GalC and \(A2B5\) antibodies, with anti-GalC and \(O4\) antibodies or with \(O4\) and \(A2B5\) antibodies, followed by the appropriate fluorescein- or rhodamine-coupled second antibodies. The results are the mean ± S.E.M. of a minimum of 3 separate experiments. In each experiment, 3 individual cultures were examined per immunolabelling. \(O-2A\) progenitor cells were defined as cells that were \(A2B5^+GalC^-\). As can be deduced from Fig. 5, virtually all of the \(A2B5^+GalC^-\) cells were \(GFAP^+\) after 3 days when kept in DMEM-BS/0.5%FCS. The proportion of all the \(O-2A\) progenitor cells that were radiolabelled is shown in A, while in B is shown the proportion of the \(O^4^-\) and \(O^4^+\) \(O-2A\) progenitor cells derived from the optic nerves of either newborn or adult rats that incorporated \([^{3}H]\)thymidine during a 20h pulse. Finally, the proportion of all the radiolabelled \(O-2A\) lineage cells in the newborn or adult optic nerve cultures that were \(A2B5^+O4^-\), \(O^4^+\) or \(GalC^+\) is shown in C.

In \textit{vitro}, the number of \(A2B5^+GalC^-GFAP^-O-2A\) progenitor cells in the cultures decreased and the number of \(GalC^+\) oligodendrocytes or \(A2B5^+GFAP^+\) type-2 astrocytes increased when the adult optic nerve cells were grown in, respectively, DMEM-BS or DMEM + 10%FCS (Fig. 7). During the culture period, the number of \(O-2A\) lineage cells kept in DMEM-BS remained constant, while the number of such cells grown in DMEM + 10%FCS increased significantly, suggesting that adult \(O-2A\) progenitor cells were stimulated to divide in response to growth factor(s) present in FCS (data not shown).

It took 4–5 days for 50% of the \(O-2A\) progenitor cells in cultures of adult optic nerve cells to become \(GalC^+\) oligodendrocytes or to become \(A2B5^+GFAP^+\) type-2 astrocytes (Fig. 7). In contrast, 50% of \(O-2A\) progenitor cells from the optic nerves of perinatal rats differentiate in less than 2 days (Raff \textit{et al.} 1983).

There were several other aspects in which differentiation of adult \(O-2A\) progenitor cells was dissimilar from differentiation of perinatal \(O-2A\) progenitors.
Fig. 7. The time course of differentiation of O-2A progenitor cells derived from the optic nerves of adult rats is very slow. Optic nerves cells isolated from adult optic nerves were grown on PLL-coated coverslips in either DMEM-BS or DMEM + 10% FCS. At various time points, cultures were immunolabelled with anti-GalC, A2B5 and anti-GFAP antibodies, followed by goat anti-mouse IgG-FITC (to visualize the binding of the anti-GalC antibodies), goat anti-mouse IgMFITC (to visualize the binding of the A2B5 antibodies) and sheep anti-rabbit IgG-FITC (to visualize the binding of the rabbit anti-GFAP antibodies). In these experiments, cells that were surface fluorescein-positive (i.e. were GalC⁺) were easily distinguishable from cells that were internally labelled by the fluorescein conjugate (i.e. were GFAP⁺). Similar results were obtained when cells were labelled with either A2B5 and anti-GalC antibodies or with A2B5 and anti-GFAP antibodies. Each point is the mean ± s.e.m. of three separate experiments and at least three coverslips were examined in each experiment. The results were expressed as percentage of total number of O-2A lineage cells. The graphs show that it takes about 5 days for 50% of the A2B5⁺GalC⁺GFAP⁻ O-2A progenitor cells to become GalC⁺ oligodendrocytes (in DMEM-BS) or A2B5⁺GFAP⁺ type-2 astrocytes (in DMEM + 10% FCS). Since, after 7 days in 10% FCS, most A2B5⁺GFAP⁺ cells were present in colonies of 2-4 cells, the O-2A progenitor cells derived from adult optic nerves that were differentiating into type-2 astrocytes may also have divided in response to growth factor(s) present in fetal calf serum. In contrast, the GalC⁺ cells that developed in DMEM-BS were mostly present as single cells.

so more slowly as compared with their counterparts in perinatal optic nerve cultures (G. Wolswijk, unpublished observations). In addition, when adult optic nerve cells were grown on coverslips in DMEM-BS for 7 days and immunolabelled with a different anti-GalC antibody, called O1 [an IgM antibody, used to allow double labelling with anti-vimentin antibodies (which were IgG1 antibodies); Sommer & Schachner, 1981], and anti-vimentin antibodies, none of the O1⁺ cells (i.e. GalC⁺ cells) contained vimentin IFs, suggesting that the oligodendrocytes generated by differentiating O-2A progenitor cells isolated from adult optic nerves do not acquire vimentin IFs in vitro. In contrast, oligodendrocytes generated by O-2A progenitor cells in cultures of perinatal optic nerve cells, and probably also in vivo, initially contain vimentin IFs, which are lost with further maturation (Raff et al. 1984b).

There were also some fundamental differences between the differentiation of perinatal and adult O-2A progenitors into type-2 astrocytes. Unlike perinatal O-2A progenitor cells, which are vimentin⁺, adult O-2A progenitors acquire vimentin whilst differentiating into type-2 astrocytes. After 7 days of growth in DMEM + 10% FCS, a stage at which over 95% of the A2B5⁺ cells expressed GFAP (Fig. 7), we found that over 65% of such cells were also labelled with anti-vimentin antibodies. FCS thus seems to induce the expression of both GFAP and vimentin in adult O-2A progenitor cells, and it seems likely that these cells had acquired first GFAP filaments and then vimentin IFs. Unlike the typical stellate morphology of type-2 astrocytes found in cultures of perinatal optic nerve cells grown in similar culture conditions, newly generated type-2 astrocytes in cultures of adult optic nerve cells generally had a more flattened morphology (data not shown).

The adult optic nerve appears not to contain perinatal O-2A progenitor cells

Although a small proportion (<3%) of the O-2A progenitor cells found in cultures of adult optic nerve were A2B5⁺O4⁻, and in this respect similar to perinatal O-2A progenitor cells, these O4⁺ adult O-2A progenitors appeared to be more like adult progenitor cells than perinatal progenitors. (1) The adult A2B5⁺O4⁻ adult optic nerve cells lacked vimentin IFs (Table 1), like O4⁺ adult progenitor cells but unlike perinatal progenitor cells. (2) The adult A2B5⁺O4⁻ O-2A progenitors only generated small colonies of O-2A lineage cells over time courses where perinatal optic nerve-derived O-2A progenitor cells frequently generated large colonies, suggesting that these cells divided at a rate similar to that of the O4⁺ O-2A progenitor cells in these adult-derived cultures. In these experiments, we first treated adult optic nerve cultures with O4 antibodies and complement to eliminate the O4⁺ adult O-2A progenitors. When the remaining cells were grown on monolayers of type-1 astrocytes, small colonies of O-2A lineage cells developed, but only at a frequency of <5% of that seen when O4⁺ adult O-2A progenitor cells had not been depleted. The colonies that developed from the A2B5⁺O4⁻ adult O-2A pro-
Characterization of O-2A<sub>adult</sub> progenitor cells

Table 3. Distinguishing properties of O-2A progenitors derived from either perinatal or adult optic nerves

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Perinatal optic nerves</th>
<th>Adult optic nerves</th>
</tr>
</thead>
<tbody>
<tr>
<td>O4 labelling (in vitro)</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;&lt;br/&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vimentin IFs</td>
<td>bipolar&lt;sup&gt;e&lt;/sup&gt;</td>
<td>unipolar</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cycle time</td>
<td>18 ± 4 h&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65 ± 18 h</td>
</tr>
<tr>
<td>Average rate of migration (on PLL)</td>
<td>21-4 ± 1.6 µm h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4.3 ± 0.7 µm h&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time course of differentiation (50% differentiated)</td>
<td>&lt;2 days&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4-5 days</td>
</tr>
</tbody>
</table>

* Virtually all of the O-2A progenitor cells isolated from the optic nerves of newborn rats are O4<sup>c</sup>, whereas many such cells isolated from the optic nerves 7-day-old rats are initially O4<sup>c</sup>, but become O4<sup>c</sup> during the first 24 h in culture when grown in Astro-CM (Sommer et al. in preparation).

Discussion

We have found that O-2A progenitor cells isolated from the optic nerves of adult O-2A progenitors. For example, the largest colony found after 7 days consisted of ten O4<sup>c</sup>GalC<sup>c</sup> O-2A progenitors and four O4<sup>c</sup>GalC<sup>c</sup> oligodendrocytes, as compared with an expected maximum size for 7 days of growth of ≈64 cells for a colony derived from a perinatal O-2A progenitor cell (Temple & Raff, 1986).

All other colonies contained less than 14 cells (average for 7 colonies = 6 cells). Indeed, it is noteworthy that we have never seen large colonies of O-2A lineage cells developing in cultures of adult optic. Thus, we find no indication that adult optic nerves contain any perinatal-type O-2A progenitor cells.

When cultures of adult optic nerve cells growing on type-1 astrocytes were incubated with A2B5, O4 and anti-GalC antibodies and complement, no new O-2A lineage cells appeared in the cultures, even after 14 days of in vitro growth, indicating that, as with perinatal O-2A lineage cells (Noble & Murray, 1984; Raff et al., 1984b), O-2A lineage cells in the adult optic nerve cultures did not arise from A2B5<sup>c</sup> cells.

Do other tissues contain perinatal and adult forms of precursor cells?

There is suggestive evidence that other tissues also contain populations of precursor cells with differing properties in the developing and mature organism. For example, when haematopoietic stem cells derived from the embryonic yolk sac or from adult bone marrow were injected into irradiated adult mice, the embryonic cells consistently generated larger spleen colonies than the adult-derived cells (Metcalf & Moore, 1971). In addition, spleen colonies generated by the adult haematopoietic stem cells contained fewer differentiated haematopoietic cell types as compared with colonies.
generated by fetal stem cells. A third difference between fetal and adult haematopoietic stem cells is that the erythroid cells generated by fetal stem cells produce the fetal form of haemoglobin, while erythroid cells generated by adult stem cells produce the adult form of haemoglobin (Wood et al. 1985, 1988).

Embryonic myoblasts and adult muscle satellite cells also appear to be significantly different from each other in their biological properties, although both can function as precursor cells to multinucleated skeletal muscle fibres. For example, adult muscle satellite cells express acetylcholine receptors at all stages of their development, but such receptors appear in embryonic myoblasts only at the onset of differentiation (Cossu et al. 1987). Furthermore, phorbol ester tumour promoters block differentiation of embryonic myoblasts, but have no effect on adult muscle satellite cells (Cossu et al. 1983). There are also further indications that myoblasts derived from early and late embryos differ in their abilities to generate fast, slow and mixed fast/slow muscle types (Miller & Stockdale, 1986; Schafer et al. 1987).

It is not known whether different properties of perinatal and adult precursors represent intrinsic properties of these cells or are the result of exposure to dissimilar microenvironments. In the case of O2Aadult and O2Aperinatal progenitor cells, we have separately seen that these cells express the properties described in the present paper even when growing in the same culture (Wolswijk & Noble, in preparation). Our studies thus suggest that the differences between perinatal and adult O2A progenitor cells represent intrinsic properties of these cells.

Why are there fetal and adult types of progenitor cells?

It seems likely that the differences between fetal and adult progenitors are related to differing requirements of fetal and mature organisms. For example, the switch from fetal to adult haemoglobin is of clear functional importance. As fetal haemoglobin has a higher affinity for oxygen than adult haemoglobin, it allows the fetal haemoglobin to be oxygenated at the expense of the maternal adult haemoglobin in the placenta (Stryer, 1981). In contrast, adult haemoglobin has the advantage that it allows a more efficient exchange of carbon dioxide and oxygen in the lungs as compared to fetal haemoglobin (Stryer, 1981). Thus, the fetal and adult forms of haemoglobin appear to have properties that suit the needs of their environment. However, it is difficult to imagine the functional value of the differences between fetal and adult muscle precursor cells, or of the reduced abilities to generate spleen colonies and the restricted potential for differentiation of adult haematopoietic stem cells.

At least some of the differences between O2Aperinatal and O2Aadult progenitor cells appear to be clearly related to the differing needs of their particular environments. During perinatal development it is necessary to generate rapidly large numbers of oligodendrocytes (and type-2 astrocytes), and the 18 h cell-cycle time of the O2Aperinatal progenitor cells would be important in this process. In addition, it appears that O2Aperinatal progenitors populate the optic nerve as a result of migration from a germinal zone in or near the optic chiasm (Small et al. 1987), and the capacity of O2Aperinatal progenitors for rapid migration would play a critical role in this migratory process. These features of the O2Aperinatal progenitor cells would, however, be inappropriate in the adult nervous system where only small numbers of new oligodendrocytes and type-2 astrocytes are needed as part of the normal glial turnover (Kaplan & Hinds, 1980), where there is insufficient space to accommodate continually the number of cells required during embryogenesis and where extensive migration may not normally be necessary.

The slow rates of migration, division and time course of differentiation of O2Aadult progenitor cells suggest that these cells may be less effective than perinatal progenitors in carrying out oligodendrocyte replacement in a demyelinating lesion. It is particularly interesting in this regard that the recovery of children suffering from optic neuritis, a demyelinating disease of the optic nerve, usually tends to be more complete than in adults suffering from this condition (McDonald, 1983). Moreover, Wolf et al. (1986) have recently reported that, although cells can migrate out of both perinatal and adult optic nerve explants into explants of chemically demyelinated cerebella, where migrating cells produce myelin, the cells derived from the adult optic nerve explants (a) took a longer time to myelinate the cerebellar axons than their perinatal counterparts, and (b) did not migrate as far as cells migrating out of the perinatal optic nerve explants. The results of these in vivo and organ culture studies are thus consistent with, and are perhaps due to, the properties of the O2Aadult progenitor cells.

What is the source of O2Aadult progenitor cells?

In all tissues in which there are differences between perinatal and adult progenitor cells the initial times of appearance and developmental origins of the adult progenitors, and their relationship to their perinatal counterparts, are unknown. The inability to identify and distinguish unambiguously between perinatal and adult progenitors in the haematopoietic system, or between embryonic and adult muscle progenitors, makes it unlikely that the development of adult-specific progenitor cells in these tissues will be easily understood. In contrast, the differences between O2Aadult and O2Aperinatal progenitor cells of the rat optic nerve are so distinct that it should prove possible to determine the time of first appearance of O2Aadult progenitor cells during development and to determine eventually the origin of this new cell type.

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References


G. Wolswijk and M. Noble

Freeman and company.
TEMPLE, S. & RAFF, M. C. (1986). Clonal analysis of
ependymocyte development in culture: Evidence for a
developmental clock that counts cell divisions. Cell 44, 773–779.
developmental clock and hemoglobin switching. In Hemoglobin Switching,

WOOD, W. G., BUNCH, C., KELLY, S., GUNN, Y. & BRECKON, G.

(1985). Control of haemoglobin switching by a developmental

(Accepted 2 November 1988)