Plane of Cell Cleavage and Numb Distribution during Cell Division Relative to Cell Differentiation in the Developing Retina

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Progenitor cells in the early developing nervous system can divide symmetrically, giving rise to two daughter cells that divide again, or asymmetrically, giving rise to one cell that differentiates and one that divides again. It has been suggested that the orientation of the cell cleavage plane during mitosis determines the type of division. A marker of early cell differentiation, the RA4 antigen, was used to identify regions of the developing chick retina with and without differentiating cells, and the orientation of the cleavage plane was characterized for mitotic figures in each region. No difference was found in the frequency of any orientation between the regions with or without differentiating cells. Furthermore, in the region of the retina with differentiating cells, the RA4 antigen was present in mitotic figures with every possible orientation. Thus, the orientation of the cleavage plane appears to be unrelated to whether or not a division produces a cell that differentiates. It has also been suggested that the intracellular protein Numb mediates neurogenesis via asymmetric localization during cell division. Numb localization was compared with expression of markers of early cell differentiation, the RA4 antigen and Delta. Differentiating and nondifferentiating cells were found both with and without Numb expression. Cells with a cleavage plane parallel to the retinal surface were polarized, such that Numb and/or the RA4 antigen, when present, were only in the daughter cell farthest from the ventricle. These findings indicate a need to reconsider current hypotheses regarding the key features underlying symmetric and asymmetric divisions in the developing nervous system.

Key words: symmetry of cell division; neuronal differentiation; Notch; Delta; RA4 antigen; neuroepithelial cell; retina; development

The cells that comprise the mature vertebrate nervous system are produced from a small pool of progenitor cells. During development, these cells increase in number by a series of symmetric cell divisions, each of which produces two cells that divide again. As development progresses, progenitor cells switch to making a limited number of asymmetric divisions, where one daughter cell resulting from each division continues to divide, and the other ceases further division and differentiates. A fundamental question is what underlying mechanisms distinguish symmetric from asymmetric divisions.

Numerous studies have examined the nature of symmetric and asymmetric cell divisions in the developing vertebrate nervous system. These investigations led to the suggestion that the orientation of the cell cleavage plane during division determines whether a progenitor cell divides symmetrically or asymmetrically (Martin, 1967; Zamenhof, 1987; Chenn and McConnell, 1995; Cayouette et al., 2001). It was concluded that when the cleavage plane is perpendicular to the ventricular surface, the division is symmetric, with both daughter cells remaining as progenitors. When the cleavage plane is parallel to the ventricular surface, the division is believed to be asymmetric, with the cell closer to the ventricle continuing to divide and the cell farther from the ventricle differentiating. The distribution of the intracellular protein Numb has also been implicated in determining the symmetry of cell division (Zhong et al., 1996; Wakamatsu et al., 1999).

There are difficulties with the current dogma. The number of cell divisions with a cleavage plane parallel to the ventricular surface is insufficient to account for the number of neurons produced during development (Zamenhof, 1987; Chenn and McConnell, 1995). In addition, there is disagreement as to whether Numb is distributed to the daughter cell that differentiates or to the cell that continues to divide (Zhong et al., 1996; Wakamatsu et al., 1999). Part of the confusion has arisen because cells undergoing symmetric and asymmetric division overlap in most neural tissues and because no marker has been used to identify mitotic cells that are destined to differentiate. Thus, previous studies could not definitively distinguish symmetric from asymmetric divisions.

The vertebrate retina offers certain advantages for the study of cell division relative to differentiation. The retina develops from neural tube, so the fundamental mechanisms that determine the symmetry of divisions in the retina should be the same as those for other parts of the CNS. Cell differentiation begins in the central part of the retina (Fujita and Horii, 1963; Kahn, 1974; McCabe et al., 1999). Cell divisions that give rise to differentiating cells initially occur only in the central retina. During the same period, cell divisions in peripheral retina are only symmetric and are essential for increasing the pool of progenitor cells (Dutting et al., 1983). Because ganglion cells are the first cells born in the retina (Spence and Robson, 1989; Prada et al., 1991; Snow and Robson, 1994), the presence or absence of ganglion cells can be used to identify areas of the retina with and without differentiating cells. Ganglion cells can be identified with the RA4 antibody as they start to differentiate (McLoon and Barnes, 2002).
Neurons in early stages of differentiation can also be identified by their expression of Delta-1 (Ahmad et al., 1997; Bao and Cepko, 1997; Henrique et al., 1997). The primary aim of this study was to determine whether the plane of division or the distribution of Numb correlates with differentiation or continued division by cells in the developing retina.

MATERIALS AND METHODS

Animals and tissue preparation. Pathogen-free, fertilized chicken eggs (White Leghorn crossed with Rhode Island Red) from the University of Minnesota Poultry Research Center (St. Paul, MN) were incubated at 37°C and 98% relative humidity. In some cases, embryos were treated with bromodeoxyuridine (BrdU) 24 hr before fixation as described previously (Waid and McLoon, 1995). Embryos at various stages of development were removed from the shell, fixed, and staged according to Hamburger and Hamilton (1951). Stage 18 and 24 embryos were fixed whole by immersion in 4% paraformaldehyde/0.1 M phosphate buffer, pH 7.4. For older embryos, eyes were dissected and immersion fixed. After 2 hr of fixation, tissue was cryoprotected in 20% sucrose/0.1 M phosphate buffer, pH 7.4, at 4°C overnight and embedded in 10% tragacanth gum/20% sucrose/phosphate buffer. Most tissue was sectioned frozen at 15 μm, and sections were mounted on chrome alum/gelatin-coated microscope slides. Tissue from embryos treated with BrdU was sectioned at 8 μm.

Immunohistochemistry. Sections were rinsed in PBS. To prevent non-specific antibody binding, sections were incubated in 10% normal goat serum/0.3% Triton X-100/PBS for 15 min. Sections were then incubated in one or two primary antibodies for 1 hr. Primary antibodies included a polyclonal antibody to chicken Numb (Wakamatsu et al., 1999), a polyclonal antibody to chicken Delta-1 (Henrique et al., 1997), and the RA4 monoclonal antibody that labels early differentiating retinal ganglion cells (McLoon and Barnes, 1989). After incubation in primary antibody, slides were rinsed in several changes of PBS for >15 min. Sections were then incubated for 1 hr in secondary antibody. These were affinity-purified goat anti-mouse IgG or anti-rabbit IgG conjugated to fluorescein isothiocyanate or to lissamine rhodamine B sulfonyl chloride (Jackson ImmunoResearch, West Grove, PA). After another series of rinses, sections were counterstained for 60 sec with 1.5 × 10^-6 μM 4',6'-diamidino-2-phenylindole (DAPI). Coverslips were mounted with glycerin-based mounting medium containing an anti-fade reagent (Kimbergaard & Perry, Gaithersburg, MD).

Sections of eyes from embryos treated with BrdU were processed for immunohistochemistry with an antibody to BrdU and the RA4 antibody as described previously (Waid and McLoon, 1995).

Analysis. Sections were examined immediately after processing with a Leica (Deerfield, IL) DMR fluorescence microscope. The plane of cleavage was determined for mitotic figures in sections of retina stained with DAPI. Anaphase and early telophase mitotic figures with a plane of cleavage perpendicular to the plane of the tissue section, as determined by focusing through the cell, were selected for analysis. In test sections, 8% of the mitotic figures had the proper orientation for analysis. Digital images of mitotic figures to be analyzed were captured with a PhotoMetrix (Huntington Beach, CA) camera and deconvolved using Microtome (VayTek, Fairfield, IA) as a subroutine within the Image-Pro Plus program (Media Cybernetics, Silver Spring, MD). The protractor function in Image-Pro was used to measure the angle of the plane of cleavage relative to the subretinal space. Every mitotic figure was evaluated in a section. Every fourth section of a retina was examined until the required number of cells had been analyzed as specified in Results for each experiment. Data from different sections of the same retina were pooled.

Mitotic cells were examined for the presence and distribution of immunostaining for particular molecules. Cells were selected for analysis if they were judged to be entirely within the plane of the section as determined by focusing the microscope through the thickness of the section. Typically, 12 serial optical sections were collected for each cell to be analyzed, which would include the entire thickness of the cell. Each image was deconvolved, and then all the images of a cell were combined into a single image (i.e., Z-projection). The plane of cleavage was determined for each cell as described above, and the distribution of the molecule of interest was noted relative to the individual daughter cells.

The percentage of DAPI-stained nuclei labeled with BrdU was determined in randomly selected microscope fields in the RA4+ region of retinal sections. Each field spanned the entire thickness of the retina. Three fields were counted per section, which were spread approximately equally across the RA4+ region only on the nasal side of the retina. Three sections were analyzed per retina, and three retinas from three embryos were analyzed per developmental stage.

Numerical data are expressed ±SEM. Data sets were compared statistically using a paired t test or an ANOVA.

RESULTS

Orientations of the cell cleavage plane during cell division in different areas of the retina

Immunostaining with the RA4 antibody was used to distinguish areas of the retina with differentiating cells from areas without differentiation. The RA4 antibody recognizes early differentiating ganglion cells, the first cell type to differentiate in the retina. Although retinal progenitor cells have been found that express certain markers characteristic of differentiated cell types (Hernandez-Sanchez et al., 1994; Alexiades and Cepko, 1997), S-phase cells, identified by brief labeling with BrdU, have not been found to express RA4 (Waid and McLoon, 1995), indicating that progenitor cells are not RA4+. Sections of chick retina at embryonic stages 18 [embryonic day 3 (E3)], 23 (E3.5–E4), and 27 (E5) were stained with the RA4 antibody. RA4+ cells were present in central retina but were absent from the periphery of the retina (Fig. 1). With further development, the RA4+ area expanded progressively. Double staining retinal sections with DAPI revealed mitotic figures in areas with and without differentiating cells. In the area of the retina without RA4 staining, each cell division presumably generates two daughter cells that divide again. To verify this, embryos were treated with BrdU overnight to label dividing cells, and sections of retinas from these embryos were processed for BrdU and RA4 immunohistochemistry and stained with DAPI. In the RA4– area, almost all cells were BrdU+ (98 ± 1.5% for stage 18, 97 ± 2% for stage 23, and 98 ± 1% for stage 27), indicating that virtually all cells generated in this region continue to divide. In contrast to the RA4+ area,
some if not most cell divisions in the RA4+ area generate at least one daughter cell that differentiates.

The first aim of this study was to determine whether the plane of cell cleavage correlates with differentiation in the developing retina. The plane of cell cleavage during cell division was assessed histologically in areas of the retina with and without differentiating cells. Mitotic figures in anaphase or telophase were analyzed in sections of embryonic retina double stained with the RA4 antibody and with DAPI. The plane of cell cleavage with respect to the surface of the retina facing the subretinal ventricle ranged from perpendicular to parallel (Fig. 2). Previous studies defined the plane of division as perpendicular or parallel if the central axis of the cleavage plane was within 30° of a line perpendicular or parallel to the ventricular surface (Chenn and McConnell, 1995; Wakamatsu et al., 1999). Using this criterion, mitotic figures in RA4− and RA4+ regions of the retina were classified and quantified. For each age, 25 cells were analyzed in each region in each of 10 retinas. Only cells dividing perpendicular to the plane of the tissue section were included in this analysis. Mitotic figures within 100 µm of the boundary between the RA4− and the RA4+ areas were not used. There was no significant difference (p = 0.39 for stage 18, 0.46 for stage 23, and 0.15 for stage 27) in the percentage of cells with parallel or perpendicular cleavage planes between the RA4− and RA4+ areas of the retina at any of the three ages examined (Fig. 3). It is particularly significant that at all three ages, parallel planes of cell cleavage were observed in the RA4− area (Fig. 2), which indicates that symmetric divisions that yield two dividing cells can have a parallel plane of division. In addition, there was no significant change between the different ages examined in the number of mitotic cells with perpendicular or parallel cleavage planes (p values ranged from 0.28 to 0.88 for the different comparisons). This shows that the percentage of mitotic figures with a parallel cleavage plane did not increase with increasing age, although the percentage of divisions generating differentiating cells increased during this period of development (Dutting et al., 1983; Morris and Cowan, 1995). These findings suggest that no plane of cleavage is unique to divisions that generate differentiating cells.

The perpendicular and parallel cleavage plane classifications did not appear to be distinct categories, as might be expected if these orientations were linked to symmetric and asymmetric divisions, respectively. To define better the range of cleavage planes exhibited by dividing cells, the cleavage plane for 250 mitotic figures was measured in increments of 10° in stage 27 retina. In general, most cells divided with a perpendicular cleavage plane (Fig. 4). The more the plane of cleavage deviated from perpendicular, the fewer cells were found. There was no apparent clustering of cells into distinct orientation groups, and cells with
a parallel orientation were rare. Furthermore, no significant difference was found in the relative frequency of any cleavage plane between the RA4⁺ and RA4⁻ areas or between different ages when the 10° bin size was used to categorize cleavage angles rather than the 30° described above. These findings suggest that dividing cells with a parallel cleavage plane are the extreme of a continuum weighted toward a perpendicular orientation and that they do not represent a unique group of cells.

**Orientations of the cell cleavage plane relative to cell differentiation**

We subsequently asked whether mitotic figures that express a marker for differentiation exhibit a particular orientation of cell cleavage. It was shown previously that some cells in late stages of mitosis start to express the RA4 antigen (McCabe et al., 1999), a marker for cell differentiation. In mitotic bodies in anaphase and telophase, the RA4 antigen was observed to distribute to one or both daughter cells (Fig. 5). The orientation of the cleavage plane of mitotic bodies relative to expression of the RA4 antigen was examined to determine whether a particular orientation correlates with differentiation. Late anaphase and telophase mitotic cells that expressed the RA4 antigen were found with cleavage planes in all possible orientations. A similar percentage of cells with parallel or perpendicular cleavage planes in the RA4⁺ area of the retina expressed the RA4 antigen. Forty-three percent of the mitotic cells with a parallel cleavage plane were RA4⁺, and 40% of the mitotic cells with a perpendicular cleavage plane were RA4⁻ (no statistical difference; \( p = 0.77 \) based on analysis of 500 cells in the RA4⁺ area of 18 retinas from stage 23 embryos) (Fig. 6). Cells with intermediate to perpendicular cleavage planes had the RA4 antigen present in one or both daughter cells. When cells with a cleavage plane within 30° of parallel to the retinal surface had the RA4 antigen, the antigen was present only in the cell farthest from the ventricle. In a survey of hundreds of mitotic figures with an approximately parallel plane of division, not one case was observed with both daughter cells or with the one daughter cell nearest the ventricle expressing the RA4 antigen. There were also cells with all orientations of cleavage in central retina that did not express the RA4 antigen. These findings show that differentiating cells can arise from divisions with any plane of cleavage. Thus, division with a perpendicular cleavage plane can produce daughter cells with asymmetric fates. Furthermore, the results indicate that when the plane of cleavage is parallel to the retinal surface, a dividing cell is polarized so that only the daughter cell farthest from the ventricle can differentiate.

**Distribution of Numb during cell division relative to differentiation**

Because the plane of cleavage does not appear to determine whether a cell division is symmetric or asymmetric, we looked for another mechanism that might determine whether cells resulting from a division divide again or differentiate. Previous work suggested that Numb determines the symmetry of cell division in the developing nervous system (Verdi et al., 1996; Zhong et al., 1996, 2000; Wakamatsu et al., 1999; Cayouette et al., 2001). The distribution of Numb was examined relative to the plane of cell division and differentiation in the developing retina. Sections of developing retina were double stained with an antibody to Numb (Wakamatsu et al., 1999) and with the RA4 antibody. Numb staining was most abundant near the vitreal surface of the retina (Fig. 7). This staining probably was associated with the endfeet of the neuroepithelial cells, as observed previously in the developing cerebral cortex (Wakamatsu et al., 1999). Numb also was present in some M-phase cells. In Numb⁺ M-phase cells, Numb was typically restricted to a crescent at the basal pole of the cell, the side away from the subretinal ventricle (Figs. 5 and 7). In cells slightly above the mitotic layer, Numb was distributed more broadly around the cells but was still concentrated on the basal side. Numb was present in M-phase cells in both the RA4⁺ and RA4⁻ areas of the retina. There was no significant difference in the percentage of cells with Numb between the two areas (20% for RA4⁺ area and 18% for RA4⁻ area in stage 23 retina; \( p = 0.47 \)). Because cells were not differentiating in the RA4⁻ area, Numb clearly is not expressed exclusively by differentiating cells. In the RA4⁺ area of retinas, Numb was present in dividing
cells exhibiting all planes of cleavage. In M-phase cells with a cleavage plane approaching vertical to the retinal surface, Numb was present in one or both daughter cells (Fig. 7). In M-phase cells with a cleavage plane approaching parallel, Numb was present in only one daughter cell, and this was always the basal cell, the cell farthest from the subretinal ventricle.

The distribution of Numb was also compared with the distribution of a marker for differentiation, the RA4 antigen. Sections of stage 23 (E3.5–E4) retina were double stained with the RA4 antibody and the antibody to Numb using secondary antibodies with different fluorochromes. Five hundred M-phase cells in the central retina where cells were differentiating were analyzed for cleavage plane and for RA4 and Numb distribution in the daughter cells. Each cell analyzed was contained completely within the thickness of the section as determined by focusing through the tissue. Examples of the results are shown in Figure 5, and the results are summarized numerically in Figure 6. Several findings are significant. Cells with a cleavage plane parallel to the retinal surface were polarized, such that Numb and/or the RA4 antigen, when present, was only in the daughter cell farthest from the ventricle. Most RA4+ cells did not express Numb, and in fact, differentiating cells expressing RA4 were just as likely to express Numb as were cells not expressing RA4 (~20% in both cases). Numb was not restricted to RA4+ mitotic figures. When an RA4+ mitotic body expressed Numb, then Numb and RA4 always colocalized to the same daughter cell or cells. The presence of RA4+/Numb+ mitotic bodies is significant, because it suggests that expression of Numb is not required for a cell to begin differentiation. Nevertheless, RA4 and Numb always colocalized when both were expressed in the same mitotic body, indicating some relationship between Numb and differentiation.

In the area of the retina with RA4+–differentiating cells, some cells expressed Numb that were RA4−. The question remains whether these cells destined to continue dividing or were cells differentiating as a cell type other than a ganglion cell. Delta is expressed transiently in differentiating cells in the CNS, including retina shortly after their final mitosis (Ahmad et al., 1997; Bao and Cepko, 1997; Henrique et al., 1997). Delta is expressed by most if not all postmitotic RA4+ cells in the central retina and by RA4− cells as well (A. O. Silva and S. C. McLoon, unpublished observations). These Delta+/RA4− cells are presumably differentiating as a cell type other than a ganglion cell. Delta expression was used as another marker for differentiation to determine whether there is a correlation between Numb and differentiation. Sections of developing retina were double stained with antibodies to Numb and to chicken Delta-1 (Fig. 8). Delta+ cells in central retina appeared to be postmitotic, as reported by others. M-phase cells did not stain for Delta, and the intensity of DAPI-stained Delta+ cells suggested that the DNA content was that of a G1-G0 phase cell. As with RA4, only some of the Delta+ cells in or near the mitotic layer in central retina were also positive for Numb (of 38 Delta+ cells, 12 were also Numb+). There were also Numb+ cells that did not express Delta. Once again, there was no apparent correlation between the presence of Numb in the cell and differentiation, as indicated by Delta expression in this case.

**DISCUSSION**

Initially during nervous system development, neuroepithelial cells divide symmetrically, giving rise to daughter cells that divide again. This allows an exponential increase in the number of progenitor cells. As development progresses, asymmetric cell division gives rise to one cell that differentiates and one cell that
divides again. Because differentiated cells in the nervous system
typically do not divide, these asymmetric cell divisions are nec-
essary to generate cells that differentiate without depleting the
population of progenitor cells that can contribute to further
growth. The observation of dividing cells with very different
cleavage planes in the developing nervous system led to the
hypothesis that the orientation of the cleavage plane distin-
guishes symmetric from asymmetric divisions (Martin, 1967;
Zamenhof, 1987). This hypothesis is intuitively appealing. Neu-
roepithelial cells, like other types of epithelial cells, are polarized
in their apical–basal plane. Different cellular constituents are
concentrated in the apical and basal poles (Huttner and Brand,
1997; Chenn et al., 1998). One might expect cell division with a
cleavage plane perpendicular to the surface of the neuroepithe-
lium to produce two daughter cells that inherit equal amounts of
the apical and basal constituents of the mother cell. This could
account for symmetric divisions. Conversely, a division with a cell
cleavage plane parallel to the surface would be expected to
produce one daughter cell inheriting the apical constituents from
the mother cell and one inheriting the basal constituents. This
could be the basis for an asymmetric division. Furthermore, with
a vertical division, the basal cell might be free of its attachment to
the junctional complex at the apical margin of the epithelium,
which would allow it to migrate away from the mitotic layer, as do
differentiating cells in the CNS. The migratory behavior of indi-
vidual cells observed after cell division with various cleavage
planes in developing cortex was interpreted as supporting this
model (Chenn and McConnell, 1995).

We examined the cleavage plane of mitotic figures in the
developing retina relative to differentiation. Three main findings
from this study challenge the hypothesized relationship between
cleavage plane and whether a cell division is symmetric or asym-
metric. First, the frequency of any particular cleavage plane did
not correlate with the presence of differentiating cells. In areas of
the retina without differentiating cells, dividing cells were present
with every possible cleavage plane, including parallel. In fact, the
relative frequency of cells with the various cleavage planes did not
differ significantly between areas of the retina with and without
differentiating cells. Furthermore, the relative frequency of cells
with the various cleavage planes remained much the same as
development progressed, although the percentage of divisions
that give rise to differentiating cells increases during this period
(Dutting et al., 1983; Morris and Cowan, 1995). Cells dividing
with a parallel cleavage plane remained a small percentage of
divisions at all stages of development studied, making it unlikely
that these could account for the number of differentiated cells
ultimately produced. Second, there was no clear dichotomy in the
plane of cleavage of dividing cells, as would be expected if cells
with a perpendicular cleavage plane were unique to symmetric
divisions and if cells with a parallel cleavage plane were unique to
asymmetric divisions. Instead, our observations suggest that cells
with a parallel plane of division are the extreme of a continuum
weighted toward a perpendicular orientation. Third, dividing
cells expressing a marker for differentiation were observed with
every possible cleavage plane. In addition, the percentage of cells
with parallel or perpendicular cleavage planes that expressed a
marker for differentiation were virtually the same. Thus, the
plane of division does not predict whether a division is symmetric
or asymmetric. Together, these findings indicate that the plane of
cell cleavage during division in the developing retina is unrelated
to determining whether the daughter cells go on to divide again
or differentiate.

Chromatid orientation is typically dynamic in dividing cells,
which appears to hold true for the developing nervous system
(Haydar et al., 2001). This could make it difficult to determine the
final orientation of cleavage of a mitotic cell in fixed tissue. Our
analysis was restricted to cells in late anaphase and telophase. At
this point in the cell cycle, the spindle apparatus has formed,
making it likely that the chromatid orientation reflects the final
cleavage plane of the cell (Shuster and Burgess, 1999;
Kaltschmidt et al., 2000).

Although a number of studies examined the plane of cleavage
during cell division in the developing nervous system, there are
considerable discrepancies in the findings. A recent investigation
looked at this issue in developing mouse retina (Cayouette et al.,
2001). In contrast to our findings, that study reported an increase
in mitotic figures with a cleavage plane parallel to the ventricular
surface of the retina at one age of development. They speculated
that this increase was caused by an increase in cell divisions that
produce differentiating cells. However, cells with a parallel cleav-
age plane never represented a large percentage of dividing cells,
and late in development, when most divisions would be expected
to produce differentiating cells, very few mitotic figures exhibited
this cleavage plane. A number of studies examined the plane of
cell division in mammalian cortex, but they have few points of
agreement. Results range from finding very few cells with a
parallel cleavage plane and no change in this through develop-
ment (Smart, 1973; Landrieu and Goffinet, 1979) to finding many
cells with a parallel cleavage plane and significant changes
through development in the percentage of cells with the different
cleavage planes (Zamenhof, 1987; Chenn and McConnell, 1995).
Given that most studies of cell division in the nervous system
examined different regions and/or different species, it leaves open
the possibility that the plane of cleavage during cell division has
a different significance for different neural tissues and/or different
species. The present study differs from all previous studies in that
we identified cells expressing a marker for differentiation in the
late stages of mitosis and so could more reliably detect any
correlation between differentiation and the plane of cleavage of
dividing cells. We conclude from this analysis that the plane of
cleavage during cell division is unrelated to whether or not a
daughter cell will differentiate, at least in the early developing
chick retina.

We did observe that when cells divide with a cleavage plane
parallel to the ventricular surface, the basal daughter cell is the
only one that can differentiate. This is consistent with the previ-
ous suggestion that neuroepithelial cells are polarized and that
when a cell divides with a parallel cleavage plane, the constituents
that initiate differentiation are located on the basal side of the cell
(Chenn and McConnell, 1995). In Drosophila, a complex of sev-
eral proteins was shown to asymmetrically localize to the basal
daughter cell, the cell that will differentiate, during mitosis (Jan
and Jan, 1998).

Because the plane of cell cleavage does not generally correlate
with the symmetry of division, we looked for another cellular
characteristic that might account for the two patterns of division.
Previous studies linked the distribution of Numb with the sym-
metry of division. Numb was originally identified in Drosophila as
an intracellular protein that blocks Notch signaling, and for some
cell lineages, an asymmetry in cell fates after division required
Numb (Rhyu et al., 1994; Frise et al., 1996; Guo et al., 1996;
Spana and Doe, 1996). Numb is also expressed in the developing
vertebrate nervous system (Verdi et al., 1996, 1999; Zhong et al.,
1996, 1997; Wakamatsu et al., 1999). Mitotic cells were found with
Numb asymmetrically distributed, which, coupled with the Drosophila findings, led to the suggestion that Numb is responsible for asymmetry in cell fate in the developing vertebrate nervous system. However, the results from studies in which Numb expression was altered are confusing. In Drosophila, mutations of Numb affected cell fate decisions for some neuronal lineages but not for others (Spana et al., 1995). In mice, knock-out of Numb resulted in an increase in the number of neurons in the early embryonic forebrain, but the number of dividing cells was normal (Zhong et al., 2000). If the increase in neuron production in this study was caused by a change in the symmetry of divisions, then the number of dividing cells also should have changed. In the chick, overexpression of Numb had a heterogeneous effect that cannot be easily interpreted (Wakamatsu et al., 1999). We found no correlation between Numb and the symmetry of division. Cells not differentiating were as likely to express Numb as cells that were differentiating. Because Numb is neither necessary nor sufficient for initiating differentiation, Numb might have a different function than inducing differentiation via asymmetric localization in the developing vertebrate nervous system.

We observed Numb concentrated on the basal side of mitotic cells in the developing chick retina. This is in agreement with a previous study that used the same antibody to examine Numb localization in several regions of the developing chick nervous system (Wakamatsu et al., 1999). Numb localizes to the basal side of neuroblasts in Drosophila as well (Rhyu et al., 1994; Spana et al., 1995). However, our finding is in contrast to the apical concentration of Numb reported for the developing mammalian nervous system, including the retina (Zhong et al., 1996, 1997; Cayouette et al., 2001). It has been suggested that the antibody to chicken Numb that we used in the present study also cross-reacts with the related protein, Numblike (Zhong et al., 2000). It is unlikely that the explanation for the different results is this simple. Numblike, at least in mammals, is not present in dividing cells (Zhong et al., 1997). Even if the antibody used in the present study did cross-react with Numblike, it would still be expected to recognize Numb. No immunostaining, however, was observed on the apical side of dividing cells using this antibody. Numb is known to have a number of isoforms, and different isoforms have been linked to promoting cell division or differentiation (Verdi et al., 1999). Antibodies used in the different studies could selectively recognize a subset of Numb isoforms, which could account for the differences observed in Numb localization. Until this issue is resolved, the role of Numb in the developing nervous system cannot be fully understood.

In summary, we found no evidence for a relationship between the plane of cleavage or Numb distribution and the symmetry of cell division. Differentiating cells were as likely to have any particular plane of cleavage or to express Numb as were cells that continued to divide. The fundamental difference between cells in the developing retina that divide symmetrically and those that divide asymmetrically remains to be identified. Different progenitor cells in the retina can express different cyclin kinase inhibitors (CKIs), and CKIs have been linked to regulation of proliferation or differentiation (Dyer and Cepko, 2001). It could be that the specific CKI or combination of CKIs expressed by a progenitor cell determines its mode of division.

REFERENCES