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Invited Review

Müller glia cells and their possible roles during retina differentiation in vivo and in vitro

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Summary. Müller cells are astrocyte-like radial glia cells which are formed exclusively in the retina. Here we present evidence that Müller cells are crucially involved in the development of the retina's architecture and circuitry. There is increasing evidence that Müller cells are present from the very early beginning of retinogenesis. We postulate the "gradual maturation hypothesis of Müller cells". According to this hypothesis, Müller cells are continuously generated by a gradual transition of neuroepithelial stem cells into mature Müller cells. This process may be partly reversible. Müller cells, or their immature precursors, are able to subserve different functions. They are primary candidates for stabilizing the complex retinal architecture and for providing an orientation scaffold. Thereby, they introduce a reference system for the migration and correct allocation of neurons. Moreover, they may provide spatial information and microenvironmental cues for differentiating neurons, and may also be important for the segregation of cell and fibre layers. Additionally, they seem to be involved in the guidance of axonal fibres both in radial and in lateral directions, as they are involved in the support and stabilization of synapses.

Key words: Müller cells, Radial glia, Retina, Development, Pigmented epithelium, Chick, Rotation culture, Chick-quail chimeras, F11

Introduction

One of the basic requirements for the evolution of actively motile organisms was the development of an efficient nervous system to perceive information from the environment, to process this information, and to adapt and control movement and behaviour. Beginning with a relatively simple net-like nervous system in coelenterates, the nervous system progressively developed into a highly complex organ and gained more and more influence in the evolutionary success of its owners. Not surprisingly, the most complex nervous systems are found in young evolutionary groups like birds and mammals. Most of our own human comprehension depends upon the specific capacity of our brain; learning, memory, thinking or consciousness. These specific abilities emerge from the unique capacity of the brain to adapt itself to changing environmental situations. This can only be achieved by mechanisms of plasticity, self organization, and learning. However, in this case, the fine architecture of the brain can not be entirely fixed. Therefore, the efficiency of the central nervous system highly depends upon few - at first sight chiastic - requirements: a) the compartmentalization of the brain into different morphological and functional parts; b) the correct and stable arrangement and wiring of the different neuronal cell types inside each compartment, as well as the connections between related areas; and c) environmental- and activity-dependent plasticity to adapt the nervous system to changing conditions and requirements both in the internal and external environment.

The brain's remarkable plasticity is also reflected by the fact that in adult humans it consists of about 10^{12} cells and each cell has about 1000 synaptic connections (Kandel et al., 1991). It is obvious that this complexity cannot be specified in detail by gene-based information. Most probably, the main function of nervous system-related genes is to provide a general blueprint consisting of a mosaic-like pattern of specific local molecular frames. Valuable morphogenetic information then emerges from, e.g. cell-cell recognition and sorting-out processes or from specific patterns of neuronal activity enabling the system to achieve different levels of inherent self-organization and stabilization. One of the most important mediators of nervous system organization and plasticity are glia cells. Although these cells have been known for more than a century, their complex significance for embryonic development and for adult physiology has been recognized only recently. In this review, we want to focus on a specific type of glia cell which occurs exclusively in the retina: the Müller cell. Here, we will consider their possible roles during the development of the retina's architecture.

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The retina: a powerful model system in order to study neural development

The embryonic development and the gross organization of most brain parts arise from the complex interactions of few basic mechanisms: a) the lateral growth and proliferation of neuroepithelial tissue; b) the migration, dispersion and allocation of neurons to their final destinations; c) the formation of synaptic contacts; d) the separation of cells and fibres into specific layers.

One of the most suitable brain parts in order to study these mechanisms is the neural retina (Dowling, 1987). Phylogenetically, it represents an evolutionary conservative structure, only differing marginally in distinct vertebrate groups. Ontogenetically, it arises as an outgrowth from the diencephalon to which it remains connected via the optic stalk and the optic nerve, respectively (Mann, 1964; Rodieck, 1973). Compared with the whole embryo or other parts of the brain, the anlage of the retina develops very early. During early embryonic stages, about half of the head's volume is occupied by the constituents of the visual system, i.e. the eyes, the connecting fibre pathways, and the central projection areas. The retina can be easily accessed for experimental approaches; however, the most important advantage is its relatively simple organization when compared to other brain parts like the cortex. The adult retina is composed of only two plexiform layers (outer plexiform layer [OPL] and inner plexiform layer [IPL]), where exclusively fibres and synapses are found, and three nuclear layers containing a limited number of neuronal cell types: photoreceptors in the outer nuclear layer (ONL); horizontal, bipolar, amacrine, and interplexiform cells in the inner nuclear layer (INL); and ganglion cells in the ganglion cell layer (GCL) (Wässle and Boycott, 1991). However, the retina is far from being a "simple" or "fully understood" tissue. Up to now, we can only estimate the number of cell subtypes or used transmitters, for example (Strettoi and Masland, 1996). Rather than discussing the functional significance of the neuronal part of the retina, here we wish to deal with a basic constituent of its glial compartment: the radial Müller cells.

Glia cells, more than glue

Glia cells were discovered by Rudolf Virchow in 1846 and named after their putative role which Virchow assigned to them: glia (glue). Originally, they were considered as constituents of a connective-like tissue, in which the neurons are embedded and which subserves no further functions. However, half of the brains's volume is occupied by glia cells, and often they dramatically outnumber the neurons. In mammalian brains there are about 10 times more glia cells than neurons (Bignami, 1991; Steinhäuser, 1996). After a long period of neuron-centred neuroscience, glia cells have received more and more attention during the last decades (the reader is referred to a comprehensive textbook dealing with many aspects of glial biology: Kettenmann and Ransom, 1995). In the meantime, it is accepted that glia cells are multifunctional cells and important mediators of a variety of different capacities. Besides many glia-specific functions, they also have many capacities formerly addressed exclusively to neurons. It could be shown that e.g. glia cells are responsible for the structural stabilization of nervous tissue and that they serve as guiding structures for migrating neurons. Glia cells regulate the balance of relevant ions and they insulate neurons. They provide trophic substances to neurons, they remove metabolic waste, and they are responsible for the failure of the central nervous system to regenerate. Via cell recognition molecules, gap junctions or specific receptors, they can communicate with each other, with neighbouring neurons and their axons, but also with non-neuronal cells or the extracellular matrix. Since glia cells also express receptors for neurotransmitters and other neuro-active ligands, contributions of glia cells to the modulation of neuronal information processes or even independent glial information processing and extra-neuronal signalling pathways are discussed (Müller, 1992; Newman and Zahs, 1997).

Müller cells are specialized astrocytes

In contrast to the enormous number of different neuronal cell types throughout the brain, glia cells can be subdivided into three morphologically distinct groups: a) astrocytes, possessing a large nucleus and prominent processes (including Müller cells); b) oligodendrocytes, possessing a small nucleus and short processes (the myelin-producing cells of the central nervous system); c) microglia, in many aspects atypical glia cells and more related to the immune system (macrophage-like cells).

Astrocytes and oligodendrocytes are summarized as macroglia. From an ontogenetic point of view and based on studies in rat optic nerve, both groups derive from the neuroectoderm. One can distinguish two types of astrocytes: A1- and A2-type astrocytes. Whereas A1-astrocytes have a separate progenitor cell, A2-astrocytes and oligodendrocytes share a common, so-called O2A-progenitor cell (Raff et al., 1983; Miller et al., 1989; Pfeiffer et al., 1993; Miller, 1996). Evolutionary older fish seem to lack an independent astrocytic lineage; thus, all oligodendrocytes and astrocytes appear to share one common progenitor (Sivron et al., 1992; Sivron and Schwartz, 1995). With respect to their morphology and their biochemical equipment, Müller cells are astrocyte-related cells. Molecularly, they express vimentin (Dräger, 1983; Pixley and de Vellis, 1984; Schnitzer, 1985; Robinson and Dreher, 1990) and under certain conditions the astrocytic marker, glial fibrillary acidic protein (GFAP; Vaughan et al., 1990). Microglia most probably derive via the blood cell-lineage from mesodermal cells. Several authors consider these cells not as glia cells but rather as specialized cells of the

immune system (Oehmichen et al., 1979; Graeber and Streit, 1990). Another classification for macroglia cells was proposed by Roitbak (1983) and Reichenbach (1989). They introduced the term "glion", analoguous to the term "neuron", for a hypothetic, general glia cell. Based on criteria of process specializations, they subdivided the macroglia cells into three groups. Accordingly, so-called "process I glions" contact the ventricle, "process II glions" contact mesenchymal spaces, and "process III glions" contact different compartments of the neuropil. Since this classification was based on the contacts glia cells are able to make, Müller cells would belong to all three categories, since their processes contact ventricular, mesenchymal and neuropil compartments. Sometimes they are even involved in the myelination of ganglion cell axons, normally considered a classical function of oligodendrocytes (Hughes and LaVelle, 1974; Prada et al., 1989).

All types of glia cells can be found in the retina, however, only Müller cells are retina-born

All types of glia cells can be found in the retina, however, important species differences are encountered. Ontogenetically, Müller cells are the only glia cells which are retina-born. Astrocytes, oligodendrocytes and microglia immigrate into the retina via the optic stalk or the blood vessels from sources located outside the retina. Whereas Müller cells are present in all species studied so far, the equipment with other glia cells mainly depends on whether the retina is vascularized or not. In non-vascularized retinae like in birds or some mammals like guinea pig or rabbit, Müller cells are typically the only glia cell population. However, oligodendrocytes and a myelinated optic fibre layer in the chicken retina have also been described (Nakazawa et al., 1993). In vascularized retinae like in humans, typical astrocytes occur, which in this case are associated with the blood vessels. Both Müller cells and astrocytes are thought to be involved in the formation of the blood-retina barrier (Huxlin et al., 1992; Tout et al., 1993), and it was supposed that Müller cells are functionally interchangeable with astrocytes to a certain degree (Holländer et al., 1991). Oligodendrocytes can also affect retinal circuitry, since in some species like rabbits, they participate in forming the myelin sheath of the ganglion axons in the optic fibre layer and optic nerve. Microglia cells also enter the retina via the blood vessels. During development or in certain pathological states, their number steadily increases (Vrabec, 1970; Murabe and Sano, 1981, 1982; Schnitzer, 1989; Navascués et al., 1995). Due to their embryonic origin and since the major functions of these cells are phagocytosis of cellular debris and presentation of antigens (Thanos, 1991), these cells are more related to the immune system and subserve other functions than the macroglia cells.

Müller cells are regularly organized

Müller cells were first described in 1851 by Heinrich Müller, then working in Würzburg. Beginning with the innovative work of Ramon y Cajal (1893), the morphology of Müller cells was investigated with a set of appropriate classical stainings like Golgi impregnations (Ramon y Cajal, 1893; Reichenbach et al., 1989), with enzymatically dissociated cells (Reichenbach et al., 1987, 1988), or with electron microscopic studies (Meller and Tetzlaff, 1976; Uga and Smelser, 1973; Bhattacharjee and Sanyal, 1975).

Lectin binding studies using peanut agglutinin (Arregui et al., 1992) and antibody techniques are other powerful approaches to study Müller cells. In the meantime, valuable sets of specific antibodies were generated to visualize their fine morphology. Müller cells have been successfully labeled with antibodies to glia-specific antigens or widespread intermediate filament proteins like glial fibrillary acidic protein (GFAP; Erickson et al., 1987; Eng and Shiurba, 1988; Distler et al., 1993), or vimentin (Schnitzer et al., 1981; Bignami et al., 1982). Antibodies to other Müller cell- or radial glia cell-specific proteins like glutamine synthetase (Linser and Moscona, 1979, 1981), carbonic anhydrase II (Linser et al., 1984; Palatroni et al., 1990), cellular retinoic acid ligand binding protein (CRALBP; Sheedlo et al., 1995), alpha-crystallin (Moscona et al., 1985); the antigens 3A7 (Lemmon, 1985), 3F11, 4F3, 3F8 (Dreher et al., 1992, 1994), 2M6 (Schlosshauer et al., 1991), 3CB2 (Prada et al., 1995), or RC1 (Edwards et al., 1990) represent only an arbitrary selection among others.

Considering their morphology, their molecular equipment and their spatio-temporal occurrence during decisive phases of neural development, Müller cells share many similarities with other radial glia cells. Typically, radial glia are present transiently during early and middle stages of development, occuring often in evolutionary "young" parts of the brain. During these early stages, radial glia cells are often the only glia cell population. This indicates that they have to subserve many of the glia-related functions mentioned above. However, as in cerebellum or cortex, their most important function is the guidance of migrating and differentiating neurons from the ventriclar zone to their final destinations (Rakic, 1972). During tissue maturation, radial glia cells normally disappear, while other glia cells become more and more abundant to take over the non-migratory functions of the radial glia cells. Besides the Bergmann glia cells in the cerebellum (Reichenbach et al., 1995), only in proliferative "hot-spots" of the adult songbird brain (Alvarez-Buylla et al., 1987, 1988, 1990; Alvarez-Buylla, 1992), in the olfactory bulb (Graziadei and Monti Graziadei, 1985), and in the retina, radial glia cells persist throughout life. Interestingly, most of these brain parts show remarkable capacities for cell proliferation and tissue regeneration in late embryonic stages or even in adults.

Müller cells span the entire width of the retina and occupy a remarkable spatial volume. They are arranged very regularly like spokes or columns since they ensheath their accompanying neurons like a stocking. The retinae of birds and mammals contain many more cells than retinae of lower vertebrates. Thereby, the neuron-to-Müller cell ratios reach only values from about 5-8 in lower vertebrates, while they increase to values from about 18-33 in mammals or birds (Dreher et al., 1994; see Reichenbach and Robinson, 1995a). Concomitantly, the areal densities of Müller cells increase from about 1000 per square mm in amphibians or reptiles to 9,000-17,000 cells per square mm in central areas in mammals and birds due to the higher packing densities of cells in higher vertebrates (Dreher et al., 1994). Spatially, there exists a decreasing central-to-peripheral gradient of Müller cell density, i.e. in central retina Müller cell density is about twice of that in the periphery.

Fine morphology of Müller cells is adapted to the microenvironment

The general shape of Müller cells is mainly determined by their radial organization. Although there are considerable morphological variations even within one retina, among related species, and among different vertebrate groups, some features are commonly present (Fig. 1). The cell body with the nucleus is located within the INL. To the scleral and vitreal surface of the retina, the cell extends two long processes contacting the outer limiting membrane (OLM) and the inner limiting membrane (ILM). Constituents of these processes partially contribute to the structure of both "membranes". Müller cells are ideally positioned to supply neurons with nutrition and to regulate the flow of metabolic substances between the retina and the vascular system. Not surprisingly, Müller cells in avascular retinae store more glycogen and they have more sclerally located mitochondria and microtubules, necessary for efficient uptake from the subretinal space (Magalhaes and Coimbra, 1970, 1972). Significant intraretinal variations can often be observed, e.g. in the rabbit, short thick Müller cells with big endfeet prevail in the retinal periphery, whereas long Müller cells with small endfeet occur in the thicker central retina (Reichenbach et al., 1989). In fish, amphibians and mammals, the vitreal process is very thick (Robinson and Dreher, 1990; Dreher et al., 1992). In reptiles and birds, it splits into numerous thin parallel filamentous processes when entering the IPL to terminate as small vitreal expansions (Prada et al., 1989; see Fig. 1). It was proposed that this sauropsid feature represents an adaption for effective K^+-buffering (Newman et al., 1984; Eberhardt and Reichenbach, 1987) and removal of carbon dioxide, as well as for the absorption and distribution of pecten-derived nutrient substances in the metabolically highly active, yet avascular avian retinae (Dreher et al., 1994; Newman 1994). At the inner limiting membrane, characteristic endfeet structures (Dreher et al., 1988) are

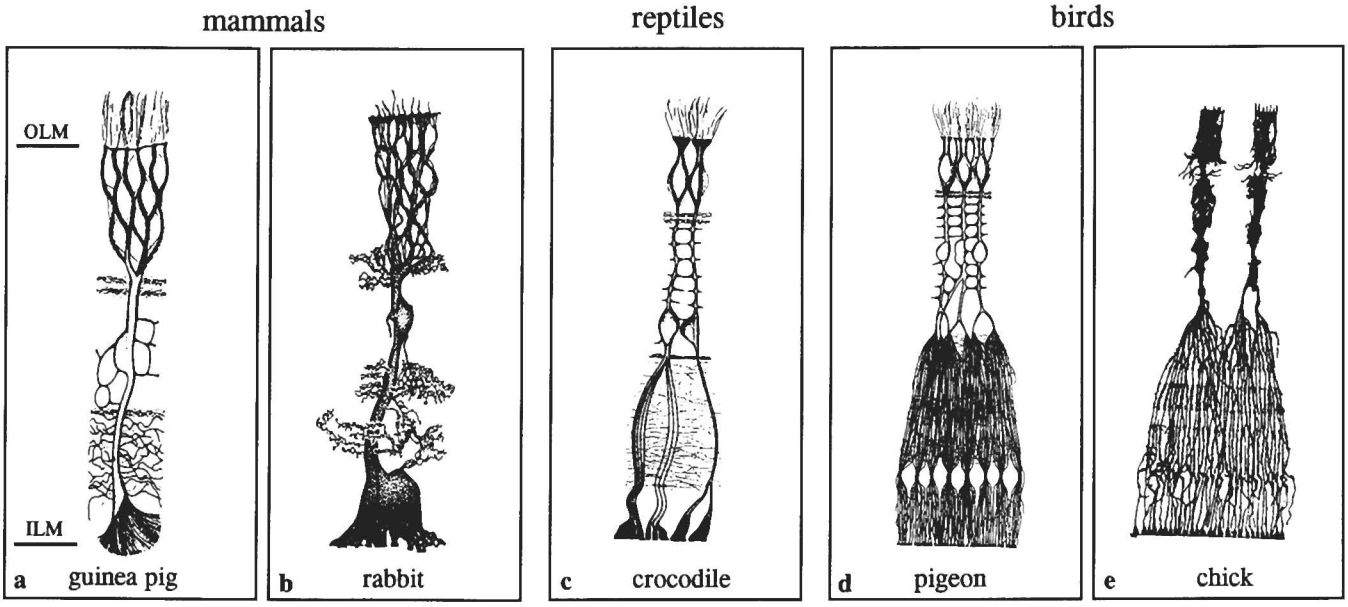

Fig. 1. *Morphology of Müller cells in different vertebrate groups.* Müller cells span the entire thickness of the retina from the outer to the ILM. At the vitreal border, they form typical endfeet structures, which are involved in the formation of the ILM. At the scleral border, their surface is enlarged by many microvilli. In the ONL and INL they ensheath the cell bodies of the photoreceptors and interneurons like a basket. In the OPL they form horizontal rootlets and in the IPL corresponding branchlets, both probably involved in the fine organization of these strata. In mammals, the scleral part of the cell in the IPL remains single and thick, whereas in birds, it splits off into many fine fibres. In the closely related reptiles, a comparable splitting can be observed. (a, after Dreher et al., 1992; b, after Robinson and Dreher 1990; c and d, after Dreher et al., 1994; e, after Ramón y Cajal, 1893).

formed which contact the basal lamina and anchor the cells. In all vertebrates except anurans, endfeet membranes carry typical so-called orthogonal arrays of particles (OAP). These are P-face-associated arrays of rectangularly arranged subunits, which are suggested to be correlated with K^+-channels (Dermietzel, 1973, 1974; Berg-von der Emde and Wolburg, 1989; Bolz and Wolburg, 1992; Wolburg, 1995). Additionally, the ILM (Halfter et al., 1983) and Müller cell endfeet (Stier and Schlosshauer, 1995) are a preferred substrate for the directed outgrowth of ganglion cell fibres towards the optic nerve head. The shape of the scleral process depends on the thickness and cell numbers of the INL and ONL. In fish, amphibians and mammals, the ONL is quite thick consisting of several rows of photoreceptors. Here, the scleral process splits into several thin processes ensheathing the photoreceptors (Collin et al., 1996a,b). In birds, the ONL consists of a single row of photoreceptors. Therefore, the scleral process remains single and thick. The scleral surface of all Müller cells carries numerous microvilli which extend into the subretinal space. Sometimes, a cilium with up to now unknown functions can be found (Ennis and Kunz, 1986). At the level of the OLM, adjacent Müller cells and photoreceptors are interconnected mostly by zonulae adhaerentes. Rarely, other types of connections can also occur like tight junctions in fish or intermediate junctions in some amphibians. In the INL, the Müller cells form filigrane basket-like processes ensheathing the neuronal somata. Comparable specialisations can also be found in the OPL and most prominently in the IPL. Fine perpendicularly oriented so-called rootlets in the OPL (Dreher et al., 1994) and so-called branchlets in the IPL (most prominently developed as "sunbursts" in cat; Stone et al., 1991) form a delicate net-like system. Since the lateral processes of neighbouring Müller cells are long enough to overlap and create contacts, this system might be a candidate to direct inner-retinal fibre outgrowth and to support the establishment and organization of the different synaptic sublayers during development (Marc, 1986; Reichenbach et al., 1989; Robinson and Dreher, 1990; see Fig. 2). In the functional retina, this system is involved in the ensheathment of neuronal processes and synapses in both plexiform layers.

In some species there is also evidence for intraretinal junctional contacts between Müller cells which would allow them some degree of lateral communication (Reale et al., 1978; for a discussion of this topic see Cook and Becker, 1995). Such a system might be comparable to the compound system of astrocytes in vascularized retinae which are coupled by gap junctions. Thus, they are forming a functional syncytium capable for long-range signalling (Yamamoto et al., 1990). However, gap junctions between Müller cells were only found in frogs and toads (Uga and Smelser, 1973) and rabbit Müller cells in vitro (Wolburg et al., 1990). Interestingly, the Müller cell system and the astrocyte system are coupled by gap junctions in rabbits (Reichenbach and Robinson, 1995b). In rat retina, a functional linkage between these two systems could be demonstrated by synchronously travelling calcium waves (Newman and Zahs, 1997) and in rat brain, transcellular radial glia labeling with retrograde tracers revealed a comparable close relation between neurons and glia (Kageyama and Robertson, 1993).

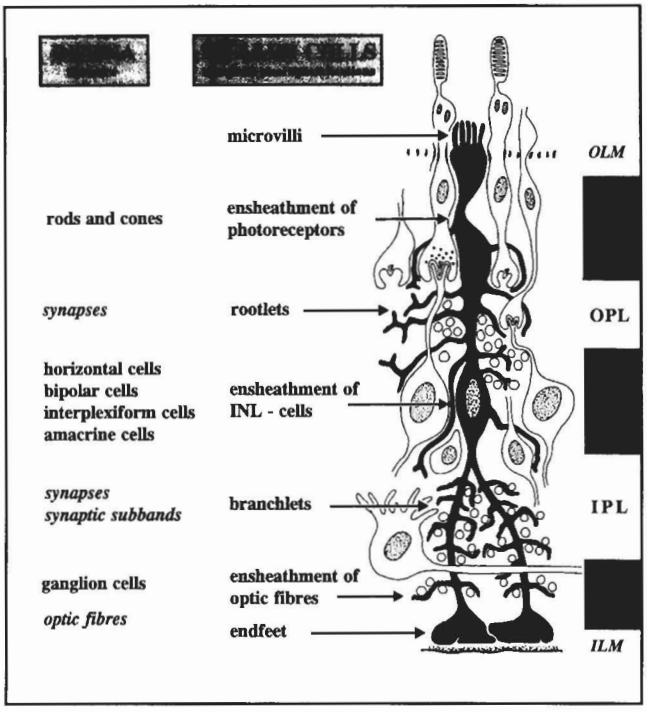

Fig. 2. *In each retinal layer, Müller cells show specific specialisations.* In the OPL and IPL typical side protrusions occur, whereas in the ONL and INL the Müller cells ensheath their related neurons. Microvilli at the scleral side and endfeet structures at the vitreal side contact the OLM and ILM (partly after Meller and Glees, 1965).

Müller cells and neurons share common progenitors

The regular arrangement and the intimate contacts between neurons and Müller cells do not only indicate their close relationship, but also reflect that they descend from a common progenitor. After invagination of the eye anlage, the outer sheet of the eye cup will form the pigmented epithelium while the inner sheet is destined to become the functional retina. All data so far indicate that the cells forming this early neuroepithelium represent an uniform population of developmentally equivalent progenitors (Nakafuku and Nakamura, 1995). Each retinal progenitor cell has the capacity to generate all types of neurons - as well as Müller cells - as could be nicely shown by studies using retrovirus-mediated gene transfer (Turner and Cepko, 1987), fluorescent dye labeling of individual cells (Wetts and Fraser, 1988; Wetts et al., 1989), or clonal-density cell culture (Jensen and Raff, 1997).

When are Müller cells born?

The question when as to Müller cells are born is crucial when dealing with possible developmental functions of Müller cells (Robinson, 1991). Although the retinal precursor cells are capable of generating both glial and neuronal cells in all species studied up to now, there exists a similar spatio-temporal pattern of cytogenesis and cellular differentiation. The first postmitotic cells are ganglion cells located in the central part of the retina (Holt et al., 1988; Altshuler et al., 1991; Jacobson, 1991). The generation of ganglion cells then proceeds in an orderly central-peripheral gradient, i.e. the last ganglion cells are born at the retinal periphery. This central-peripheral gradient is modified by a temporal-nasal gradient showing a developmental advantage of the temporal side of the retina (Fujita and Horii, 1963; Kahn, 1974; Layer and Kotz, 1983; Liu et al., 1983; Spence and Robson, 1989; Prada et al., 1991). Concomitantly, the rates of thymidine uptake and of self renewal of stem cells first diminish near the centre of the retina, while it is still pronounced towards the periphery (Dütting et al., 1983; Hernández-Sánchez et al., 1994). In most species two distinct phases of cell proliferation can be distinguished in which specific cell types are generated. It is presumed that the potential fate of the early totipotential progenitor cells is increasingly reduced during subsequent cell divisions. Additionally, the daughter cells may undergo successive states of competence, thus generating mosaic-like patterns of different progenitor subpopulations capable of generating specific limited subsets of cell types (Cepko et al., 1996; Alexiades and Cepko 1997). The temporal course of cell proliferation roughly follows a vitreal-scleral gradient, i.e. the last mitotic cells are found in the scleral part of the INL, whereby the dorsal retina is slightly developmentally advanced when compared with the ventral retina (Prada et al., 1991). During the first phase, ganglion cells, cones, horizontal cells and a subpopulation of amacrine cells (so-called phase 1-cells, according to the terminology of Reichenbach, 1993) are produced. During the second phase, Müller cells, rods, bipolar cells and some amacrine cells are born (so-called phase 2-cells; for review and further references see Reichenbach, 1993; Reichenbach and Robinson, 1995a; see also Fig. 3). These studies were mostly performed

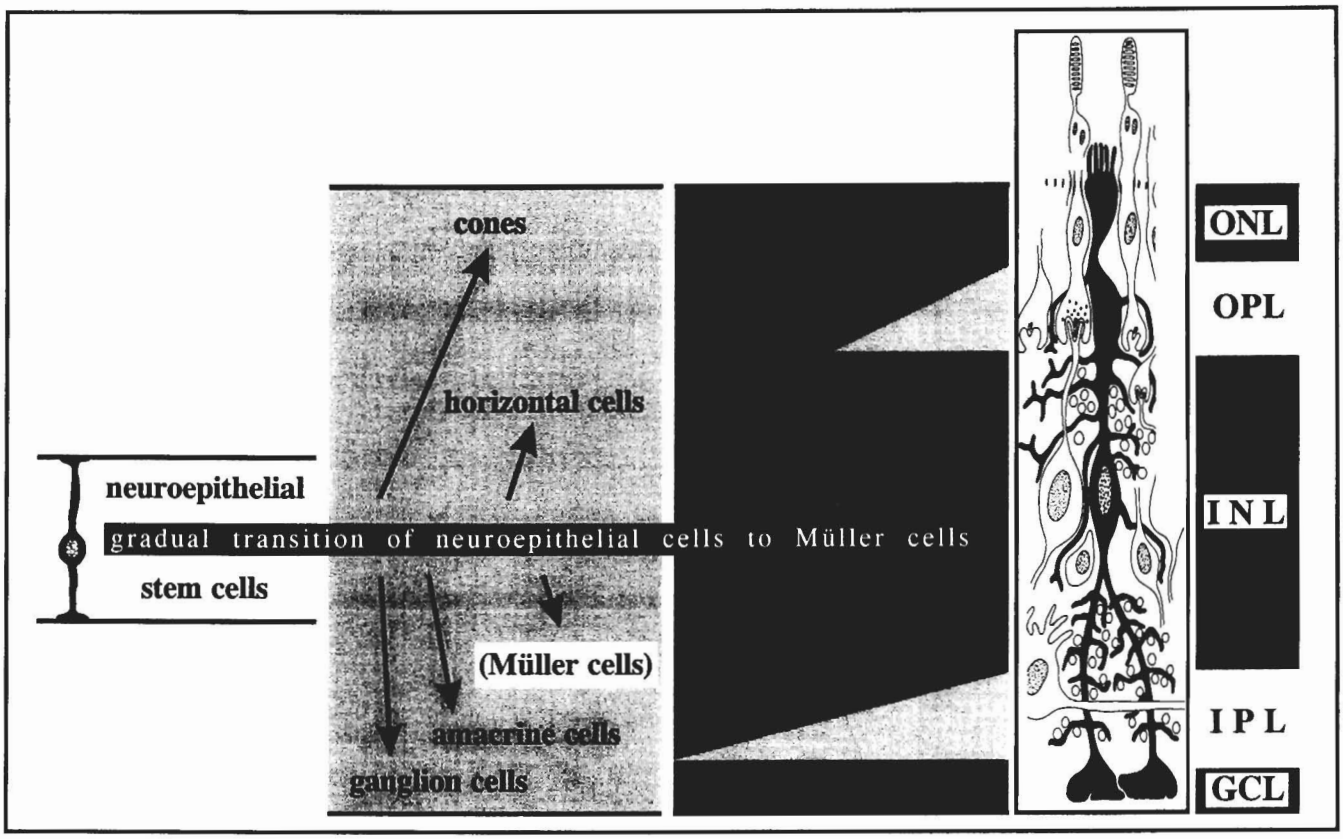

Fig. 3. *The "gradual maturation hypothesis of Müller cells".* According to this model, Müller cells can be produced at any stage of retinogenesis, dependent on environmental cues. An early phase of self-renewal of multipotential stem cells leads to an areal increase of the neuroepithelium. During a first phase of proliferation, mainly cones, horizontal, amacrine and ganglion cells are generated. They are stabilized and kept together radially into cell columns by Müller cells. During a second phase of proliferation, the remaining stem cells (possibly immature Müller cells) produce rods and bipolar and amacrine cells before the rest of them transform into mature Müller cells. Concomitantly, the separation of cell and fibre layers becomes apparent and the fine architecture of the retina is established (insert partly after Meller and Glees, 1965).

using [^{3}H]-thymidine-autoradiography; comparable results came out by retroviral studies. According to these data, Müller cells should appear only late during retinogenesis, and hence, early developmental functions of these cells would seem to be rather unlikely (Turner et al., 1990).

However, things are more complicated. Using other techniques like scanning electron microscopy (Uga and Smelser, 1973; Meller and Tetzlaff 1976), immunocytochemistry (Lemmon, 1985; Chan-Ling, 1994), fluorescent dye labeling (Reinicke et al., 1996), chick/quail chimeras (Layer et al., 1990; Willbold et al., 1995), or Golgi impregnations (Prada et al., 1989), Müller cells were shown to be present from the very beginning of retinal development, i.e. they are among the earliest retinal cells born (see also Robinson and Dreher, 1990 and some further literature therein). Can these contradictory views be explained? Although Müller cells and neurons emerge from the same progenitor cells, the crucial point seems to be that Müller cells retain a remarkable plasticity. In contrast to neurons which become comitted once, and then will never change their fate furtheron, Müller cells seem to have different functions during different phases of retinogenesis. These different functions may explain the different responses of Müller cells to specific antibodies or histochemical reactions, as well as their capacity for prolonged cell proliferation. Since Müller cells have characteristic endfeet structures forming an ILM on their vitreal sides and extending microvilli-like structures into the apical space, they share many features with ependymoglia. These cells represent an archaic type of glia cells (Reichenbach and Robinson, 1995b). Additionally, there are - compared with the overall retinal cell population - proportionally more Müller cells in cone-dominated than in rod-dominated retinae and furthermore, the density of Müller cells seems to be independent from rod but closely related to cone density (Reichenbach and Robinson, 1995a). Cones are older in evolution than rods (Okano et al., 1992). This indicates that cones were among the first cells occurring in tissues specialized for visual perception. The intimate relationship between cones and Müller cells therefore also points to a very early phylogenetic and ontogenetic appearance of Müller cells. Hence, there is no doubt that Müller cells - or immature precursors of them - must be among the first cells arising in the retina. However, their regular differentiation to adult Müller cells takes place only later. Additionally, they, or at least a subpopulation of them, retain the capacity to proliferate; therefore, they cannot be detected with [^{3}H]-thymidine- or BrdU-studies. Since Müller cells and the progenitors of the phase 2-cells share many similarities, it has already been supposed that these late progenitors are in fact immature Müller cells (Reichenbach and Robinson, 1995a). This wide range of Müller cell plasticity is also underlined by the observation that after injury of the adult retina, Müller cells re-enter the proliferation cycle to establish a glial scar (Burke and Smith, 1981).

The "gradual maturation hypothesis of Müller cells"

Based on these considerations, we here present the gradual maturation hypothesis of Müller cells (Fig. 3). We suggest that Müller cells are present from the very early beginning of retinal development and that more of them are generated throughout the period of retinal cytogenesis. However, due to their remarkable plasticity during retinal maturation, their morphological shape, their biochemical equipment and their functions all change continuously. The transition of early radial neuroepithelial precursor cells to adult Müller cells seems to be a graded process. This process does not run strictly in one direction, but - under certain conditions - may be reversible to some extent.

Possible developmental functions of Müller cells: facts and hints

Degenerative and embryological mechanisms are often closely linked. The etiology and possible repair strategies for many pathological states may be related to embryonic developmental mechanisms. Therefore, the study of developmental mechanisms may be useful for the comprehension of many pathological problems. It is known that e.g. Müller cells play a central role in some diseases like proliferative vitreoretinopathy (Machemer, 1988) or different types of retinal degeneration (DiLoreto et al., 1995; de Raad et al., 1996; Molthagen et al., 1996). This not only strengthens again the plasticity and dynamic role of Müller cells, but also underlines their important functions during development. However, what kind of roles may the Müller cells play?

It is widely accepted that in the retina only one type of progenitor cell exists which gives rise to Müller cells and all neuronal types. This issue however raises the question as to how each individual cell is destined to its final fate and location to fit into the retinal architecture and circuitry. It becomes more and more obvious that the mechanisms of retinal cell fate determination and histogenesis depend on complex interactions with environmental factors. Although genetic and cell-intrinsic factors seem to be also important (Watanabe and Raff, 1990; Williams and Goldowitz, 1992; Williams et al., 1996), it has been inferred that micro-environmental cues largely dictate the final fate of postmitotic retinal neurons, at least partly by cell-cell signalling (Turner and Cepko, 1987; Holt et al., 1988; Wetts and Fraser, 1988; Turner et al., 1990). However, only a few candidates for such cues could be identified up to now. It could be shown that, e.g. *Sonic hedgehog* protein is a putative mitogen for precursor cells (Jensen and Wallace, 1997), that thyroid hormone is able to induce cone photoreceptors (Kelley et al., 1995), and that ganglion cells are committed from progenitor cells by the *Notch*-pathway (Austin et al., 1995; Kopan and Turner, 1996; Bao and Cepko, 1997). It could be also shown that the level of expression of epidermal growth factor (EGF) receptor (Gospodarowicz et al., 1986)

contributes to the regulation of cell fate (Lillien, 1995). Basic fibroblast growth factor (bFGF) is generally involved in the control of cellular proliferation (Lewis et al., 1992) and also in retinal differentiation and regeneration (Park and Hollenberg, 1989; Pittack et al., 1991, 1997; Opas and Dziak, 1994). It is discussed that early committed cell populations such as ganglion cells or photoreceptors represent master cells which initiate the consequent determination of other cell types within a developmental hierarchy. Not surprisingly, ganglion cells and photoreceptors are the most vitreally and sclerally located cell populations. Such a scheme would be open to the question whether retinal differentiation is an autochthonic process or whether it is initiated and triggered from the outside.

Müller cells as candidates for providing positional information

The multitude of potential factors affecting retinogenesis indicates that factors from the outside and retina-born factors are involved in the regulation of progenitor cell proliferation and determination of the final cell fates. A prerequisite for their complex interactions and signalling cascades is a suitable transport system for the efficient distribution of cues. Due to their architecture and molecular equipment, Müller cells are now well-suited candidates for providing such a system, because: 1) the microvilli-system and the filter-system of the zonulae adherentes at the level of the OLM point to specific uptake mechanisms at the scleral surface of Müller cells; and 2) due to their columnar radial organization and intraretinal specialisations, Müller cells represent a three-dimensional reference system for their related adjacent neurons. These capabilities are independent from the origin of the signals; however, glia-derived signals initiate rather than determine the final levels of differentiation.

Another crucial requirement for possible developmental functions of Müller cells is the fine-tuned spatio-temporal expression of molecules enabling them to efficiently communicate with other cells or with the environment. All cytotopographical spezialisations of Müller cells are accompanied by specific biochemical properties of the membrane (for a comprehensive review see Reichenbach, 1989), altogether enabling Müller cells to form complex and fine-tune spatial interactions with their environment. It has already been shown that Müller cells are equipped with receptors to respond to a wide range of autocrine and paracrine signals deriving from the retina (Hicks et al., 1992), the pigmented epithelium (Tanihara et al., 1993a,b; Jaynes and Turner, 1995; see below), the vascular system, and inflammatory cells (see Puro, 1995). Müller cells have receptors to nerve growth factor (NGF; Chakrabarti et al., 1990), and epidermal growth factor (EGF) and transforming growth factor (TGF) alpha are able to induce c-fos gene expression (Sagar et al., 1991). Early Müller cells express insulin-like growth factor (IGF) binding proteins as well as transferrin in their endfeet (Zeevalk and Hyndman, 1987). The latter process is highly coordinated with the expression of IGF-receptors on ganglion cells (Lee et al., 1992). Such observations are in line with the fact that the survival of early ganglion cells (a presumed master cell population) transiently depends on Müller cells. Later they become dependent on their target cells in the superior colliculus. (Raju and Bennett, 1986; Armson et al., 1987). Müller cells also play an important role during the final maturation of photoreceptors (another presumed master cell population) as could be shown by using the specific glia toxin, DL-α-aminoadipic acid (Rich et al., 1995). Most of these capacities are associated with adult or maturing Müller cells; however, there might also exist comparable capacities during early development.

Do Müller cells mediate pigment epithelium-derived retinogenetic signals?

One of the most important non-retinal sources for factors determining retinal development is the pigmented epithelium. After retinectomy or under specific experimental conditions, cells of the pigmented epithelium have the remarkable capacity to trans-differentiate themselves into neural retina (Orts-Llorca and Genís-Gálvez, 1960; Coulombre and Coulombre, 1965; Okada, 1980; Stroeva and Mitashov, 1983; Reh et al., 1987). The progression of retinal degeneration in the RCS rat (which has a pigmented epithelium defect) can be halted by the transplantation of normal pigmented epithelium (Li and Turner, 1988; López et al., 1989), and retinal grafts are better differentiated in the presence of co-grafted pigmented epithelium (Aramant et al., 1990). When pigmented epithelium is explanted on a nitro-cellulose filter, it strongly promotes cell proliferation and tissue lamination of retinal explants (Liu et al., 1988). Pigmented epithelium-deficient transgenic mice show a distorted retinal differentiation (Raymond and Jackson, 1995) and dispersed retinal cells re-arrange histotypically into cellular spheres (so-called stratoids) only in the presence of a monolayer of pigmented epithelium (Rothermel et al., 1997). Molecularly, proteins of the *hedgehog* gene family (Levine et al., 1997) and several pigmented epithelium-derived retinogenetic factors (Campochiaro et al., 1989; Tombran-Tink et al., 1991; Campochiaro, 1993) have been shown to promote neurite outgrowth and retinal progenitor cell production (Sheedlo and Turner, 1996a).

These observations are in line with studies using pigmented epithelium-derived factors secreted into a defined medium. These conditioned media promoted photoreceptor and Müller cell proliferation and differentiation (Jaynes and Turner, 1995) as well as the up-regulation of mRNA for the immediate early genes NGFI-A and c-fos in both Müller cells and pigmented epithelium (Jaynes et al., 1995). These factors also seem to promote the formation and stabilisation of Müller cell endfeet at the ILM (Wolburg et al., 1991). It seems

highly possible that Müller cells are involved in the mediation of pigmented epithelium-derived factors, and it remains a fascinating possibility that these factors act - at least partly - via the production and differentiation of Müller cells.

Müller cells express different types of cell recognition molecules

How can Müller cells contribute to cell-cell communication? During the last two decades, the different families of cell recognition molecules have been recognized as important mediators for cell-cell and cell-environment communication. Recognition molecules are involved in different processes like, e.g. sorting out processes or the regulation and guidance of migrating cells and fibres. Müller cells have been shown to express some of these molecules, like L1/NgCAM (Drazba and Lemmon, 1990) or 5A11 (Fadool and Linser, 1993). Besides these types of molecules, barrier-associated proteins like EAP-300 (embryonic avian polypeptide of 300 KDa) or claustrin (McCabe and Cole, 1992) have also been found. Both cell recognition molecules and barrier-associated molecules often act in a complementary manner as positive (i.e. supporting guidance and migration of cells and fibres), and negative (i.e. forming repulsive barriers) regulators of development. To further elucidate these questions, we searched for additional Müller cell-expressed recognition molecules. We were able to localize F11 (Rathjen et al., 1987), a member of the immunoglobulin superfamily, on the surface of Müller cells (Willbold et al., 1997a).

One of the most obvious features of the retina is its columnar organization, comparable to other brain areas like the cortex (Rakic, 1972; Luskin et al., 1988), the spinal cord (Leber et al., 1990) or the optic tectum (Puelles and Bendala, 1978; Senut and Alvarado-Mallart, 1987; Gray and Sanes, 1991). In these brain areas, a scaffold of radial glia cells acts as a guiding structure for migrating neurons (Hatten 1990, 1993; Rakic et al., 1994; Rakic, 1995). First of all, one could speculate that Müller cells may have a similar function. Nevertheless, this should occur in a somehow modified fashion, since in the retina neuronal migration by perikaryal translocation seems to prevail (Morest, 1970; Snow and Robson, 1995). Radial migration may also occur, however, mainly at later stages of retinogenesis. This would primarily concern the migration of the offspring of phase 2-cells, after the developing retina has already achieved a considerable thickness.

Therefore, we focused our interest onto possible repulsive barrier functions to better understand the organizing function of Müller cells. Müller cells are likely candidates for forcing the clonal offsprings of the retinal progenitor cells into radial cell columns. To investigate this issue, we used rotation-incubated so-called spheroids of dissociated retinal cells. Using gliotoxins and chimeras of chick and quail cells, we were able to follow the developmental fate of Müller cells and to look at their functions in organizing the complex retinal architecture.

Retinospheroids as model systems to study retinogenesis in vitro

How can possible developmental functions of Müller cells be followed and untangled? It is obvious that in vivo studies are indispensable, since only they can yield the final proof of any real biological significance. However, these experiments are often difficult to carry out, disturbing influences of neighbouring tissues cannot be excluded, and the developing structures can hardly be observed. To overcome most of these problems, a number of powerful in vitro culture techniques have been developed. Especially for developmental studies, the retinospheroid technology is most useful. This system is based upon the capacity of dissociated embryonic chicken retinal cells to aggregate, to proliferate, to differentiate and to re-organize themselves into histotypic structures (Adler, 1973; Honegger and Richelson, 1976; Akagawa et al., 1987). These processes are very similar to those observed during normal development. In fact, in vitro regenerates are morphologically and physiologically almost indistinguishable from a normal retina. Technically, tissues from defined retinal origins are dissociated into single cells and small cell clusters, respectively. After reaggregation under rotation culture conditions, these cells will then form so-called retinospheroids (Fig. 4). We use two different systems, one system (called rosetted spheroids) has been introduced by Moscona and his colleagues (Moscona, 1952, 1974; Garber and Moscona, 1972a, b; Hausman et al., 1976; Vardimon et al., 1991), a second system (called stratospheroids) was introduced by us (Vollmer et al., 1984; Layer and Willbold, 1989).

Rosetted spheroids are inversely laminated retinal aggregates

This system starts with dissociated cells from the central part of the functional embryonic retina. The reaggregates thus obtained are called rosetted spheroids. They show the formation of patterns similar but far from identical to a normal retina. Noticeably, a rosette structure arises which represents the centre for the organization of histotypic tissue. Rosettes can be observed in the retina both in vitro and under some pathological or experimental conditions in vivo (i.e. real Flexner-Wintersteiner rosettes; Shimada et al., 1973; Mayerson and Moscona, 1979; Hibbard and Ehrlich, 1982), but have not been shown in other brain regions (however, in some neuronal tumours so-called Homer Wright rosettes occur frequently; yet this type of rosette shows a completely different morphology and organization. It is not homologous to the Flexner-Wintersteiner rosette type Paulus, 1995). Rosettes are the very first and initial recognizable structures when

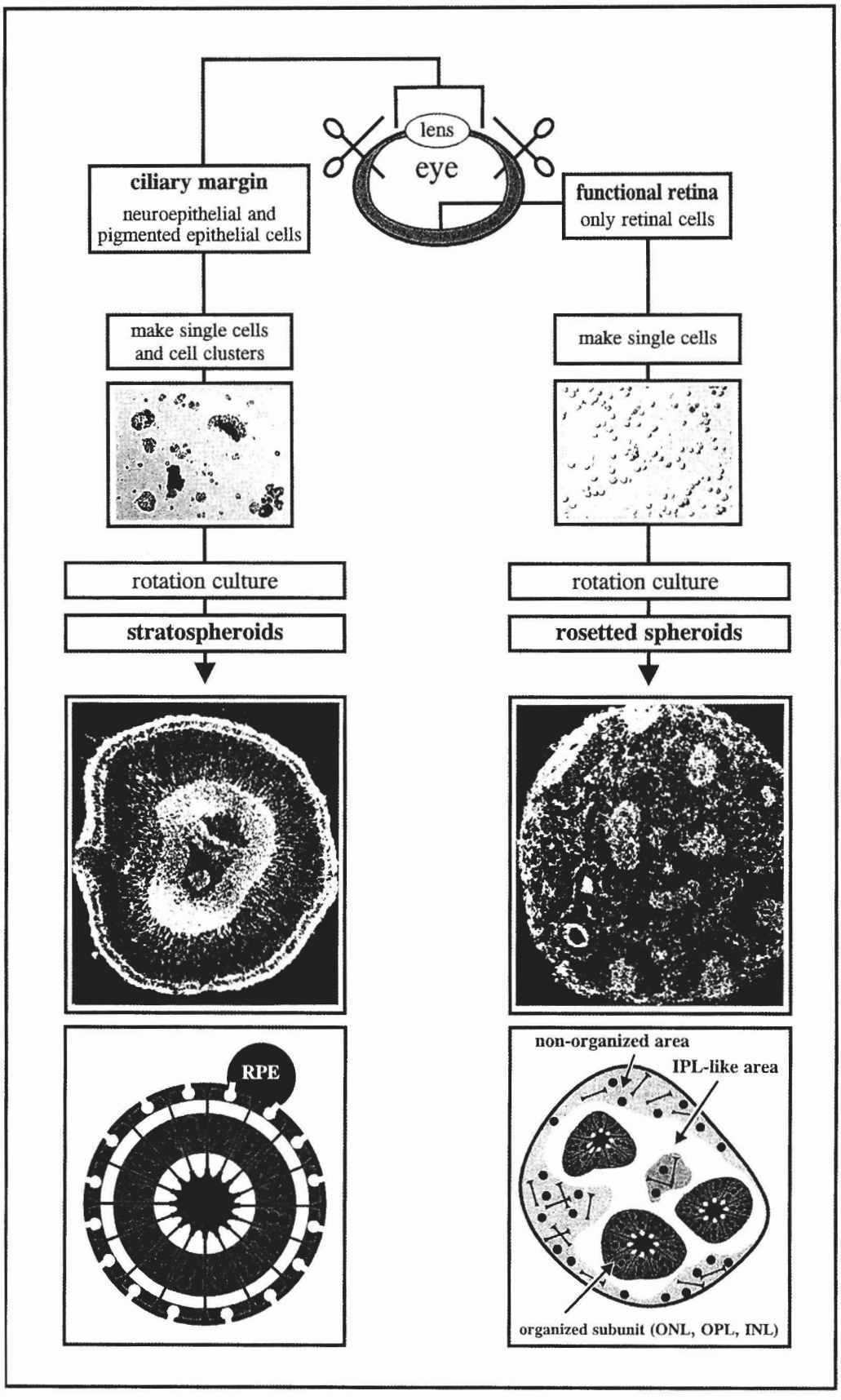

Fig. 4. *Retinospheroids as developmental model systems to study retinogenesis.* Stratospheroids (left) and rosetted spheroids (right) are two independent in vitro systems to study mechanisms of retinogenesis. Stratospheroids emerge from neuroepithelial and pigmented epithelial cells located in the ciliary margin. These cells are dissociated into single cells and cell clusters and rotation-incubated for up to 2-3 weeks. Especially by proliferation, a three-dimensional spherical structure is built up with a correct orientation of the retinal layers. Rosetted spheroids emerge exclusively from cells originally located in the central part of the retina. They are completely dissociated into single cells and are also rotation-incubated for up to 2-3 weeks. Rosetted spheroids are composed of several so-called subunits, each with a rosette in its centre. In their mature state, these rosettes hold photoreceptors. They are surrounded by an OPL, a radially organized INL and an IPL. IPL-like areas and so-called non-organized areas interconnect the retinal subunits.

dissociated single cells are put into rotation culture (Sheffield and Moscona, 1970; BenShaul and Moscona, 1975). Three-dimensionally, an initial rosette is a hollow spherical structure, whose wall is formed by a single row of mitotic cells (Layer et al., 1990). Thus, about 80% of all cells are produced in culture during the first 3-5 days (Vollmer and Layer, 1987; Willbold and Layer, 1992). Later, the wall of the mature rosette is formed by photoreceptors. They are separated by fibres of Müller cells which form an OLM sealing off the rosette's inner lumen.

By forming an OLM, and since rosettes are spherical structures, the Müller cells are radially arranged like the spokes of a wheel. In contrast to the in vivo situation, an ILM is not formed and the Müller cells end freely with their vitreal processes in the IPL (Wolburg et al., 1991). This entity structure consisting of a rosette, a radial Müller cell scaffold and accompanying neurons represents a subunit (Fujisawa, 1973). During early spheroid formation, several of these subunits fuse. Then, in each subunit, OPL- and INL-homologous areas develop and all main retinal cell types such as photoreceptors, bipolar, horizontal, amacrine or ganglion cells can be identified. The different subunits are interconnected either by matrix areas corresponding to an IPL or by areas composed of cells and fibres with no obvious organization ("non-organized areas", Layer et al., 1997b). Noticeably, the orientation of the cellular layers in the subunits is inverted as compared to the in vivo situation. In vivo, the photoreceptors are facing outwards towards the pigmented epithelium; in rosetted spheroids they are facing towards the inside of the rosette.

Stratospheroids are correctly laminated retinal regenerates

In contrast to rosetted spheroids which emerge from central retinal cells, stratospheroids develop from cells and small cell clusters of the pigmented epithelium together with non-pigmented neuroepithelial cells derived from the ciliary margin (Layer and Willbold, 1989). Cells of the pigmented epithelium support and strongly influence processes of retinal regeneration both in vivo (Coulombre and Coulombre, 1965; Stroeva and Mitashov, 1983; Park and Hollenberg, 1989) and in vitro (Pittack et al., 1991; Zhou and Opas, 1994; Zhao et al., 1995; Sheedlo and Turner, 1996b; Rothermel et al., 1997). Stratospheroids are not composed of rosettes and their corresponding retinal subunits, but instead, each spheroid represents a coherent spherical structure, expressing all three retinal cell layers in a correct polarity. Thus, the ganglion cells are located inside the spheroid, while the photoreceptors are facing the culture medium. Stratospheroids are generated in a totally different way to rosetted spheroids. Aggregation of individual cells plays only a minor role since the culture predominantly starts from cell clusters containing pigmented and nonpigmented cells. The pigmented cells induce neuroblasts to proliferate and differentiate by up to now unknown factors; concomitantly they also induce a correct laminar orientation of the newly produced cells. In the mature stratospheroid, a core of pigmented cells typically remains near the centre or at the periphery. Thereby, the pigmented epithelium core remains intimately connected with the retinal part of the spheroid (Vollmer and Layer, 1986). Both rosetted spheroids and stratospheroids allow us to study normal retinogenesis under constant and controlled conditions. Recently, we introduced a third system; so-called stratoids. These stratoids are correctly laminated like stratospheroids but develop from fully dispersed cells of the central retina under the influence of a pigmented epithelium monolayer (Rothermel et al., 1997).

Most importantly, the retinospheroid technology offers a number of advantages when compared to other in vivo or in vitro techniques:
- When compared to monolayer or slice cultures, growth of the tissues is not restricted to two dimensions, but is allowed to proceed into the third spatial dimension as well, due to the free floating of cells and spheroids in the culture medium.
- Starting from dissociated cells, the complex retinal architecture of retinospheroids is gradually built up and the final histological structure and physiological capacities are comparable to a normal embryonic retina.
- In retinospheroids, artificial cell-substrate interactions are absent and disturbing environmental factors are negligible.

Possible developmental functions of Müller cells tested in vitro

The cell recognition molecule F11 is expressed on Müller cells

A powerful communication system between Müller cells and their environment is a prerequisite for developmental interactions. What molecular equipment enables Müller cells to communicate with their neighbouring cells? Since Müller cells have already been shown to express 5A11, a cell recognition molecule involved in neuron-glia interactions (Fadool and Linser, 1993), we focused our interest onto the spatio-temporal expression of the cell recognition molecule F11, a member of the immunoglobulin superfamily. F11 is primarily expressed in brain but also weakly in lung and pancreas. F11 is involved in cell contact-dependent modulation of neuronal differentiation, for instance in neurite outgrowth and fasciculation (Brümmendorf and Rathjen, 1995). The role of F11 in specific cell-cell interactions during development is context-dependent: it can play the role of a ligand (Morales et al., 1993), or the role of a receptor component (Peles et al., 1995); or it can interact with extracellular matrix molecules (Rathjen et al., 1991; Zisch et al., 1992; Brümmendorf et al., 1993) or with axonal receptors (Brümmendorf et al., 1993). In brain, F11 is mainly associated with extending

neuronal processes. In the retina in vivo, F11 is expressed in the inner and outer plexiform layer (Layer and Willbold, 1989, 1994; Rathjen et al., 1991), as well as in a system of radially organized structures. Noticeably, this feature is much more pronounced in vitro. Using immunocytochemistry and Western blot analysis in stratospheroids, we could unequivocally show that these radially organized structures are identical to Müller cells (Willbold et al., 1997a; see Fig. 5). Since the F11-molecule has been studied primarily in the context of its neuronal expression, this glial expression may help to explain a migration-supportive function of Müller cells. Additionally, it makes new interactions between glial and neuronal structures possible. Interestingly, F11-expression seems to be a unique feature of Müller cells, since we were not able to localize F11 on other radial and non-radial glia types in the central nervous system. This may be explained by the complex functions of Müller cells if compared with the more restricted functions of other radial glia cell types. Which potential functions of F11 on Müller cells are in line with this finding? It could be shown that L1/NgCAM, a F11-ligand, is involved in the radial migration of neuronal precursors in the forebrain and in the cerebellum (Chuong et al., 1987; Barami et al., 1994). In the retina, L1/NgCAM was shown to be involved in retinal ganglion cell neurite outgrowth on Müller cells (Drazba and Lemmon, 1990). Therefore, it seems conceivable that the expression of F11 on Müller cells is related to the radial migration of phase-2 cells and to the directed guidance of outgrowing fibres.

Müller cells retain the capacity to promote retinal lamination in vitro: only retinal spheroids develop a high degree of laminar and cellular organization

It is obvious that Müller cells differ in many aspects from other radial glia cells occuring in the brain; e.g. in

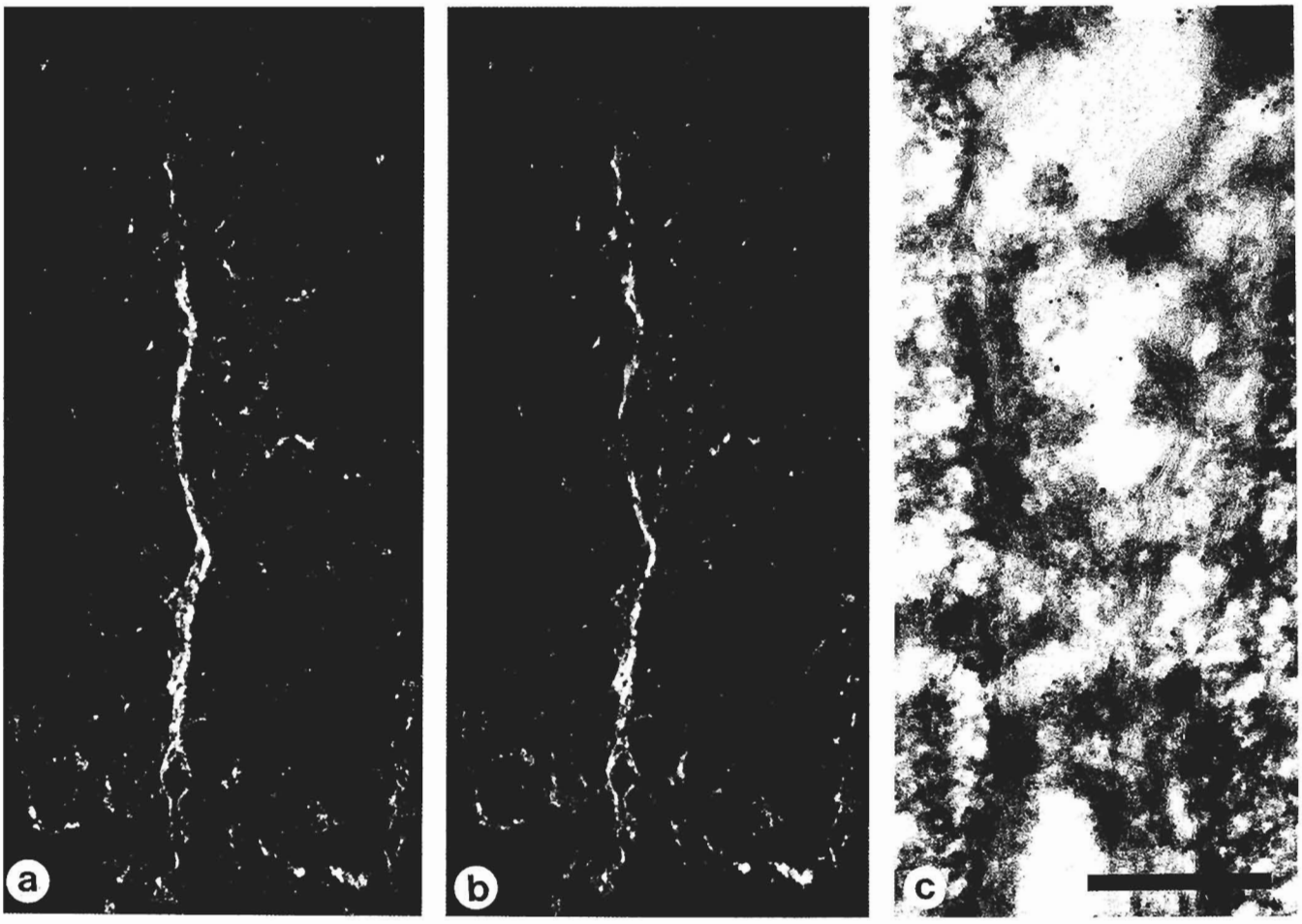

Fig. 5. *Müller cells express the cell recognition molecule F11.* F11 co-localizes with vimentin, a specific marker for Müller cells. Fluorescence optical view (a, b) and transmission electronmicroscopic view of semithin cryosections (0.3 μm) of a stratospheroid after 12 days in culture, double-labeled with F11 (a, detected with FITC; c, detected with 10 nm colloidal gold) and vimentin (b, detected with Cy3; c detected with 18 nm colloidal gold), respectively. In both preparations, partial views of a Müller cell are seen. Note the strict parallel and radial orientation of the cells (a, b) as well as the co-localization of both antigens on the Müller cells (c). Bar: a and b, 20 μm; c, 1 μm.

cortex, tectum, or cerebellum. This is also reflected by their specific behaviour in spheroids. Comparable to retinospheroids, dissociated cells from other brain regions are capable of forming spheroids as well. Tectal, telencephalic and cerebellar spheroids also show significant amounts of cell and fibre differentiation. However, only in retinal spheroids can a histotypic organization be detected with clearly separated cell and fibre layers as well as radially running cell columns. We suggest that this pattern is promoted by Müller cells which are able to reorganize an early radial scaffold. We observe a strict congruency between these radial scaffolds, the presence of rosettes, and the distribution of radially organized areas in rosetted spheroids. Tectal, telencephalic and cerebellar spheroids do not show organized radial glia scaffolds. Instead, the radial glia cells are randomly arranged and the spheroids do not show a histotypical organization (see Fig. 6). This organizing capacity of Müller cells in vitro also strongly suggests the existence of a comparable function in vivo. Moreover, since non-retinal radial glia cells are not able to reorganize a histotypic organization in vitro, Müller cells seem to be qualitatively different from other radial glia cells (Willbold et al., 1997b).

Müller cells stabilize retinal cell columns

From their radial architecture, from their regular arrangement and from their close ontogenetic relationship to their neuronal counterparts, Müller cells are primary candidates for establishing, organizing, stabilizing and sustaining the retinal architecture. To test these stabilizing functions of Müller cells, chimeric stratospheroids were prepared by mixing chick and quail cells at the beginning of the culture. Chick-quail chimeras have the advantage that the fate of quail cells can be selectively followed. They have an additional nucleolus which can be visualized by classical nuclear stainings (Le Douarin, 1973). Alternatively, whole cells and tissues can be detected by quail-specific antibodies (Lance-Jones and Lagenaur, 1987; Aoyama et al., 1992). In chimeric stratospheroids, chick and quail cells are not distributed randomly. They are arranged in a variable but nevertheless highly specific pattern (Layer et al., 1990). Each spheroid is composed of species-specific radial sectors whose lateral borders run through the entire width from the ONL to the GCL. The border between adjacent sectors is formed by a Müller cell (Willbold et al., 1995), and sometimes, a sector can be composed of a single Müller cell and its associated cell column. The most important point is that cells from one species cannot cross the interspecies border and invade a neighbouring sector. Therefore, Müller cells form a strict barrier inhibiting lateral migration of cells and forcing the individual neurons to be aligned in their column (see Fig. 7). Concerning fibre growth, another important observation was that processes of neurons or glia cells within the IPL can traverse the interspecies borders, as revealed by the extension of plexiform quail material into chick sectors. This might be due to the failure of Müller cells to express chondroitin sulphate proteoglycan (MacLaren, 1996). This is a repulsive molecule expressed by reactive astrocytes. It has also been shown to control the sequence of ganglion cell differentiation and the initial direction of axons both in the embryo and during injuries (Silver, 1994). Although we do not know the exact nature of this border-crossing material, we suppose that the major contribution comes from fibrous

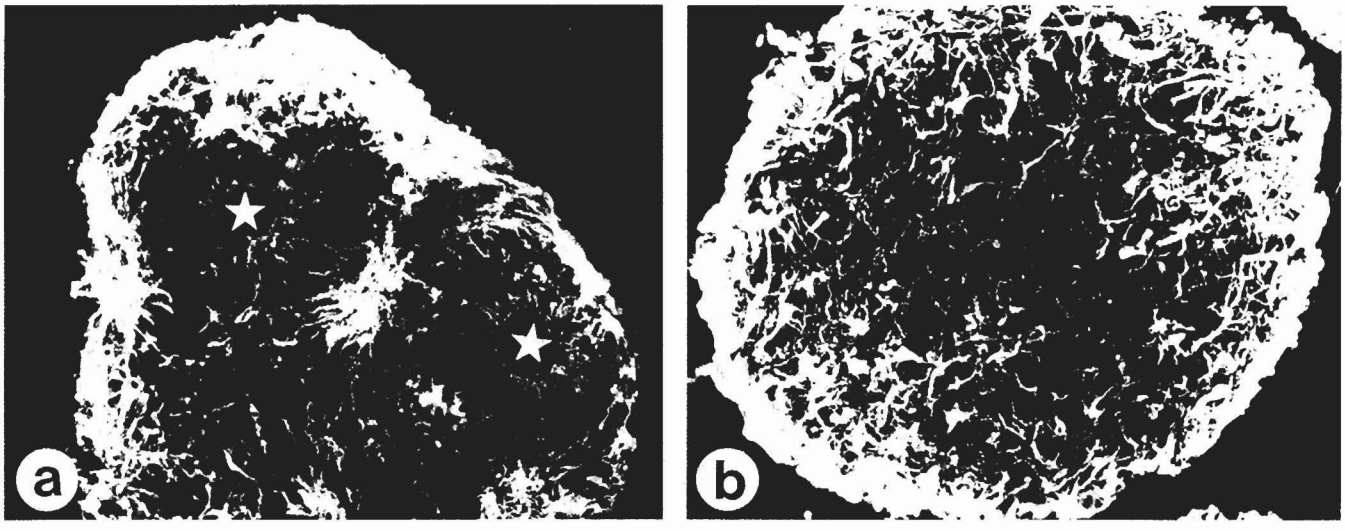

Fig. 6. *Müller cells reorganize radial scaffolds in rosetted spheroids.* Cryosections of a rosetted **(a)** and a tectal **(b)** spheroid after 14 days in culture were stained with antibodies against vimentin to detect radial glia cells. In the retinal spheroid two radial organized subunits can be seen (rosettes are marked by a white star). In the tectal spheroid, the radial glia cells show only a random meshwork. Bar: 50 μm.

branchlets of Müller cells, which have been nicely visualized by others to project into the IPL (Robinson and Dreher, 1990, Stone et al., 1991). This indicates that Müller cells are generally involved in subdividing the neuroepithelium in distinct cellular compartments and in establishing, further subdividing, and promoting the fine architecture of the IPL. It seems possible, that the fibrous branchlets are important structures for the guidance of axonal fibres projecting into specific synaptic sublaminae (Layer et al., 1997a). Whereas Müller cells allow outgrowing fibres to migrate tangentially, they represent strict barriers for laterally migrating cells - at least at later stages of development - allowing only the allocation of neurons in radial direction. To test such a border-function of Müller cells, we selectively damaged Müller cells with the glia-specific toxin DL-α-aminoadipic acid (Olney et al., 1971; Lund Karlsen 1978; Kato et al., 1990; Sugawara et al., 1990). It could be shown that high concentrations of this toxin induced total damage of Müller cells, leading to a complete breakdown of histogenesis (Reinicke and Layer, 1992; Kühnel et al., 1994; Germer et al., 1997). After applying a lower dosage causing a sensitive damage, the ensheathment of cell columns becomes permeable, neurons leave their cell column, cross the interspecies border and migrate laterally into the next sector (Willbold et al., 1995). Disturbed migration patterns of displaced amacrine cells were also observed in a comparable study in vivo (Prada et al., 1989). These experiments show that Müller cells are intimately involved both in mechanisms of radial migration and in the stabilization of the retinal columns. From these observations several important principles of retinogenesis and Müller cell functions can be deduced: a) The

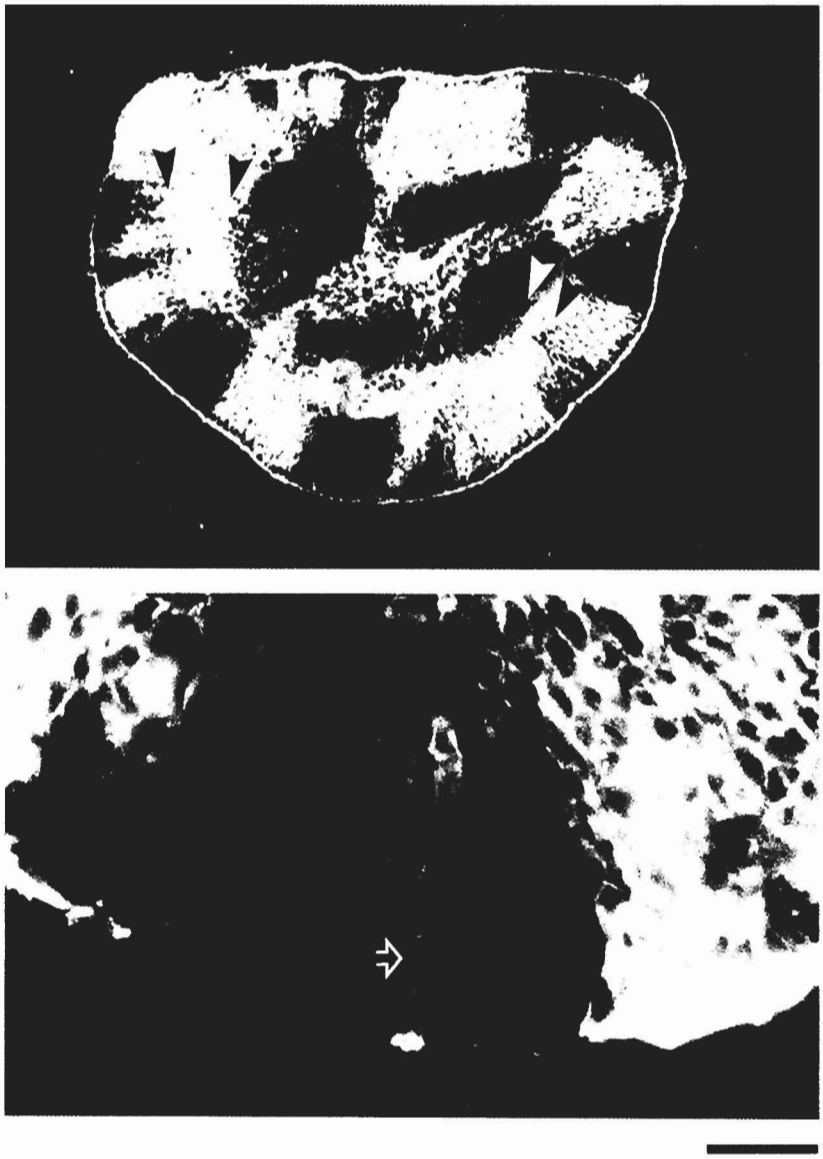

Fig. 7. *Müller cells stabilize and segregate the retinal cell columns in chimeric spheroids, composed of chick and quail cells.* **a.** Müller cells separate adjacent chick and quail cell columns, but allow lateral growth of fibres within the IPL (borders of IPL are marked by arrowheads). A quail antibody-labeled paraffin section of a chimeric stratospheroid after 8 days in culture shows chick and quail sectors (quail stained). Quail-derived fibres in IPL grow laterally into chick sectors. Note also staining of the membrana limitans externa in chick sectors. **b.** A single quail column (arrow) is embedded in a chick sector. Bar: a, 50 μm; b, 25 μm.

presence of multipotential stem cells giving rise to all retinal cell types including Müller cells. b) The radial alignment of the offspring and their ensheathment by a Müller cell. c) The failure of individual cells to leave their related cell column and to cross the interspecies borders.

In contrast to other studies using retroviral vectors (Fekete et al., 1994), chimeric mouse retina (Williams and Goldowitz, 1992), or X chromosome-inactivated transgenic mice (Reese et al., 1995), tangentially migrating single cells could not be detected in chimeric stratospheroids. These cells were assumed to be early-born. We cannot exclude that dispersed cells also occur in spheroids and have escaped our attention; however, they could also be eliminated very early by apoptosis or targeted cell death. Since in stratospheroids all cell types are present in sufficient numbers (Layer and Willbold, 1994), the reasons for this difference remain elusive.

Interestingly, the cells which are generated late in development, i.e. rods, bipolar cells and some amacrine cells, seem to share more similarities inside their group than with cells born earlier in development. These cells differentiate into cones, horizontal, amacrine or ganglion cells (for review see Reichenbach et al., 1993). Moreover, both groups seem to differ also in their migratory capacities. Perhaps, the failure of late-born-cells to disperse tangentially might be caused by Müller cells. It seems possible that during early stages of development, Müller cells (or their precursors) allow migration both in lateral and in radial direction. Further, one has to keep in mind that during early stages, these cells reach their final position not by real migration, but by perikaryal translocation (Morest, 1970; Snow and Robson, 1995). At later stages, when the retina has already achieved a relevant thickness, Müller cell-guided migration gains more importance, while lateral migrations are no longer observable. Therefore, the developmentally decisive switch from predominantly lateral growth to strict radial growth may be explained by the increased inhibition of lateral and the support of radial migration paths by Müller cells. Therefore, we recently proposed a scheme according to which retinogenesis can be subdivided into three distinct, yet overlapping phases. In a first phase which we call "lateralization", an areal increase of the early neuroepithelium is achieved by the continuous adding of a sufficient number of progenitor cells. In a second phase which we call "radialization", the thickness of the retina increases by radial growth and formation of columnar units. In a third phase which we call "lamination" the cell and fibre layers are formed (Layer and Willbold, 1993; Willbold et al., 1996).

A central role for radial glia cells during brain evolution?

The developmental switch from lateral to radial migration paths is an interesting hint towards an important role of radial glia cells during evolution of higher brain areas. Comparable to the structure of the retina, the cerebral cortex is also organized into horizontal and vertical arrays of neurons that form morphologically and functionally distinct laminar and columnar compartments (Creutzfeld, 1993). This pattern is consistent in a wide number of species. However, there are dramatic differences concerning the surface of the cortex. The so-called "radial-unit hypothesis" is able to fashionably explain this increase in cortical surface without changing its thickness and organization. It predicts that mutations in the regulatory genes that control the number and mode of cell divisions in the proliferation zone are responsible for the increase of columnar compartments without changing the number and arrangement of cells within each compartment. It further implies that this increase is coupled to regulatory mechanisms determining the lateral and radial distribution of the migrating neurons (Rakic, 1988, 1995). It seems feasible to assign the latter issue to radial glia cells. Although the "radial-unit hypothesis" was developed to explain neocortical expansion, comparable mechanisms seem to be also present in the retina. This would indicate that these mechanisms are evolutionary very conservative, and have already been used in "older" radially organized parts of the brain. Here also, an areal increase of the early neuroepithelium is achieved by the continuous adding of progenitors for new columnar units. This process has been arrested in birds and mammals, but may be active lifelong in lower vertebrates to fit the eye's volume to the growing animal. Additionally, during early phases of retinogenesis, some lateral dispersion of cells can occur, whereas later only radial migration is observable. This is in agreement with findings in other radially organized brain parts. Using the retrovirus technique and chick/quail chimeras, some laterally migrating cells could be observed, similar to the telencephalon (Luskin, 1993), or the avian tectum (Gray and Sanes, 1991; Martinez et al., 1992). However, these data do not really challenge the "radial-unit hypothesis", since laterally migrating cells rather represent another (evolutionary older?) mode of migration, and one may speculate that they are the founders of specific cell populations subserving specific functions within a coherent columnar structure (Doetsch and Alvarez-Buylla, 1996).

Taken together, data from many different studies indicate that Müller cells are multifunctional cells which gradually acquire their adult morphology and physiology. Beginning from the very early neuroepithelium and until the adult stage, Müller cells retain a remarkable degree of plasticity and capacities, assigning them an important and indispensable role during retinal development.

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References

Adler R. (1973). Cell interactions and histogenesis in embryonic neural aggregates. Exp. Cell Res. 77, 367-375.

Akagawa K., Hicks D. and Barnstable C.J. (1987). Histiotypic organization and cell differentiation in rat retinal reaggregate cultures. Brain Res. 437, 298-308.

Alexiades M.R. and Cepko C.L. (1997). Subsets of retinal progenitors display temporally and distinct biases in the fates of their progeny. Development 124, 1119-1131.

Altshuler D.M., Turner D.L. and Cepko C.L. (1991). Specification of cell type in the vertebrate retina. In: Development of the visual system. Lam D. and Shatz C.J. (eds.) Proceedings of the Retina Foundation Symposia Volume 3; A Bradford Book. The MIT Press. Cambridge, Massachusetts, London. pp 37-58

Alvarez-Buylla A. (1992). Neurogenesis and plasticity in the CNS of adult birds. Exp. Neurol. 115, 110-114.

Alvarez-Buylla A., Buskirk D.R. and Nottebohm F. (1987). Monoclonal antibody reveals radial glia in adult avian brain. J. Comp. Neurol. 264, 159-170.

Alvarez-Buylla A., Theelen M. and Nottebohm F. (1988). Mapping of radial glia and of a new cell type in adult canary brain. J. Neurosci. 8, 2707-2712.

Alvarez-Buylla A., Theelen M. and Nottebohm F. (1990). Proliferation "hot spots" in adult avian ventricular zone reveal radial cell division. Neuron 5, 101-109.

Aoyama H., Asamoto K., Nojyo Y. and Kinutani M. (1992). Monoclonal antibodies specific to quail embryo tissues: their epitopes in the developing quail embryo and their application to identification of quail cells in quail-chick chimeras. J. Histochem. Cytochem. 40, 1769-1777.

Aramant R., Seiler M., Ehinger B., Bergström A., Gustavii B., Brundin P. and Adolph A.R. (1990). Transplantation of human embryonic retina to adult rat retina. Restor. Neurol. Neurosci. 2, 9-22.

Armson P.F., Bennett M.R. and Raju T.R. (1987). Retinal ganglion cell survival and neurite regeneration requirements: the change from Müller cell dependence to superior colliculi dependence during development. Brain Res. 429, 207-216.

Arregui C., Barra H.S. and Landa C.A. (1992). Peanut agglutinin binding glycoproteins in the chick retina - their presence in Müller glia cells. J. Neurosci. Res. 31, 532-542.

Austin C.P., Feldman D.E., Ida J.A.Jr. and Cepko C.L. (1995). Ganglion cells in the vertebrate retina are selected from an equivalence group regulated by notch. Development 121, 3637-3650.

Bao Z.-Z. and Cepko C.L. (1997). The expression and function of notch pathway genes in the developing rat eye. J. Neurosci. 17, 1425-1434.

Barami K., Kirschenbaum B., Lemmon V. and Goldman S.A. (1994). N-cadherin and Ng-CAM/8D9 are involved serially in the migration of newly generated neurons into the adult songbird brain. Neuron 13, 567-582.

BenShaul Y. and Moscona A.A. (1975). Scanning electron microscopy of aggregating embryonic neural retinal cells. Exp. Cell Res. 95, 191-204.

Berg-von der Emde K. and Wolburg H. (1989). Müller (glial) cells but not astrocytes in the retina of the goldfish possess orthogonal arrays of particles. Glia 2, 458-469.

Bhattacharjee J. and Sanyal S. (1975). Developmental origin and early differentiation of retinal Müller cells in mice. J. Anat. 120, 367-372.

Bignami A. (1991). Glial cells in the central nervous system. Disc. Neurosci. 8, 1-45.

Bignami A., Raju T. and Dahl D. (1982). Localization of vimentin, the nonspecific intermediate filament protein, in embryonal glia and in early differentiating neurons. Dev. Biol. 91, 286-295.

Bolz S. and Wolburg H. (1992). First appearance of orthogonal arrays of particles in Müller cell membranes depends on retina maturation. Dev. Neurosci. 14, 203-209.

Brümmendorf T. and Rathjen F.G. (1995). Cell adhesion molecules 1: immunoglobulin superfamily. Protein Profile 2, 963-1108.

Brümmendorf T., Hubert M., Treubert U., Leuschner R., Tárnok A. and Rathjen F.G. (1993). The axonal recognition molecule F11 is a multifunctional protein: specific domains mediate interactions with Ng-CAM and restrictin. Neuron 10, 711-727.

Burke J.M. and Smith J.M. (1981). Retinal proliferation in response to vitreous hemoglobin or iron. Invest. Ophthalmol. Vis. Sci. 20, 582-592.

Campochiaro P.A. (1993). Cytokine production by retinal pigmented epithelial cells. Int. Rev. Cytol. 146, 75-82

Campochiaro P.A., Sugg R., Grotendorst G. and Hjelmeland L. (1989). Retinal pigment epithelial cells produce PDGF-like proteins and secretes them into their media. Exp. Eye Res. 49, 217-227.

Cepko C.L., Austin C.P., Yang X., Alexiades M. and Ezzeddine D. (1996). Cell fate determination in the vertebrate retina. Proc. Natl. Acad. Sci. USA 93, 589-595.

Chakrabarti S., Sima A.A.F., Lee J., Brachet P. and Dicou E. (1990). Nerve growth factor (NGF), proNGF and NGF receptor-like immunoreactivity in BB rat retina. Brain Res. 523, 11-15.

Chan-Ling T. (1994). Glial, neuronal and vascular interactions in the mammalian retina. Prog. Ret. Eye Res. 13, 357- 389.

Chuong C.M., Crossin K.L. and Edelman G.M. (1987). Sequential expression and differential function of multiple adhesion molecules during the formation of cerebellar cortical layers. J. Cell Biol. 104, 331-342.

Collin S.P., Collin H.B. and Ali M.A. (1996a). Fine structure of the retina and pigment epithelium in the creek chub, *Semotilus atromaculatus* (Cyprinidae, Teleostei). Histol. Histopathol. 11, 41-53.

Collin S.P., Collin H.B. and Ali M.A. (1996b). Ultrastructure and organisation of the retina and pigment epithelium in the cutlips minnow, *Exoglossum maxillingua* (Cyprinidae, Teleostei). Histol. Histopathol. 11, 55-69.

Cook J.E. and Becker D.L. (1995). Gap junctions in the vertebrate retina. Microsc. Res. Tech. 31, 408-419.

Coulombre J.L. and Coulombre A.J. (1965). Regeneration of neural retina from pigmented epithelium in the chick embryo. Dev. Biol. 12, 79-92.

Creutzfeld O.D. (1993). Cortex cerebri. Performance, structural and functional organization of the cortex. Göttingen, 1993.

de Raad S., Szczesny P.J., Munz K. and Remé C.E. (1996). Light damage in the rat retina: glial fibrillary acidic protein accumulates in Müller cells in correlation with photoreceptor damage. Ophthalmic Res. 28, 99-107.

Dermietzel R. (1973). Visualization by freeze fracturing of regular structures in glial cell membranes. Naturwissenschaften 60, 208.

Dermietzel R. (1974). Junctions in the central nervous system of the cat. III. Gap junctions and membrane associated orthogonal particle complexes (MOPC) in astrocytic membranes. Cell Tissue Res. 149, 121-135.

DiLoreto D.A.Jr., Martzen M.R., del Cerro C., Coleman P.D. and del

Cerro M. (1995). Müller cell changes precede photoreceptor cell degeneration in the age-related retinal degeneration of the Fischer 344 rat. Brain Res. 698, 1-14.

Distler C., Weigel H. and Hoffmann K.-P. (1993). Glia cells of the monkey retina. I. Astrocytes. J. Comp. Neurol. 333, 134-147.

Doetsch F. and Alvarez-Buylla A. (1996). Network of tangential pathways for neuronal migration in adult mammalian brain. Proc. Natl. Acad. Sci. USA 93, 14895-14900.

Dowling J.E. (1987). The vertebrate retina: an approachable part of the brain. Harvard University Press. Cambridge.

Dräger U.C. (1983). Coexistence of neurofilaments and vimentin in a neurone of adult mouse retina. Nature 303, 169-172.

Drazba J. and Lemmon V. (1990). The role of cell adhesion molecules in neurite outgrowth on Müller cells. Dev. Biol. 138, 82-93.

Dreher Z., Egner M. and Stone J. (1988). Müller cell endfeet at the inner surface of the retina: light microscopy. Vis. Neurosci. 1, 169-180.

Dreher Z., Robinson S.R. and Distler C. (1992). Müller cells in vascular and avascular retinae: a survey of seven mammals. J. Comp. Neurol. 323, 59-80.

Dreher Z., Distler C. and Dreher B. (1994). Vitread filamentation of avian Müller cells and its putative functional correlates. J. Comp. Neurol. 350, 96-108.

Dütting D., Gierer A. and Hansmann G. (1983). Self-renewal of stem cells and differentiation of nerve cells in the developing chick retina. Dev. Brain Res. 10, 21-32.

Eberhardt W. and Reichenbach A. (1987). Spatial buffering of potassium by retinal Müller (glial) cells of various morphologies calculated by a model. Neuroscience 22, 687-696.

Edwards M.A., Yamamoto M. and Caviness V.S. Jr. (1990). Organisation of radial glia and related cells in the developing murine CNS. An analysis based upon a new monoclonal antibody marker. Neuroscience 36, 121-144.

Eng L.F. and Shiurba R.A. (1988). Glial fibrillary acidic protein: a review of structure, function, and clinical application. In: Neuronal and glial proteins: structure, function, and clinical application. Academic Press. New York.

Ennis, S. and Kunz Y.W. (1986). Differentiated retinal Müller cells are ciliated - ultrastructural evidence in the teleost *Poecilia reticulata* P. Cell Biol. Int. Rep. 10, 611-622.

Erickson P.A., Fisher S.K., Guérin C.J., Anderson D.H. and Kaska D.D. (1987). Glial fibrillary acidic protein increases in Müller cells after retinal detachment. Exp. Eye Res. 44, 37-48.

Fadool J.M. and Linser P.J. (1993). 5A11 antigen is a cell recognition molecule which is involved in neuronal-glial interactions in avian neural retina. Dev. Dyn. 196, 252-262.

Fekete D.M., Perez-Miguelsanz J., Ryder E.F. and Cepko C.L. (1994). Clonal analysis in the chicken retina reveals tangential dispersion of clonally related cells. Dev. Biol. 166, 666-682.

Fujisawa H. (1973). The process of reconstruction of histological architecture from dissociated retinal cells. Wilhelm Roux Arch. Entwicklungsmech. Org. 171, 312-330.

Fujita S. and Horii M. (1963). Analysis of cytogenesis in chick retina by tritiated thymidine autoradiography. Arch. Histol. 23, 359-366.

Garber B.B. and Moscona A.A. (1972a). Reconstruction of brain tissue from cell suspensions. I. Aggregation patterns of cells dissociated from different regions of the developing brain. Dev. Biol. 27, 217-234.

Garber B.B. and Moscona A.A. (1972b). Reconstruction of brain tissue from cell suspensions. II. Specific enhancement of aggregation of embryonic cerebral cells by supernatant from homologues cell cultures. Dev. Biol. 27, 235-24.

Germer A., Kühnel K., Grosche J., Friedrich A., Wolburg H., Price J., Reichenbach A. and Mack A.F. (1997). Development of the neonatal rabbit retina in organ culture. 1. Comparison with histogenesis in vivo, and the effect of a gliotoxin (α-aminoadipic acid). Anat. Embryol. 196, 67-79.

Gospodarowicz D., Neufeld G. and Schweigerer L. (1986). Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells. Cell Differ. 19, 1-17.

Graeber M.B. and Streit W.J. (1990). Microglia: immune network in the CNS. Brain Pathol. 1, 2-5.

Gray G.E. and Sanes J.R. (1991). Migratory paths and phenotypic choices of clonally related cells in the avian optic tectum. Neuron 6, 211-225.

Graziadei P.P. and Monti Graziadei G.A. (1985). Neurogenesis and plasticity of the olfactory sensory neurons. Ann. NY Acad. Sci. 457, 127-142.

Halfter W., Newgreen D.F., Sauter J. and Schwarz U. (1983). Oriented axon outgrowth from avian embryonic retinae in culture. Dev. Biol. 95, 56-64.

Hatten M.E. (1990). Riding the glial monorail: a common mechanism for glialguided neuronal migration in different regions of the developing mammalian brain. Trends Neurosci. 13, 179-184.

Hatten M.E. (1993). The role of migration in central nervous system neuronal development. Curr. Opin. Neurobiol. 3, 38-44.

Hausman R.E., Knapp L.W. and Moscona A.A. (1976). Preparation of tissue specific cell-aggregating factors from embryonic neural tissues. J. Exp. Zool. 198, 417-422.

Hernández-Sánchez C., Frade J.M. and de la Rosa E.J. (1994). Heterogeneity among neuroepithelial cells in the chick retina revealed by immunostaining with monoclonal antibody PM1. Eur. J. Neurosci. 6, 105-114.

Hibbard E. and Ehrlich D. (1982). Laser-induced defects of retinal development in chick embryos. Neurosci. Lett. 34, 221-226.

Hicks D., Bugra K., Faucheux B., Jeanny J.C., Laurent M., Malecaze F., Mascarelli F., Raulais D., Cohen Y. and Courtois Y. (1992). Fibroblast growth factors in the retina. Prog. Ret. Res. 11, 333-375.

Holländer H., Makarov F., Dreher Z., van Driel D., Chan-Ling T. and Stone J. (1991). Structure of the macroglia of the retina: sharing and division of labour betwen astrocytes and Müller cells. J. Comp. Neurol. 313, 587-603.

Holt C.E., Bertsch T.W., Ellis H.M. and Harris W.A. (1988). Cellular determination in the Xenopus retina is independent of lineage and birth date. Neuron 1, 15-26.

Honegger P. and Richelson E. (1976). Biochemical differentiation of mechanically dissociated mammalian brain in aggregating cell culture. Brain Res. 109, 335-354.

Hughes W.F. and LaVelle A. (1974). On the synaptogenic sequence in the chick retina. Anat. Rec. 179, 297-301.

Huxlin K.R., Sefton A.J. and Furby J.H. (1992). The origin and development of retinal astrocytes in the mouse. J. Neurocytol. 21, 530-544.

Jacobson M. (1991). Developmental neurobiology. 3rd edition. Plenum Press. New York.

Jaynes C.D. and Turner J.E. (1995). Müller cell survival and proliferation in response to medium conditioned by the retinal pigment epi-

thelium. Brain Res. 678, 55-64.

Jaynes C.D., Sheedlo H.J., Agarwal N., O'Rourke K. and Turner J.E. (1995). Müller and retinal pigmented epithelial (RPE) cell expression of NGFI-A and c-fos in response to medium conditioned by the RPE. Mol. Brain Res. 32, 329-337.

Jensen A.M. and Raff M.C. (1997). Continuous observation of multipotential retinal progenitor cells in clonal density culture. Dev. Biol. 188, 267-279.

Jensen A.M. and Wallace V.A. (1997). Expression of sonic hedgehog and its putative role as a precursor cell mitogen in the developing mouse retina. Development 124, 363-371.

Kageyama G.H. and Robertson R.T. (1993). Transcellular retrograde labeling of radial glial cells with WGA-HRP and DiI in neonatal rat and hamster. Glia 9, 70-81.

Kahn A.J. (1974). An autoradiographic analysis of the time of appearance of neurons in the developing chick neural retina. Dev. Biol. 38, 30-40.

Kandel E.R., Schwartz J.H. and Jessell T.M. (1991). Principles of neural sciences. 3rd edition. Elsevier. New York, Amsterdam, Oxford.

Kato S., Sugawara K., Matsukawa T. and Negishi K. (1990). Gliotoxic effects of alpha-aminoadipic acid isomers on the carp retina: a longterm observation. Neuroscience 36, 145-153.

Kelley M.W., Turner J.K. and Reh T.A. (1995). Ligands of steroid/thyroid receptors induce cone photoreceptors in vertebrate retina. Development 121, 3777-3785.

Kettenmann H. and Ransom B.R. (1995). Neuroglia. Oxford University Press. New York, Oxford.

Kopan R. and Turner D.L. (1996). The notch pathway: democracy and aristocracy in the selection of cell fate. Curr. Opin. Neurobiol. 6, 594-601.

Kühnel K., Grosche J., Härtig W., Wolburg H., Schumacher U. and Reichenbach A. (1994). Müller cells in organ cultures of the developing rabbit retina. In: Göttingen neurobiology report 1994. Elsner N. and Breer H. (eds). Georg Thieme Verlag. Stuttgart, New York. p 776.

Lance-Jones C.C. and Lagenaur C.F. (1987). A new marker for identifying quail cells in embryonic avian chimeras: a quail-specific antiserum. J. Histochem. Cytochem. 35, 771-780.

Layer P.G. and Kotz S. (1983). Asymmetrical developmental pattern of uptake of Lucifer Yellow into amacrine cells in the embryonic chick retina. Neuroscience 9, 931-941.

Layer P.G. and Willbold E. (1989). Embryonic chicken retinal cells can regenerate all cell layers in vitro, but ciliary pigmented cells induce their correct polarity. Cell Tissue Res. 258, 233-242.

Layer P.G. and Willbold E. (1993). Histogenesis of the avian retina in reaggregation culture: from dissociated cells to laminar neuronal networks. Int. Rev. Cytol. 146, 1-47.

Layer P.G. and Willbold E. (1994). Regeneration of the avian retina by retinospheroid technology. Prog. Ret. Eye Res. 13, 197-229.

Layer P.G., Berger J. and Kinkl N. (1997a). Cholinesterase precede "ON-OFF" channel dichotomy in the embryonic chick retina before onset of synaptogenesis. Cell Tissue Res. 288, 407-416.

Layer P.G., Alber R., Mansky P., Vollmer G. and Willbold E. (1990). Regeneration of a chimeric retina from single cells in vitro: cell-lineage-dependent formation of radial cell columns by segregated chick and quail cells. Cell Tissue Res. 259, 187-198.

Layer P.G., Rothermel A., Hering H., Wolf B., deGrip W.J., Hicks D. and Willbold E. (1997b). Pigmented epithelium sustains cell proliferation and decreases expression of opsins and acetylcholinesterase in reaggregated chicken retinospheroids. Eur. J. Neurosci. 9, 1795-1803.

Le Douarin N.M. (1973). A biological cell labelling technique and its use in experimental embryology. Dev. Biol. 30, 217-222.

Leber S.M., Breedlove S.M. and Sanes J.R. (1990). Lineage, arrangement and death of clonally related motoneurons in chick spinal cord. J. Neurosci. 10, 2451-2462.

Lemmon V. (1985). Monoclonal antibodies specific for glia in the chick nervous system. Dev. Brain Res. 23, 111-120.

Lee W.H., Javedan S. and Bondy C.A. (1992). Coordinate expression of insulin-like growth factor system components by neurons and neuroglia during retinal and cerebellar development. J. Neurosci. 12, 4737-4744.

Levine E.M., Roelink H., Turner J. and Reh T.A. (1997). Sonic hedgehog promotes rod photoreceptor differentiation in mammalian retinal cells in vitro. J. Neurosci. 17, 6277-6288.

Lewis G.P., Erickson P.A., Guérin C.J., Anderson D.H. and Fisher S.K. (1992). Basic fibroblast growth factor: a potential regulator of proliferation and intermediate filament expression in the retina. J. Neurosci. 12, 3968-3978.

Li L. and Turner J.E. (1988). Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. Exp. Eye Res. 47, 911-916.

Lillien L. (1995). Changes in retinal cell fate induced by overexpression of EGF receptor. Nature 377, 158-162.

Linser P.J. and Moscona A.A. (1979). Induction of glutamine synthetase in embryonic neural retina: localization of Müller fibers and dependence on cell interactions. Proc. Natl. Acad. Sci. USA 76, 6476-6480.

Linser P.J. and Moscona A.A. (1981). Induction of glutamine synthetase in embryonic neural retina: its suppression by the gliatoxic agent α-aminoadipic acid. Dev. Brain Res. 1, 103-119.

Linser P.J., Sorrentino M. and Moscona A.A. (1984). Cellular compartmentalization of carbonic anhydrase C and glutamine synthetase in developing and mature mouse neural retina. Brain Res. 315, 65-71.

Liu L., Layer P.G. and Gierer A. (1983). Binding of FITC-coupled peanut-agglutinin (FITC-PNA) to embryonic chicken retinae reveals developmental spatio-temporal patterns. Dev. Brain Res. 8, 223-229.

Liu L., Cheng S.H., Jiang L.Z., Hansmann G. and Layer P.G. (1988). The pigmented epithelium sustains cell growth and tissue differentiation of chicken retinal explants in vitro. Exp. Eye Res. 46, 801-812.

López R., Gouras P., Kjeldbye H., Sullivan B., Reppucci V., Brittis M., Wapner F. and Goluboff E. (1989). Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. Invest. Ophthalmol. Vis. Sci. 30, 586-588.

Lund Karlsen R. (1978). The toxic effect of sodium glutamate and DL-α-aminoadipic acid on rat retina: changes in high affinity uptake of putative transmitters. J. Neurochem. 31, 1055-1061.

Luskin M.B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11, 173-189.

Luskin M.B., Pearlman A.L. and Sanes J.R. (1988). Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. Neuron 1, 635-647.

Machemer R. (1988). Proliferative vitreoretinopathy (PVR): a personal account of its pathogenesis and treatment. Proctor lecture. Invest.

Ophthalmol. Vis. Sci. 29, 1771-1783.

MacLaren R.E. (1996). Development and role of retinal glia in regeneration of ganglion cells following retinal injury. Br. J. Ophthalmol. 80, 458-464.

Magalhaes M.M. and Coimbra A. (1970). Electron microscope radioautographic study of glycogen synthesis in the rabbit retina. J. Cell Biol. 47, 263-275.

Magalhaes M.M. and Coimbra A. (1972). The rabbit retina Müller cell. A fine structural and cytochemical study. J. Ultrastruct. Res. 39, 310-326.

Mann I.C. (1964). The development of the human eye. 3rd edition. Grune & Stratton, Inc., New York.

Marc R.E. (1986). Neurochemical stratification in the inner plexiform layer of the vertebrate retina. Vision Res. 26, 223-238.

Martinez S., Geijo E., Sánchez-Vives M.V., Puelles L. and Gallego R. (1992). Reduced junctional permeability at interrhombomeric boundaries. Development 116, 1069-1076.

Mayerson P.L. and Moscona A.A. (1979). Malformation of embryonic neural retina elicited by BrdU. Differentiation 13, 173-184.

McCabe C.F. and Cole G.J. (1992). Expression of the barrier associated proteins EAP-300 and claustrin in the developing central nervous system. Brain Res. Dev. Brain Res. 70, 9-24.

Meller K. and Glees P. (1965). The differentiation of neuroglia-Müller-cells in the retina of chick. Z. Zellforsch. 66, 321-332.

Meller K. and Tetzlaff W. (1976). Scanning electron microscopic studies on the development of the chick retina. Cell Tissue Res. 170, 145-159.

Miller R.H. (1996). Oligodendrocyte origins. Trends Neurosci. 19, 92-96.

Miller R.H., Ffrench-Constant C. and Raff M.C. (1989). The macroglial cells of rat optic nerve. Annu. Rev. Neurosci. 12, 517-534.

Molthagen M., Schachner M. and Bartsch U. (1996). Apoptotic cell death of photoreceptor cells in mice deficient for the adhesion molecule on glia (AMOG, the beta 2-subunit of the Na, K-ATPase). J. Neurocytol. 25, 243-255.

Morales G., Hubert M., Brümmendorf T., Treubert U., Tárnok A., Schwarz U. and Rathjen F.G. (1993). Induction of axonal growth by heterophilic interactions between the cell surface recognition proteins F11 and NrCAM/Bravo. Neuron 11, 1113-1122.

Morest D.K. (1970). The pattern of neurogenesis in the retina of the rat. Z. Anat. Entwickl. Gesch. 131, 45-67.

Moscona A.A. (1952). Cell suspensions from organ rudiments of chick embryos. Exp. Cell Res. 3, 535-539.

Moscona A.A. (1974). Surface specification of embryonic cells: lectin receptors, cell recognition, and specific cell ligands. In: The cell surface in development. Moscona A.A. (ed). Wiley & Sons. New York. pp 67-99

Moscona A.A., Fox L., Smith J. and Degenstein L. (1985). Antiserum to lens antigens immunostains Müller glia cells in the neural retina. Proc. Natl. Acad. Sci. USA 82, 5570-5573.

Murabe Y. and Sano Y. (1981). Thiaminepyrophosphatase activity in the plasma membrane of microglia. Histochemistry 71, 45-52.

Murabe Y. and Sano Y. (1982). Morphological studies on neuroglia. VI. Postnatal development of microglial cells. Cell Tissue Res. 225, 469-485.

Müller C.M. (1992). A role for glial cells in activity-dependent central nervous plasticity? Review and hypothesis. Int. Rev. Neurobiol. 34, 215-281.

Nakafuku M. and Nakamura S. (1995). Establishment and characterization of a multipotential neural cell line that can conditionally generate neurons, astrocytes, and oligodendrocytes in vitro. J. Neurosci. Res. 41, 153-168.

Nakazawa T., Tachi S., Aikawa E. and Ihnuma M. (1993). Formation of the myelinated nerve fiber layer in the chicken retina. Glia 8, 114-121.

Navascués J., Moujahid A., Almendros A., Marin-Teva J.L. and Cuadros M.A. (1995). Origin of microglia in the quail retina: central-to-peripheral and vitreal-to-scleral migration of microglial precursors during development. J. Comp. Neurol. 354, 209-228.

Newman E.A. (1994). A physiological measure of carbonic anhydrase in Müller cells. Glia 11, 291-299.

Newman E.A. and Zahs K.R. (1997). Calcium waves in retinal glial cells. Science 275, 844-847.

Newman E.A., Frambach D.A. and Odette L.L. (1984). Control of extracellular potassium levels by retinal glial cell K^+ siphoning. Science 225, 1174-1175.

Oehmichen M., Wiethölter H. and Greaves M.F. (1979). Immunological analysis of human microglia: lack of monocytic and lymphoid membrane differentiation antigens. J. Neuropathol. Exp. Neurol. 38, 99-103.

Okada T.S. (1980). Cellular metaplasia or transdifferentiation as a model for retinal cell differentiation. In: Current topics in developmental biology. Denis-Donini S. and Augusti-Tocco D. (eds). pp 349-380.

Okano T., Kojima D., Fukada Y., Shichida Y. and Yoshizawa T. (1992). Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc. Natl. Acad. Sci. USA 89, 5923-5936.

Olney J.W., Ho O.I. and Rhee V. (1971). Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp. Brain Res. 14, 61-76.

Opas M. and Dziak E. (1994). bFGF-induced transdifferentiation of RPE to neuronal progenitors is regulated by the mechanical properties of the substratum. Dev. Biol. 161, 440-454.

Orts-Llorca F. and Genís-Gálvez J.M. (1960). Experimental production of retinal septa in the chick embryo. Differentiation of pigment epithelium into neural retina. Acta Anat. 42, 31-70.

Palatroni P., Gabrielli M.G. and Taborro R. (1990). Carbonic anhydrase activity in mammalian retina. Developmental aspects in altricial and precocial species. Acta Histochem. 88, 187-198.

Park C.M. and Hollenberg M.J. (1989). Basic fibroblast growth factor induces retinal regeneration in vivo. Dev. Biol. 134, 201-205.

Paulus W. (1995). Tumoren des Nervensystems. In: Neuropathologie - Morphologische Diagnostik der Krankheiten des Nervensystems, der Skelettmuskulatur und der Sinnesorgane. Springer-Verlag. Berlin, Heidelberg, New York.

Peles E., Nativ M., Campbell P.L., Sakurai T., Martinez R., Lev S., Clary D.O., Schilling J., Barnea G., Plowman G.D., Grumet M. and Schlessinger J. (1995). The carbonic anhydrase domain of receptor tyrosine phosphatase ß is a functional ligand for the axonal cell recognition molecule contactin. Cell 82, 251-260.

Pfeiffer S.E., Warrington A.E. and Bansal R. (1993). The oligodendrocyte and its many cellular processes. Trends Cell Biol. 3, 191-197.

Pittack C., Grunwald G.B. and Reh T.A. (1997). Fibroblast growth factors are necessary for neural retina but not pigmented epithelium differentiation in chick embryos. Development 124, 805-816.

Pittack C., Jones M. and Reh T.A. (1991). Basic fibroblast growth factor induces retinal pigment epithelium to generate neural retina in vitro. Development 113, 577-588.

Pixley S.K.R. and de Vellis J. (1984). Transition between immature radial glia and mature astrocytes studies with a monoclonal antibody to vimentin. Dev. Brain Res. 15, 201-210.

Prada F.A., Magalhaes M.M., Coimbra A. and Genís-Gálvez J.M. (1989). Morphological differentiation of the Müller cell: Golgi and electron microscopy study in the chick retina. J. Morphol. 201, 11-22.

Prada F.A., Dorado M.E., Quesada A., Schwarz U. and de la Rosa E. (1995). Early expression of a novel radial glia antigen in the chick embryo. Glia 15, 389-400.

Prada C., Puga J., Perez-Mendez L., Lopez R. and Ramirez G. (1991). Spatial and temporal patterns of neurogenesis in the chick retina. Eur. J. Neurosci. 3, 559-569.

Puelles L. and Bendala M.C. (1978). Differentiation of neuroblasts in the chick optic tectum up to eight days of incubation: a Golgi study. Neuroscience 3, 307-325.

Puro D.G. (1995). Growth factors and Müller cells. Prog. Ret. Eye Res. 15, 89-101.

Raff M.C., Miller R.H. and Noble M. (1983). A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature 303, 390-396.

Raju T.R. and Bennett M.R. (1986). Retinal ganglion cell survival requirements: a major but transient dependence on Müller glia during development. Brain Res. 383, 165-176.

Rakic P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. 145, 61-84.

Rakic P. (1988). Specification of cerebral cortical areas. Science 241, 170-176.

Rakic P. (1995). A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends Neurosci. 18, 383-388.

Rakic P., Cameron R.S. and Komuro H. (1994). Recognition, adhesion, transmembrane signaling and cell motility in guided neuronal migration. Curr. Opin. Neurobiol. 4, 63-69.

Ramón y Cajal S. (1893). La rétine des vertébrés. La Cellule 9, 17-257; reprinted in Rodieck R.W. (1973). The vertebrate retina. W.H. Freeman and Co. San Francisco.

Rathjen F.G., Wolff J.M., Frank R., Bonhoeffer F. and Rutishauser U. (1987). Membrane glycoproteins involved in neurite fasciculation. J. Cell Biol. 104, 343-353.

Rathjen F.G., Wolff J.M. and Chiquet-Ehrismann R. (1991). Restrictin: a chick neural extracellular matrix protein involved in cell attachment co-purifies with the cell recognition molecule F11. Development 113, 151-164.

Raymond S.M. and Jackson I.J. (1995). The retinal pigmented epithelium is required for development and maintenance of the mouse neural retina. Curr. Biol. 5, 1286-1295.

Reale E., Luciano L. and Spitznas M. (1978). Communicating junctions of the human sensory retina. A freeze-fracture study. Albrecht von Graefes Arch. Klin. Exp. Opthalmol. 208, 77-92.

Reese B.E., Harvey A.R. and Tan S.-S. (1995). Radial and tangential dispersion patterns in the mouse retina are cell-class specific. Proc. Natl. Acad. Sci. USA 92, 2494-2498.

Reh T.A., Nagy T. and Gretton H. (1987). Retinal pigmented epithelial cells induced to transdifferentiate to neurons by laminin. Nature 330, 68-71.

Reichenbach A. (1989). Attempt to classify glial cells by means of their process specialization using the rabbit retinal Müller cell as an example of cytotopographic specialization of glial cells. Glia 2, 250-259.

Reichenbach A. (1993). Two types of neuronal precursor cells in the mammalian retina - a short review. J. Hirnforsch. 34, 335-341.

Reichenbach A. and Robinson S.R. (1995a). Phylogenetic constraints on retinal organisation and development. Prog. Ret. Eye Res. 15, 139-171.

Reichenbach A. and Robinson S.R. (1995b). Ependymoglia and ependymoglia-like cells. In: Neuroglia. Kettenmann H. and Ransom B.R. (eds) Oxford University Press. New York, Oxford.

Reichenbach A., Reichelt W. and Schümann R. (1987). Use of Pappenheim's panoptic staining method on enzymatically isolated cells for demonstration of postnatal development of the rabbit retina. Z. Mikrosk. Anat. Forsch. 101, 597-608.

Reichenbach A., Hagen E., Schippel K., Brückner G., Reichelt W. and Leibnitz L. (1988). Cytotopographical specialization of enzymatically isolated rabbit retinal Müller (glial) cells: structure, ultrastructure, and [^{3}H]-ouabain binding sites. Z. Mikrosk. Anat. Forsch. 102, 897-912.

Reichenbach A., Schneider H., Leibnitz L., Reichelt W., Schaaf P. and Schümann R. (1989). The structure of rabbit retinal Müller (glial) cells is adapted to the surrounding retinal layers. Anat. Embryol. 180, 71-79.

Reichenbach A., Siegel A., Rickmann M., Wolff J.R., Noone D. and Robinson S.R. (1995). Distribution of Bergmann glial somata and processes: implications for function. J. Brain Res. 36, 509-517.

Reichenbach A., Stolzenburg J.-U., Eberhardt W., Chao T.I., Dettmer D. and Hertz L. (1993). What do retinal Müller (glial) cells do for their neuronal "small siblings"? J. Chem. Neuroanat. 6, 201-213.

Reinicke M.K.W. and Layer P.G. (1992). The pharmacological injury of Müller glia interferes with chicken retinogenesis in vitro: retino-spheroids as developmental assay systems. In: Rhythmogenesis in neurons and networks. Elsner N. and Richter D.W. (eds). Georg Thieme Verlag. Stuttgart, New York. p 397.

Reinicke M., Layer P.G. and Willbold E. (1996). Contact fluorescence dye staining of immature Müller cells and photoreceptors in developing retina. In: Göttingen neurobiology report 1996. Elsner N. and Schnitzler H.-U. (eds). Georg Thieme Verlag. Stuttgart, New York. p 441.

Rich K.A., Figueroa S.L., Zhan Y. and Blanks J.C. (1995). Effects of Müller cell disruption on mouse photoreceptor cell development. Exp. Eye Res. 61, 235-248.

Robinson S.R. (1991). Development of the mammalian retina. In: Neuroanatomy of the visual pathways and their development. Dreher B. and Robinson S.R. (eds). Vision and visual dysfunction, Vol. 3 Cronly-Dillon J.R. (series-ed). Macmillan. UK. pp 69-128.

Robinson S.R. and Dreher Z. (1990). Müller cells in adult rabbit retinae: morphology, distribution and implications for function and development. J. Comp. Neurol. 292, 178-192.

Rodieck R.W. (1973). The vertebrate retina. W.H. Freeman and Co. San Francisco.

Roitbak A.J. (1983). Neuroglia. Eigenschaften-Funktionen-Bedeutung. Gustav Fischer Verlag. Jena.

Rothermel A., Willbold E., DeGrip W.J. and Layer P.G. (1997). Pigmented epithelium induces complete retinal reconstitution from dispersed embryonic chick retinae in reaggregation culture. Proc. R. Soc. Lond. B 264, 1-10.

Sagar S.M., Edwards R.H. and Sharp F.R. (1991). Epidermal growth factor and transforming growth factor alpha induce c-fos gene expression in retinal Müller cells in vivo. J. Neurosci. Res. 29, 549-559.

Schlosshauer B., Grauer D., Dütting D. and Vanselow J. (1991). Expression of a novel Müller glia specific antigen during development and after optic nerve lesion. Development 111, 789-799.

Schnitzer J. (1985). Distribution and immunoreactivity of glia in the retina of the rabbit. J. Comp. Neurol. 240, 128-142.

Schnitzer J. (1989). Enzyme-histochemical demonstration of microglial cells in the adult and postnatal rabbit retina. J. Comp. Neurol. 282, 249-263.

Schnitzer J., Franke W.W. and Schachner M. (1981). Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. J. Cell Biol. 90, 435-447.

Senut M.C. and Alvarado-Mallart R.M. (1987). Cytodifferentiation of quail tectal primordium transplanted homotopically into the chick embryo. Dev. Brain Res. 32, 187-205.

Sheedlo H.J. and Turner J.E. (1996a). Effects of retinal pigment epithelial cell-secreted factors on neonatal rat retinal explant progenitor cells. J. Neurosci. Res. 44, 519-531.

Sheedlo H.J. and Turner J.E. (1996b). Influence of a retinal pigment epithelial cell factor(s) on rat retinal progenitor cells. Dev. Brain Res. 93, 88-99.

Sheedlo H.J., Jaynes D., Bolan A.L. and Turner J.E. (1995). Mullerian glia in dystrophic rodent retinas: an immunocytochemical analysis. Brain Res. Dev. Brain Res. 85, 171-180.

Sheffield J.B. and Moscona A.A. (1970). Electron microscopic analysis of aggregation of embryonic cells: the structure and differentiation of aggregates of neural retina cells. Dev. Biol. 23, 36-61.

Shimada M., Wakaizumi S., Kasubuchi Y., Kusunoki T. and Nakamura T. (1973). Cytosine arabinoside and rosette formation in mouse retina. Nature 246, 151-152.

Silver J. (1994). Inhibitory molecules in development and regeneration. J. Neurol. 242 (Suppl. 1), S22-24.

Sivron T. and Schwartz M. (1995). Glial cell types, lineages, and response to injury in rat and fish: implications for regeneration. Glia 13, 157-165.

Sivron T., Jeserich G., Nona S. and Schwartz M. (1992). Characteristics of fish glial cells in culture: possible implications as to their lineage. Glia 6, 52-66.

Snow R.K.L. and Robson J.A. (1995). Migration and differentiation of neurons in the retina and optic tectum of the chick. Exp. Neurol. 134, 13-24.

Spence S.G. and Robson J.A. (1989). An autoradiographic analysis of neurogenesis in the chick retina in vitro and in vivo. Neuroscience 32, 801-812.

Steinhäuser C. (1996). In situ-Eigenschaften zentraler Gliazellen: Konsequenzen für das Konzept der Informationsverarbeitung im ZNS? Biologie in unserer Zeit 26, 78-84.

Stier H. and Schlosshauer B. (1995). Axonal guidance in the chicken retina. Development 121, 1443-1454.

Stone J., Holländer H. and Dreher Z. (1991). "Sunbursts" in the inner plexiform layer: a spectacular feature of Müller cells in the retina of the cat. J. Comp. Neurol. 303, 400-411.

Strettoi E. and Masland R.H. (1996). The number of unidentified amacrine cells in the mammalian retina. Proc. Natl. Acad. Sci. USA 93, 14906-14911.

Stroeva O.G. and Mitashov V.I. (1983). Retinal pigment epithelium: proliferation and differentiation during development and regeneration. Int. Rev. Cytol. 83, 221-293.

Sugawara K., Torigoe K., Okoyama S., Negishi K. and Kato S. (1990). Neurotoxic effects of L-α-aminoadipic acid on the carp retina: a long term observation. Neuroscience 36, 155-163.

Tanihara H., Yoshida M., Matsumoto M. and Yoshimura N. (1993a). Identification of transforming growth factor-beta expressed in cultured human retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 34, 413-419.

Tanihara H., Yoshida M. and Yoshimura N. (1993b). Tumor necrosis factor-alpha gene is expressed in stimulated retinal pigment epithelial cells in culture. Biochem. Biophys. Res. Commun. 187, 1029-1034.

Thanos S. (1991). The relationship of microglial cells to dying neurons during natural cell death and axotomy-induced degeneration of the rat retina. Eur. J. Neurosci. 3, 1189-1207.

Tombran-Tink J., Chader G.G. and Johnson L.V. (1991). PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. Exp. Eye Res. 53, 411-414.

Tout S., Chan-Ling T., Holländer H. and Stone J. (1993). The role of Müller cells in the formation of the blood-retinal barrier. Neuroscience 55, 291-301.

Turner D.L. and Cepko C.L. (1987). A common progenitor for neurons and glia persists in rat retina late in development. Nature 328, 131-136.

Turner D.L., Snyder E.Y. and Cepko C.L. (1990). Lineage-independent determination of cell type in the embryonic mouse retina. Neuron 4, 833-845.

Uga S. and Smelser G.K. (1973). Electron microscopic study of the development of the retinal Müllerian cells. Invest. Ophtalmol. 12, 295-307.

Vardimon L., Fox L.E., Cohen-Kupiec R., Degenstein L. and Moscona A.A. (1991). Expression of v-src in embryonic neural retina alters cell adhesion, inhibits histogenesis, and prevents induction of glutamine synthetase. Mol. Cell. Biol. 11, 5275-5284.

Vaughan D.K., Erickson P.A. and Fisher S.K. (1990). Glial fibrillary acidic protein (GFAP) immunoreactivity in rabbit retina: effect of fixation. Exp. Eye Res. 50, 385-392.

Vollmer G. and Layer P.G. (1986). An in vitro model of proliferation and differentiation of the chick retina: coaggregates of retinal and pigment epithelial cells. J. Neurosci. 6, 1885-1896.

Vollmer G. and Layer P.G. (1987). Cholinesterases and cell proliferation in "nonstratified" and "stratified" cell aggregates from chicken retina and tectum. Cell Tissue Res. 250, 481-487.

Vollmer G., Layer P.G. and Gierer A. (1984). Reaggregation of embryonic chick retina cells: pigment epithelial cells induce a high order of stratification. Neurosci. Lett. 48, 191-196.

Vrabec F. (1970). Microglia in the monkey and rabbit retina. J. Neuropathol. Exp. Neurol. 29, 241-252.

Wässle H. and Boycott B.B. (1991). Functional architecture of the mammalian retina. Physiol. Rev. 71, 447-480.

Watanabe T. and Raff M.C. (1990). Rod photoreceptor development in vitro: intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. Neuron 2, 461-467.

Wetts R. and Fraser S.E. (1988). Multipotent precursors can give rise to all major cell types of the frog retina. Science 239, 1142-1145.

Wetts R., Serbedzija G.N. and Fraser S.E. (1989). Cell lineage analysis reveals multipotent precursors in the ciliary margin of the frog retina. Dev. Biol. 136, 254-263.

Willbold E. and Layer P.G. (1992). A hidden retinal regenerative capacity from the chick ciliary margin is reactivated in vitro, that is

accompanied by down-regulation of butyrylcholinesterase. Eur. J. Neurosci. 4, 210-220.

Willbold E., Reinicke M., Lance-Jones C., Lagenaur C., Lemmon V. and Layer P.G. (1995). Müller glia stabilizes cell columns during retinal development: lateral cell migration but not neuropil growth is inhibited in mixed chick-quail retinospheroids. Eur. J. Neurosci. 7, 2277-2284.

Willbold E., Mansky P. and Layer P.G. (1996). Lateral and radial growth uncoupled in reaggregated retinospheroids of embryonic avian retina. Int. J. Dev. Biol. 40, 1151-1159.

Willbold E., Brümmendorf T., Rathjen F.G., Schwarz H. and Weiss B. (1997a). The neural cell recognition molecule F11 is expressed on Müller cells and Schwann cells in vitro. J. Brain Res. 38, 71-80.

Willbold E., Berger J., Reinicke M. and Wolburg, H. (1997b). On the role of Müller glia cells in histogenesis: only retinal spheroids, but not tectal, telencephalic and cerebellar spheroids develop histotypical patterns. J. Brain Res. 38, 383-396.

Williams R.W. and Goldowitz D. (1992). Structure of clonal and polyclonal cell arrays in chimeric mouse retina. Proc. Natl. Acad. Sci. USA 89, 1184-1188.

Williams R.W., Strom R.C., Rice D.S. and Goldowitz D. (1996). Genetic and environmental control of variation in retinal ganglion cell number in mice. J. Neurosci. 16, 7193-7205.

Wolburg H. (1995). Orthogonal arrays of intramembranous particles: a review with specific reference to astrocytes. J. Brain Res. 36, 239-258.

Wolburg H., Willbold E. and Layer P.G. (1991). Müller glia endfeet, a basal lamina and the polarity of retinal layers form properly in vitro only in the presence of marginal pigmented epithelium. Cell Tissue Res. 264, 437-451.

Wolburg H., Reichelt W., Stolzenburg J.U., Richter W. and Reichenbach A. (1990). Rabbit retinal müller cells in cell culture show gap and tight junctions which they do not express in situ. Neurosci. Lett. 11, 58-63.

Yamamoto T., Ochalski A., Hertzberg E.L. and Nagy J.I. (1990). On the organization of astrocytic gap junctions in rat brain as suggested by LM and EM immunohistochemistry of connexin 43 expression. J. Comp. Neurol. 302, 853-863.

Zeevalk G.G and Hyndman A.G. (1987). Transferrin in chick retina: distribution and location during development. Brain Res. 465, 231-241.

Zhao S., Thornquist S.C. and Barnstable C.J. (1995). In vitro trans-differentiation of embryonic rat retinal pigment epithelium to neural retina. Brain Res. 677, 300-310.

Zhou Y. and Opas M. (1994). Cell shape, intracellular pH, and fibroblast growth factor responsiveness during transdifferentiation of retinal pigment epithelium into neuroepithelium in vitro. Biochem. Cell Biol. 72, 257-265.

Zisch A.H., D'Alessandri L., Ranscht B., Falchetto R., Winterhalter K.H. and Vaughan L. (1992). Neuronal cell adhesion molecule contactin/F11 binds to tenascin via its immunoglobulin-like domains. J. Cell Biol. 119, 203-213.