Introduction

The vertebrate retina, like other regions of the central nervous system (CNS), developmentally derives from the neural tube at the end of the neurular stage of embryogenesis (Fig. 1A). In early embryonic life, the neural tube evaginates to form two optic vesicles in the region of diencephalon. Each optic vesicle subsequently invaginates to form the two-layered optic cup (Fig. 1B). The inner layer of the optic cup forms a multi-layered neural retina. The neural retina is specialized for the reception of the light energy from environment and for the generation and integration of the neural responses (for reviews, see Dowling, 1987; Rodieck, 1998). The outer layer of the optic cup differentiates into a mono-layered retinal pigmented epithelium (RPE) that lines the back of the neural retina. RPE supports many of the physiological requirements of the neural retina, such as nutrient exchange, phagocytosis of photoreceptor disc shedding and absorption of stray light (for reviews, see Young, 1976; Zinn and Marmor, 1979; Dowling, 1987; Tombran-Tink et al., 1992).

As a consequence of its embryological origin, the adult vertebrate retina shows a laminar organization comparable to that of the cerebral cortex of the brain (Ramón y Cajal, 1892; Dowling, 1987; Rodieck, 1998). All vertebrate retinas consist of at least five basic types of neurons (photoreceptor cells, bipolar cells, horizontal cells, amacrine cells, and ganglion cells) and nonneuronal glial cells (Fig. 2). The constituent cells are arranged in penta-lamina array: three nuclear layers [outer nuclear later (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL)] and two synaptic layers [outer plexiform layer (OPL) and inner plexiform layer (IPL)]. The ONL is formed by the somata of photoreceptor cells. Photoreceptors absorb light energy by their outer segments, convert this to an electrophysiological response and transmit the visual signals to second order neurons, such as bipolar and horizontal

cells. The OPL is the first synaptic zone of the retina where photoreceptors, bipolar and horizontal cells are connected. The INL contains the somata of bipolar, horizontal and amacrine cells. The bipolar cells receive input from photoreceptors and transmit electrical signals to ganglion cells whose somata are located in the GCL. The region of the communications between bipolar, amacrine and ganglion cells is the IPL, the second synaptic zone. Ganglion cells integrate the different aspects of the visual information, encode them into action potentials, and transmit them to the brain via the optic nerve that is composed of ganglion cell axons. Horizontal and amacrine cells are involved in lateral interactions in the OPL and the IPL, respectively.

In the CNS, including the retina, cell to cell communication can be divided into two general types: chemical and electrical synapses. Chemical synapses enable communication via the secretion of neurotransmitters; in this case, chemical agents released by the presynaptic neurons bind to specific receptors on the membrane of the postsynaptic neurons. The binding of neurotransmitters to the receptors causes synaptic potential which then alters electrical activity of the postsynaptic cell (for reviews, see Dowling, 1987; Rodieck, 1998). Electrical synapses, formed by intercellular contacts called gap junctions, permit direct, passive flow of electrical current from one neuron to another, thus making it possible to propagate electrical signals very rapidly. In addition, a variety of small molecules can freely diffuse the cytoplasm of the pre- and postsynaptic neurons. In the retina, electrical coupling through gap junctions is found in all cell classes that comprise the mature retina (Cook and Becker, 1995). A large number of morphological, electrophysiological, and pharmacological studies have been carried out on gap junctions between retinal horizontal cells, because of their large cell size.

The vertebrate retina is superbly suited for studying cell differentiation, synaptic transmission, information processing, and laminar organization of the CNS. These studies are facilitated because all vertebrate retinas consist of a limited number of cell classes, because the entire retina can be removed intact from the back of the eye, and

because the anatomical organization of retinal cells has been conserved among different vertebrate species.

My overall research interest is to understand the mechanisms of the differentiation of retinal neurons and their synaptic formation on the basis of functional aspects. At this point of view, teleost fish retina is an attractive tissue, because it grows throughout the animal's life by adding new cells of all types from progenitor cells that are clustered at the peripheral edge of the retina (Müller, 1952; Johns, 1977; Wolburg, 1978; Maier and Wolburg, 1979; Sharma and Ungar, 1980; Johns and Fernald, 1981; Kästner and Wolburg, 1982; Raymond et al., 1988; Negishi et al., 1990; Hitchcock and Raymond, 1992). This property offers a particular technical advantage for investigating the differentiation of retinal neurons, synaptic formation and establishment of neural networks, because the peripheral retinas have a spatial arrangement of progenitor cells and mature cells, progenitor cells being most peripheral and differentiating cells being more central.

As yet, studies of goldfish retinal development and regeneration have concentrated mainly on morphological and histochemical changes (Müller, 1952; Johns, 1977; Wolburg, 1978; Maier and Wolburg, 1979; Sharma and Ungar, 1980; Johns and Fernald, 1981; Wolburg, 1982; Raymond et al., 1988; Negishi, et al., 1990; Hitchcock, et al., 1992). Recent studies have also started to focus on molecular mechanisms that lead to differentiation of retinal neurons (Levine, et al., 1994; Hitchcock et al., 1996; Levine, et al., 1997; Passini et al., 1997). However, studies of functional differentiation of retinal neurons during development and regeneration of goldfish have been limited (cf. Olson et al., 1999, 2000).

Therefore, in the present study, to take an advantage of the peripheral retina of adult goldfish as a model of development, I have developed living slice preparation of the peripheral retina which contains a continuous gradient of developmental stages. Since ganglion cells are the first retinal neurons to differentiate during development in almost all vertebrate embryos (Hinds and Hinds, 1974; McLoon and Barnes, 1989;

Altshuler et al., 1991; Cepko, et al., 1996) and during regeneration of newt retina (Cheon et al., 1998), I have investigated how marginal progenitor cells differentiate into retinal neurons, especially ganglion cells in this system. For this purpose, I have started here to examine the appearance and maturation of voltage-gated Na^+ current with whole-cell patch-clamp methods. This current was targeted because it is found in all adult retinal ganglion cells (Ishida, 1995), because it enables ganglion cells to encode sensory information into action potentials, and because it is larger in amplitude than Na^+ currents in the few other retinal cells that it has been found in (Shingai and Christensen, 1983; Boos et al., 1993; Feigenspan et al., 1998).

As described above, gap junctions are one of the pathways mediating cell-to-cell communication. In the CNS including the retina, cells in the early developmental stage are coupled via gap junctions, and uncoupled as differentiation proceeds (Dixon and Cronly-Dillon, 1972; Warner, 1973; Fujisawa et al., 1976; Spray et al., 1981; Spitzer, 1982; Connors et al., 1983; Sakaguchi, et al., 1984; LoTurco and Kriegstein, 1991; Peinado, et al., 1993; Penn et al., 1994; Bittman et al., 1997). To the best of my knowledge, however, no direct evidence has thus far been provided that gap junctions are present among progenitor cells in the adult goldfish retina. Therefore, I have also investigated in whether marginal progenitor cells are coupled through gap junctions. Lastly, I have discussed together with the result of development of Na^+ channels whether the neuronal differentiation requires uncoupling from marginal progenitor cells.