II General Introduction

1. Development of the vertebrate eye and structure of the retina

The vertebrate eye, 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, eye rudiments, in the region of the diencephalon (Fig. 1B, top). Each optic vesicle subsequently invaginates to form the two-layered optic cup (Fig. 1B, middle). The inner layer of the optic cup forms a multi-layered neural retina (Fig. 1B, bottom). The neural retina is specialized for the reception and transduction of light energy from the visual image of the environment, and for the generation and integration of the neural responses (for review, see 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. The RPE supports many of the physiological requirements of the neural retina, such as nutrient exchange, phagocytosis of photoreceptor discs after 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 known vertebrate retinas are organized according to the same basic plan. Figure 2 is a schematic diagram of the typical cell types of retinal neurons and organization of retinal layers. The retina consists of at least five major types of retinal neurons (photoreceptor, bipolar, horizontal, amacrine, and ganglion cells) and non-neuronal glial cells (Müllar cells). The constituent cells are arranged in a penta-laminar array; three nuclear layers [outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL)] and two synaptic layers

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[outer plexiform layer (OPL) and inner plexiform layer (IPL)]. Light, entering the eye, passes through the retina and is captured by photoreceptor cells [rod (R) and cone (C)], whose somata are located at the ONL in a layer of photoreceptor cell (RCL). Photoreceptor cells absorb light energy, convert this to electrical responses, and transmit them as visual signals to second-order neurons, such as bipolar cells (B) and horizontal cells (H). The OPL is the first synaptic zone of the retina where photoreceptor, bipolar and horizontal cells are connected. The bipolar cells receive input from photoreceptor cells and transmit electrical signals to third-order neurons, such as amacrine cells (A) and ganglion cells (G). The INL contains somata of horizontal, bipolar and amacrine cells. The IPL is the second synaptic zone of the retina where bipolar, amacrine and ganglion cells interact. Ganglion cells whose somata located in the GCL encode electrical signals into action potentials, and transmit them to the brain via their axons, which are bundled together in the optic nerve. Horizontal cells extend processes widely in the OPL, and mediate lateral interactions within this first synaptic zone. Amacrine cells, like horizontal cells, extend processes widely in the IPL, and mediate lateral interactions within it. The predominant type of glial cell in the vertebrate retina is called the Müllar cell. These cells extend vertically through the retina from the distal margin of the ONL to the inner margin of the retina.

2. Retinal synapses

In the CNS including the retina, cell to cell communication can be divided into two general types: chemical and electrical synapses (Fig. 3). In chemical synapses, a chemical agent known as a neurotransmitter, which is stored in synaptic vesicles, is released by exocytosis as the presynaptic cell is depolarized. The neurotransmitter rapidly diffuses across the synaptic cleft and binds to specific receptors on the membrane of the postsynaptic cell. The binding of the

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neurotransmitter to the receptor causes ion channels in the postsynaptic membrane to open and/or close and the resulting neurotransmitter-induced currents alter the membrane potential of the postsynaptic cell (for reviews, see Dowling, 1987; Rodieck, 1998). Two main classes of synaptic pathways, excitatory and inhibitory synapses, are generally recognized because neurotransmitters released from presynaptic terminals can generate excitatory or inhibitory effects in the postsynaptic neurons (for review, see Scheller and Hall, 1992). The main excitatory transmitters are the acidic amino acids, L-glutamate and acetylcholine (Ach). These transmitters cause membrane potential toward action potential threshold (Fig. 3A, left). The main inhibitory transmitters are γ -aminobutyric acid (GABA) and glycine. These transmitters can shift membrane potential away from action potential threshold (Fig. 3A, right). In the vertebrate retina, L-glutamate has been identified at the terminals of photoreceptor cells and bipolar cells, and Ach has been identified in a subpopulation of amacrine cells (cholinergic amacrine cells) (for review, see Hutchins, 1987; Cheon and Saito, 1999). GABA has been identified in a subpopulation of horizontal cells (GABAergic horizontal cells) and a subpopulation of amacrine cells (GABAergic amacrine cells), and glycine has been identified in a subpopulation of amacrine cells (glycinergic amacrine cells) (Vaughn et al., 1981; Ball, 1987; Yang and Yazulla, 1988; Araki and Kimura, 1991; Pourcho and Owczarzak, 1991).

Electrical synapses work by allowing ionic current to flow passively through a special kind of intercellular contacts called gap junctions (Fig. 3B). Gap junctions contain precisely aligned, paired channels in the membrane of each neuron, such that each channel pair forms a pore. The pore of a gap junction channel is large enough to pass inorganic ions and other small water-soluble molecules less than 1.5 kDa between the cytoplasm of pairs of neurons, thereby coupling the cells both electrically and metabolically. In the adult

vertebrate retina, gap junctions are found between some cells of the same morphological subtype, as well as between cells of different classes (for reviews, see Vaney, 1994; Cook and Becker, 1995).

3. Regeneration of the vertebrate retina

Many vertebrates can regenerate their neural retina following damage of the original retina (for reviews, see Reyer, 1977; Hitchcock and Raymond, 1992; Mitashov, 1996; Raymond and Hitchcock, 1997; Tsonis, 2000, 2002). However, there is considerable variability in this capacity among species. In birds and mammals, for example, retinal regeneration is restricted to early embryonic life (Stroeva, 1960; Coulombre and Coulombre, 1965, 1970; Machemer and Norton, 1968; Anderson et al., 1981; Fisher et al., 1991). In anuran amphibians, this ability persists up to metamorphosis (Lopashov and Sologub, 1972; Reh et al., 1987). On the other hand, certain species of fish and urodele amphibians retain the ability to regenerate a functional retina even in adult life. In the adult goldfish, for example, two cellular sources of the regenerated retina have been described: (1) intrinsic progenitor cells existing at the ciliary marginal zone (CMZ); and (2) progenitor cells within the ONL, termed rod precursor cells (Raymond and Rivlin, 1987; Raymond et al., 1988; for review, see Hitchcock and Raymond, 1992). In adult newts and salamanders, cellular sources of their retinal regeneration are retinal pigment epithelial (RPE) cells and intrinsic progenitor cells at the CMZ (Wachs, 1920; Stone, 1950a,b; Hasegawa, 1958; Keefe, 1973a,b). The process of retinal regeneration in adult newts and salamanders has been well studied morphologically (for reviews, see Reyer, 1977; Mitashov, 1996; Reymond and Hitchcok, 1997; Saito, 1999; Tsonis, 2000). After surgical removal of neural retinas from adult newt eyes, the remaining RPE cells lose their pigment granules, transdifferentiate into retinal progenitor cells, which further differentiate into various neurons, and then

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finally reform a functional neural network. Such a retinal regeneration, as well as retinal development, promises to be a useful tool for understanding metaplasia as well as examining the mechanisms of the neural differentiation and the formation of neural networks in the CNS.

4. Aims of the present study

One of my research interests is to understand the mechanisms of differentiation of various retinal neurons and their synapse formation on the basis of functional aspects. As noted above, the regeneration of adult newt retina is a useful model system for this purpose. Studies of newt retinal regeneration have concentrated mainly on morphological and histochemical changes (Klein et al., 1990; Negishi et al., 1992; Saito et al., 1994; Chiba et al., 1997; Cheon and Saito, 1999; Cheon et al., 1998, 2001; Umino and Saito, 2002). Recent studies have also started to focus on molecular mechanisms that lead to differentiation of retinal neurons (Park and Hollenberg, 1989; Kaneko et al., 1999a, 2001; Marquardt et al., 2001; Sakakibara et al., 2002; Saito and Chiba, 2003). However, studies of functional differentiation of retinal neurons during regeneration of adult newt retina have been limited (Sarthy and Lam, 1983; Kaneko and Saito, 1992; Chiba and Saito, 1995b; Umino, 2003).

The process of retinal regeneration from a functional perspective includes genesis of neurotransmitter systems, and of voltage- or ligand-gated ion channels. In the present study, I focus on the functional differentiation of retinal ganglion cells, which process and convey information from the retina to visual centers in the brain. Ganglion cells are the first retinal neurons to differentiate during retinal regeneration (Cheon et al., 1998), as well as during retinal development in most vertebrate embryos (for review, see Altshuler et al., 1991). They express voltage-gated ion channels, such as Na^+ channels, and both excitatory and inhibitory inputs from other retinal neurons (Chiba and Saito,

1995a; Chiba et al., 1997; Cheon et al., 1998; Chiba, 1998) which are easily identified by electrophysiological recording techniques.

This thesis is composed of two chapters. In Chapter 1, I report a decay of gap junctions between progenitor cells associated with ganglion cell differentiation during retinal regeneration. In the early regenerating newt retina, it has been reported that progenitor cells were electrically inexcitable and strongly coupled to each other through gap junctions (Chiba and Saito, 2000). However, it is not yet known when ganglion cells start to differentiate and whether gap-junction channels correlate with ganglion cell differentiation.

In Chapter 2, I report the time course of the appearance and maturation of neurotransmitter receptors. This kind of experiment has been done on solitary ganglion cells dissociated from newt retinas at different stages of regeneration (Chiba and Saito, 1995b). However, the use of dissociated cells provides certain technical disadvantages, such as possible contamination by other neurons and loss of dendritic processes by the dissociation procedures. To solve these problems, in this study, I prepare living slice preparation from the retina at different stages of regeneration. This enables us to directly correlate the morphological differentiation of ganglion cells with their functional differentiation.