Ⅰ Abstract

Adult newt (*Cynops phyrrhogaster*) possesses the ability to regenerate a new functional retina following complete removal of the original retina even in adult life. Regenerating retinas were divided into five stages ('early', 'intermediate-I, -II and -III', and 'late' stages) on their basis of the morphological appearance. In the present study, I prepared living slice preparations of the adult newt retina at different stages of regeneration, and investigated the functional differentiation of retinal neurons, especially ganglion cells, during regeneration using whole-cell patch-clamp methods. Cells from which currents were recorded were identified by Lucifer Yellow and/or biocytin fills. In the 'early' regenerating newt retina, it has been reported that mitotically active progenitor cells were electrically inexcitable and strongly coupled each other through gap junction. In Chapter 1, I raised a question whether loss of the gap junctional coupling correlates with ganglion cell differentiation. For this purpose, I measured changes in the gap-junction currents and appearance and maturation of voltage-gated Na^+ currents during retinal regeneration.

All progenitor cells examined in the 'intermediate-I and-II' regenerating retinas, like those in the 'early' regenerating retina (Chiba & Saito, 2000), were characterized by oval or slender shape and did not exhibit voltage-gated Na^+ currents. They showed gap-junction currents under conditions that suppressed nonjunctional currents flowing through the plasma membrane. The presence of gap junctions between progenitor cells was confirmed by tracer coupling following intracellular injection of biocytin. Voltage-gated Na^+ currents were first detected in premature ganglion cells with rounded somata located at the most proximal level of the 'intermediate-II' regenerating retina. On average, the maximum Na^+ current amplitude was 432 pA and the activation threshold of the current was about –45 mV. These premature ganglion cells did not exhibit

tracer coupling. In the 'late' regenerating retina, both the maximum Na^+ current and the activation threshold increased in amplitude up to about 795 pA and –55 mV respectively. Electrical and tracer coupling were not observed between these cells. Mature ganglion cells revealed a maximum Na^+ current of about 953 pA and an activation threshold of about –56 mV. They did not exhibit significant gap-junction currents, but showed tracer coupling. I provide evidence suggesting that the loss of gap junctions correlates with the appearance of voltage-gated Na^+ currents in ganglion cells.

Since the time course of the appearance of voltage-gated Na^+ channels was clarified in Chapter 1, I investigated the appearance and maturation of excitatory and inhibitory neurotransmitter-sensitivity of ganglion cells in Chapter 2. Progenitor cells in regenerating retinas did not express any voltage-gated Na^+ currents or responsiveness to excitatory amino acid analogues (AMPA and NMDA) and inhibitory amino acids (GABA and glycine). Voltage-gated Na^+ currents were first detected in premature ganglion cells with round cell body located at the most proximal level of the 'intermediate-II' regenerating retina. AMPA- GABA- and glycine-induced currents were simultaneously observed in many premature ganglion cells expressing Na^+ channels, but not all, suggesting that the onset of Na^+ channels is slightly earlier than that of excitatory and inhibitory amino acid receptors during regeneration. NMDA-evoked currents were first observed in the 'intermediate-III' regenerating retina just before synaptogenesis. Pharmacological properties and reversal potential values of the excitatory and inhibitory amino acid responses did not change substantially between regenerating ganglion cells and mature ganglion cells, while rectification properties of current-voltage relations for AMPA and NMDA responses were more voltage dependent in premature ganglion cells than mature ganglion cells.