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EST analysis of regenerating newt retina

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A vertebrate retina is an organ belonging to the central nerve system (CNS), and is usually difficult to regenerate except at an embryonic stage in life. However, certain species of urodele amphibians, such as newts and salamanders, possess the ability to regenerate a functional retina from retinal pigment epithelial (RPE) cells even as adults. 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 retinal neurons, and then finally reform a functional neural network.

To understand the molecular mechanisms of CNS regeneration, we attempted to investigate the genes expressing in regenerating newt retina. mRNAs were isolated from regenerating retinas at 18-19 days after the surgical removal of the normal retina, and a cDNA library (regenerating retinal cDNA library) were constructed. Our EST analysis of 112 clones in the regenerating cDNA library revealed that about 70% clones are closely related to the genes previously identified. About 40% clones are housekeeping genes, and about 15% clones encode proteins related to the regulation of gene expression and to the proliferation of the cells. Sequences similar to neural retina- and RPE-specific genes were not detected at all. These results led us to suppose that the regenerating retinal cells are in a state considerably different from those of neither neural retina nor RPE cells.

Key words: Retina; Regeneration; Differentiation; Expression sequence tag; Newt (*Cynops pyrrhogaster*);

INTRODUCTION

A vertebrate retina is an organ belonging to the central nerve system (CNS), and is usually difficult to regenerate except at an embryonic stage in life. However, certain species of urodele amphibians, such as newts and salamanders, possess the ability to regenerate a functional retina from retinal pigment epithelial (RPE) cells even as adults [1,2]. After surgical removal of neural retinas from adult newt eyes, the remaining RPE cells lose their pigment granules (depigmentation), transdifferentiate into retinal progenitor cells, which further differentiate into various retinal neurons, and then finally reform a functional neural network.

Vigorous studies using molecular biological approaches have been carried out to investigate the development mechanisms. However, the molecules so far characterized in newt retina are limited [2-5]. In the present study, we isolated mRNA from regenerating newt retinas. Then, a cDNA library, regenerating retinal cDNA library, were constructed, and genes expressing in regenerating retinas were analyzed. Our results suggested that our library is available for EST analysis of regenerating newt retina.

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MATERIALS AND METHODS

Adult newts (*Cynops pyrrhogaster*) were purchased from a local supplier and handled according to the Guidelines for Animal Experimentation of Osaka University. Neural retinas and lens were surgically removed from adult newts anesthetized with 0.1 % FA 100 (Tanabe Pharmacy, Osaka, Japan) as described previously [5,6]. Eyeballs were enucleated under anesthesia at 18 or 19 days after operation. Retinas were homogenized in ISOGEN (Nippon Gene, Tokyo, Japan), and total RNAs were isolated according to the manufacturer's protocol. mRNAs were purified using a Quick Prep Micro mRNA Purification Kit (Amercham Pharmacia Biotech).

Construction of the cDNA libraries were carried out by using Uni ZAP XR Library Construction Kit (Stratagene). Briefly, double-stranded cDNA with an additional XhoI site at the end of the polyA tail was ligated with an EcoRI adapter. After digestion of EcoRI and XhoI, cDNA was inserted between the EcoRI and XhoI sites of Lambda-ZAPII, and packaged with Giga-Pack III gold. After an amplification of the cDNA libraries, clones were mass excised, and transformed into plasmids by an ExAssist-SOLR system (Stratagene). Plasmids were isolated and sequenced using a dye primer sequencing kit (Amersham) and a DNA sequencer (Hitachi SQ-5500).

RESULTS AND DISCUSSION

We isolated 112 clones from the regenerating retinal cDNA library, and digested with EcoRI and XhoI endonucleases. Inserted cDNAs that appeared to have 0.5-6 kb in length were sequenced with T3 primer (EcoRI direction). Our EST analysis revealed that closely related sequences were found in the databases for about 70% clones: 43 clones (ca. 40% of 112) are likely housekeeping genes, and 17 clones (ca. 15%) encode proteins related to the regulation of gene expression and to the proliferation of the cells. About 30% sequences did not hit any sequences in the database, but open reading frames (ORFs) were found in 13 (ca. 12%) sequences. In 112 sequences, only a couple of sequences are identical, suggesting that our library is available for EST analysis of regenerating retina.

Regenerating retinas were divided into three stages (early, intermediate, and late) based on their morphological appearance, and intermediate-regenerating retina was further divided into three substages, I, II and III as described by Cheon and Saito [7]. In our histochemical analysis, the regenerating retinas at 18 or 19 days after operation are in the stages from early to intermediate II, which are before the expression of rhodopsin [5]. Clones encoding neural retina- and RPE-specific proteins were not detected in 112 clones at all. These results led us to suppose that the regenerating retinal cells are in a state considerably different from those of neither neural retina nor RPE cells.

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