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OIST Principal Investigator Dr. Ichiro MASAI elucidates Mechanism underlying lens fiber differentiation: Contribution to therapy of cataract eye disease

Okinawa, Japan, September 13, 2010 — In vertebrates, the lens of the eye consists of epithelial cells and fiber cells. During the differentiation of lens fiber cells in the creation of the eye, subcellular organelles including the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus are degraded, resulting in the transparency of lens fiber cells. However, the mechanism underlying the differentiation of lens fiber cells remains to be elucidated.

Researchers of the OIST Developmental Neurobiology Unit, led by Principal Investigator Dr. Ichiro Masai, have reported their research on the mechanism underlying lens fiber cell differentiation in the scientific journal *Development*. A cataract is a condition where the lens of the eye becomes increasingly opaque, and is a common eye disease causing vision loss. Cataract formation may be caused by various cellular defects, but the majority (90%) of causes are associated with aging. A survey in 2005 by the Ministry of Welfare in Japan showed that almost 1.3 million people suffer from cataracts, making it the most common eye disease in Japan. In current therapy of cataracts, it is common to surgically remove the lens. However, the increasing opacity of the remaining lens epithelial cells, a process called “posterior cataract opacification”, is a serious complication of cataract surgery. The mechanism underlying lens fiber cell differentiation that we have elucidated is associated with posterior cataract opacification. Their investigations will continue to reveal more about this process and thus improve our ability to treat cataract disease.

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http://dev.biologists.org/content/137/19/3257.abstract

*** Please see the attachment for the summary of Dr. Masai’s research paper.

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The ubiquitin proteasome system is required for cell proliferation of the lens epithelium and for differentiation of lens fiber cells in zebrafish
～Contribution to improvement of cataract therapy～

1. Background

In vertebrates, the lens consists of epithelial cells and fiber cells. During the differentiation of lens fiber cells, subcellular organelles including the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus are degraded, resulting in the transparency of lens fiber cells. However, the mechanism underlying the differentiation of lens fiber cells remains to be elucidated.

The lens placode delaminates from the epidermal ectoderm and forms the lens vesicle. In the lens vesicle, posterior epithelial cells differentiate into lens fiber cells, whereas anterior lens epithelial cells remain as proliferative lens progenitor cells. At the interface between anterior lens epithelial cells and the posterior lens fiber core, called the equator, epithelial cells differentiate into lens fiber cells, which elongate towards both the anterior and posterior poles of the lens sphere, become flat, and cover the old lens fiber core, similar to an onion (Fig. 1). Because of these developmental profiles, the lens is a good model with which to study the mechanism underlying cell differentiation and morphogenesis. In addition, lens fiber cells lose subcellular organelles including the nucleus, endoplasmic reticulum, and Golgi apparatus, resulting in their transparency. It is commonly known that mammalian red blood cells have no nuclei because they are removed at the final stage of erythroid cell differentiation. Macrophages engulf the nuclei that are expelled from erythroid precursor cells. On the other hand, the denucleation of lens fiber cells is considered to occur in a cell-autonomous manner.

During the denucleation process, nuclear DNA is degraded. In mice, DNase II-like acid DNase (DLAD, DNase2b) was identified as a DNase involved in the denucleation of lens fiber cells (Noshimoto et al., 2003 Nature 424, 1071-1074.). Because DLAD is an acid DNase homologous to DNase II, which is found in lysosomes, that has an essential role in the degradation of nuclear DNA expelled from erythroid precursor cells, a possible model is that the degradation of lens fiber cell nuclei is mediated by the cell’s own lysosomes through a process called autophagy. However, it was reported that organelle degradation in the lens occurs normally in autophagy-deficient Atg5 knockout mice, suggesting that organelle degradation during lens differentiation is independent of autophagy. The mechanism underlying lens fiber cell denucleation remains to be elucidated.

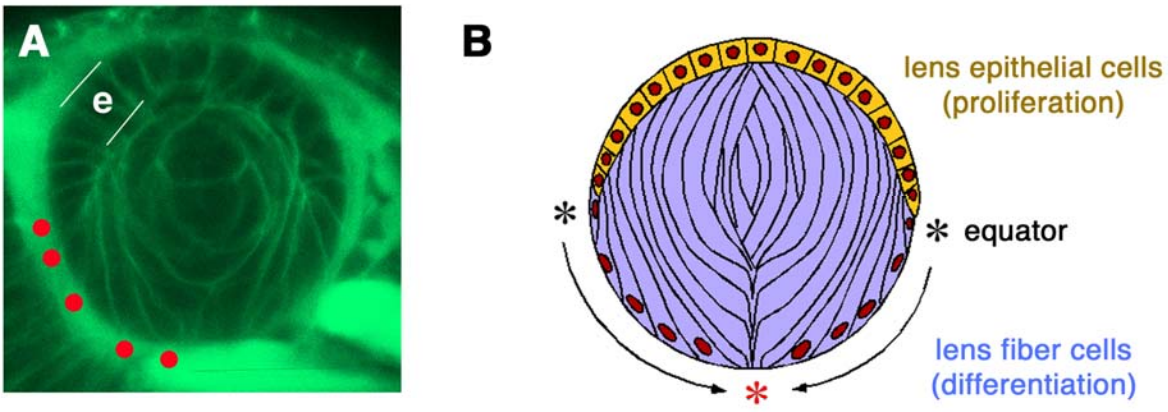

Figure 1

Figure 1

(A) Zebrafish lens at 31 hours-post-fertilization (hpf) labeled with fluorescent lipid. e: lens epithelial cells. The red circle indicates the basal foot of lens fiber cells, which migrate to the posterior region of the lens fiber sphere.

(B) Schematic drawing of lens fiber cell differentiation. Anterior lens cells become lens epithelial cells (yellow), whereas posterior lens cells differentiate into lens fiber cells (light blue), which form the lens fiber core. Newly differentiating lens fiber cells are born at the equator (asterisk), extend processes bidirectionally towards the anterior and posterior poles of the lens fiber core, and cover the old lens fiber core similar to an onion. Differentiating lens fiber cells become flat and lose their subcellular organelles including the nuclei. The red asterisk indicates the position where the basal feet of opposing lens fiber cells meet and are sutured.

2. Research findings and significance

To elucidate the mechanism underlying the differentiation of lens fiber cells, we screened zebrafish mutants showing defects in this process. We identified a zebrafish mutant, *volvox* (*vov*), in which the nuclei of lens fiber cells fail to be eliminated and the morphogenesis of lens fiber cells is abnormal (Fig. 2).

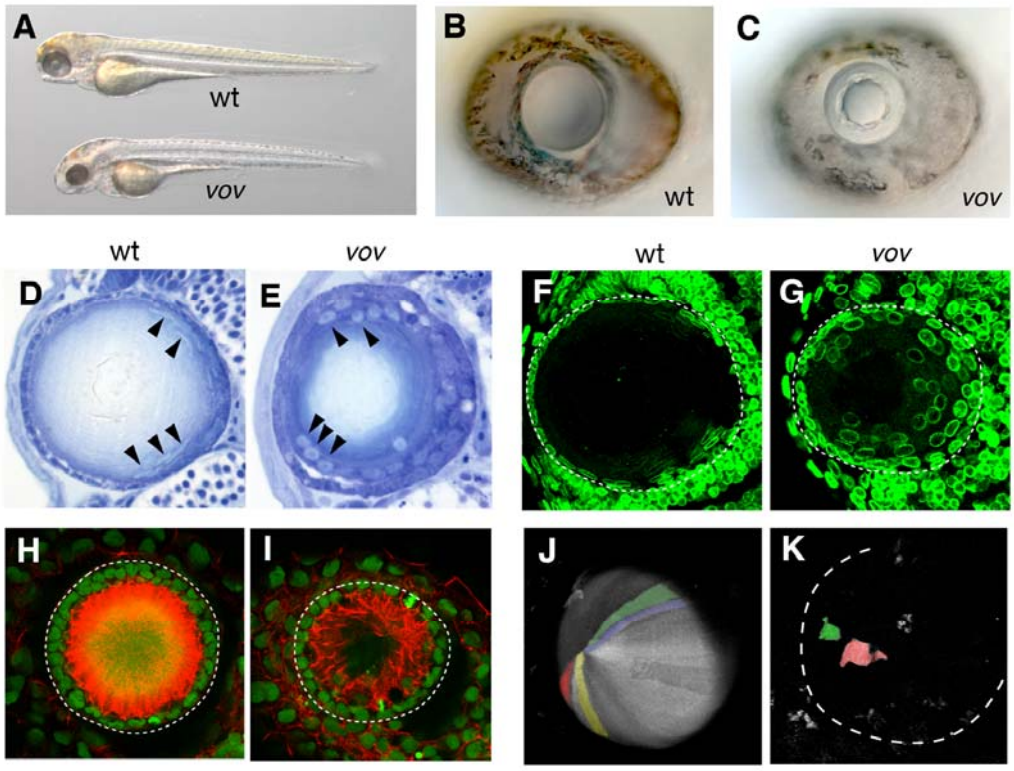

Figure 2

Figure 2

(A) Wild-type and vov mutant embryos at 72 hpf.

(B-C) Lateral view of 72 hpf wild-type (B) and vov mutant (C) lenses. In the vov mutant lens, a small lens core is surrounded by less transparent and more irregularly shaped cells.

(D, E) Sections of 72 hpf wild-type (D) and vov mutant (E) lenses. The nuclei are flat and positioned along the posterior edge of the wild-type lens sphere (arrowheads in D), whereas the nuclei are swollen and located in the anterior region surrounding a small lens fiber core in the vov mutant (arrowheads in E).

(F, G) Expression of LaminB1 in 72 hpf wild-type (F) and vov mutant (G) lenses. LaminB1 demarcates the nuclear membrane (green). White dashed lines indicate the outline of the lens sphere.

(H, I) Anterior view of 72 hpf wild-type (H) and vov mutant (I) lenses labeled with Rhodamine-conjugated phalloidin (red) and Sytox green (green), which stain Filamentous actin (F-actin) and nucleus, respectively. F-actin is dense in the wild-type lens fiber region, whereas F-actin is sparsely and irregularly distributed in the vov mutant lens.

(J, K) Confocal images of lens of 72 hpf wild-type (J) and vov mutant (K) zebrafish expressing the SAGFF168A; UAS:EGFP transgene. Individual lens fiber cells are indicated by pseudocolors. In the vov mutant, GFP-expressing cells fail to elongate correctly, do not maintain a fiberlike structure, and appear to undergo degradation.

We cloned the *vov* mutant gene and found that it encodes a regulatory subunit of a proteasome, *psmd6* (Fig. 3A and 3B). A proteasome is an approximately 2,500kDa protein complex and selectively degrades polyubiquitinated proteins. This protein degradation system is called as the ubiquitin proteasome system (UPS). UPS is important for the removal of unnecessary proteins and regulates normal cellular functions such as cell proliferation, cell differentiation, and immunoresponses through the degradation of various regulatory proteins. We found that the UPS-mediated protein degradation is compromised in the *vov* mutant (Fig. 3C). In eukaryote cells, protein degradation is mediated selectively by UPS or nonselectively by autophagy. Our findings suggest that UPS is required for lens fiber cell differentiation including lens denucleation.

Furthermore, we found that an E3 ubiquitin ligase, which is Anaphase promoting complex/cyclosome (APC/C), is involved in lens nucleation. It was reported that, in a mouse lens cell culture, TGF-β promotes the APC/C-dependent degradation of a transcription corepressor, snoN, which inhibits the transcription of a Cdk inhibitor, p57, resulting in the promotion of lens fiber cell differentiation. Taken together, these findings suggest that the APC/C-dependent polyubiquitination of proteins and the subsequent degradation by proteasomes are important for the differentiation of lens fiber cells (Fig. 3D).

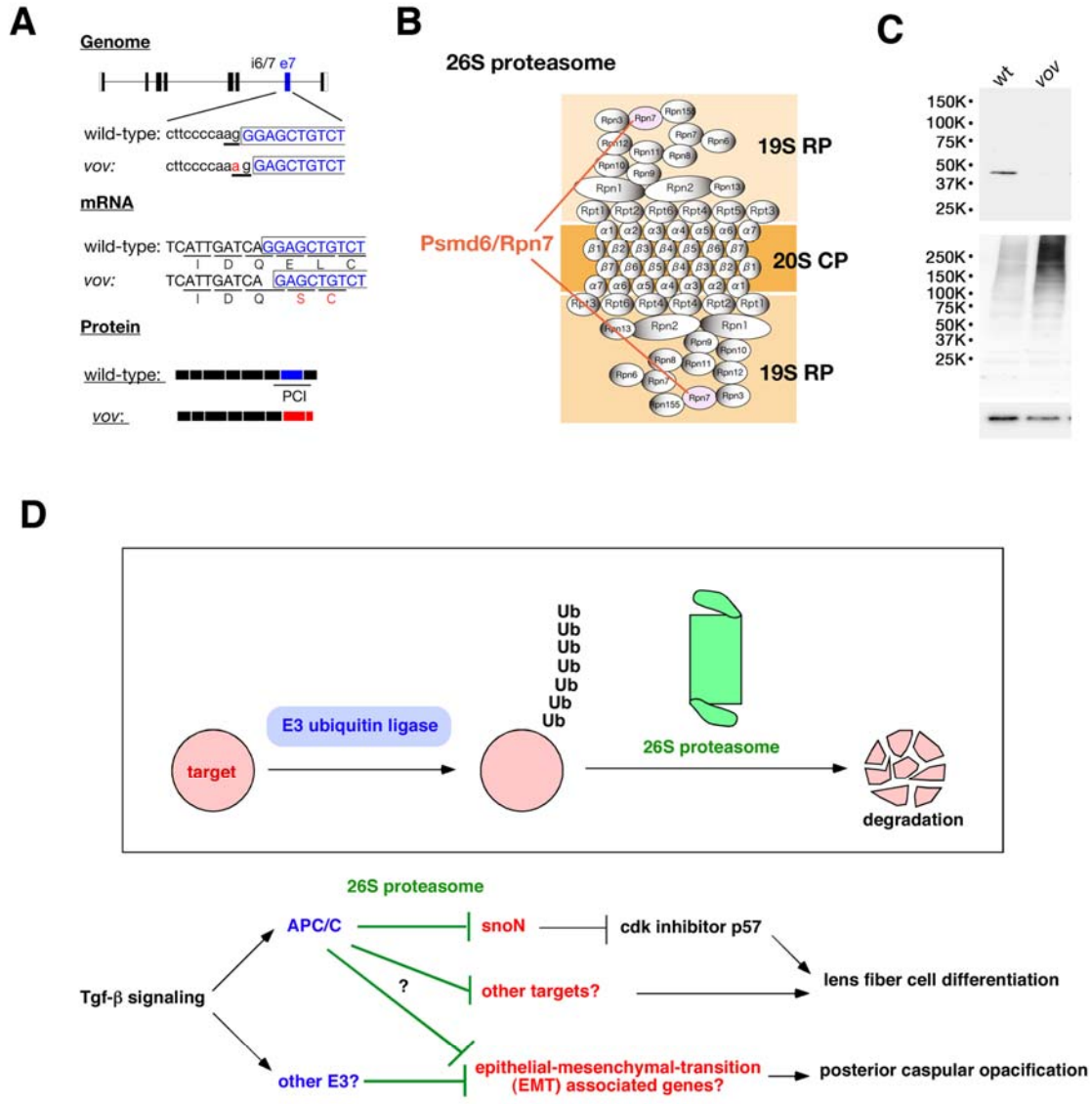

Figure 3

Figure 3

(A) The vov mutant gene encodes Psmd6. Nucleotide substitution occurs in a splicing acceptor site of the intron boundary causing a one-base deletion in psmd6 mRNA (top) that results in a frameshift within the coding region (middle) and loss of the PCI domain, which is necessary for proteasome activity (bottom).

(B) Schematic drawing of 26S proteasome, which consists of 20S CP and 19S RP. Psmd6 is a subunit of RP.

(C) Western blotting of 72 hpf wild-type and vov mutant with anti-Psmd6 (top) and anti-polyubiqutinated protein (FK2) (bottom) antibodies. The level of Psmd6 is reduced and that of polyubiquitinated proteins is increased in the vov mutant.

(D) Role of UPS in lens fiber cell differentiation.

Top panel: Target proteins (red) are polyubiquitinated by E3-ubiquitin ligase (light blue) and degraded by the proteasome (green). In differentiating lens fiber cells, TGF- signaling activates APC/C, which polyubiqutinates snoN. The proteasome-dependent degradation of snoN promotes lens fiber cell differentiation by activating the Cdk inhibitior p57. We show that APC/C-dependent polyubiquitination and proteasome-dependent degradation of proteins are important for lens fiber cell differentiation. It was reported that TGF- signaling and UPS promote posterior capsular opacification, which is a common complication with vision loss after cataract surgery, suggesting that the understanding of the role of UPS in lens fiber cell differentiation will contribute to the improvement of the therapy of cataracts.

3. Future perspective

In humans, posterior capsular opacification (PCO) is the most common postoperative complication of cataract surgery that causes vision loss. Previous studies using human lens cell culture suggested that PCO is mediated by the activation of TGF-β and UPS (Fig. 3D) (Hosler et al., 2006 INVOS 47, 2569-2575.). In the future, further study on the role of UPS in lens fiber cell differentiation will contribute to the improvement of the therapy of cataracts.

<Research Article>

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2) Article Title: The ubiquitin proteasome system is required for cell proliferation of the lens epithelium and for differentiation of lens fiber cells in zebrafish

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