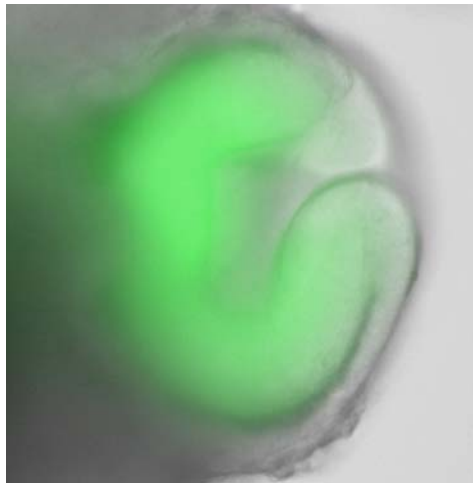


The self-made eye: Formation of optic cup from ES cells

April 7, 2011 – Developmental processes are increasingly well-characterized at the molecular and cell biological levels, but how more complex tissues and organs involving the coordinated action of multiple cell types in three dimensions is achieved remains something of a black box. One question of particular interest and importance is whether signaling interactions between neighboring tissues are essential to guiding organogenesis, or whether these can arise autonomously from developmental routines inherent to a given primordial tissue. Finding answers to these questions will be critical both to a better understanding of embryonic phenomena and to the ability to control the differentiation of cell populations into desired configurations.



Optic cup derived from mouse ES cells. GFP shows expression of Rx.

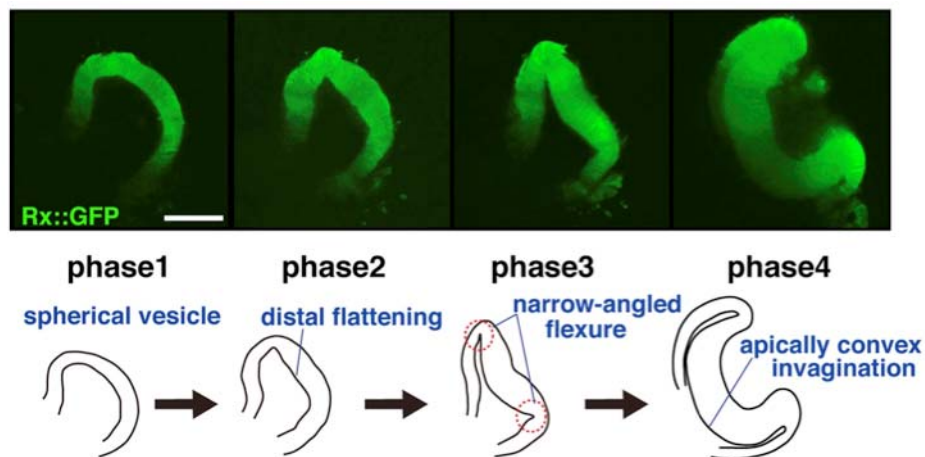
A breakthrough new report by Mototsugu Eiraku, deputy leader of the Four-dimensional Tissue Analysis Unit and colleagues in the Laboratory for Neurogenesis and Organogenesis (Group Director, Yoshiki Sasai), as well as the RIKEN VCAD Program, and Kyoto and Osaka Universities, describes how mouse embryonic stem cells (ESCs) are able to differentiate and assemble into an optic cup, capable of giving rise to a tissue exhibiting the stratified structure characteristic of the retina *in vivo*. Published in *Nature*, the study used a cutting-edge three-dimensional tissue culture system not only to demonstrate this self-organizing capacity of pluripotent stem cells, but the underlying cell dynamics as well.

The mechanistic basis for the formation of the optic cup, with its complex two-walled structure, has been a longstanding question in embryology. The retina, with its origins in the lateral midbrain, is part of the central nervous system. Its development begins with the formation of the optic vesicle, a pocket of epithelium that deepens and pinches to form the optic cup, which develops a double layer of cells, with pigment epithelium on the outer, and neural retina on the inner wall. It has generally been thought that this transformation is triggered by chemical and physical influences from other tissues, such as lens or cornea, but some, including the father of experimental embryology, Hans Spemann, have suggested that perhaps external induction or force is not necessary.

To resolve this question, Eiraku et al. built on a series of techniques and findings emerging from the use of the SFEBq (serum-free culture of embryoid body-like aggregates) ES cell culture system developed by the Sasai lab, which had previously been used to differentiate these pluripotent stem cells into a wide range of neuronal cell types, including, recently, structurally organized cerebral cortical neurons. By adding extracellular matrix proteins to

the SFEBq medium, the group was able to epithelially-organized retinal precursors at high efficiencies by day 7 of culture. One day later, optic vesicle-like structure began to form, followed by bi-layered optic cup-like structures by day 10. The pigmented and neuronal character of the outer and inner layers of cells in these spontaneously formed tissues were confirmed by gene expression, indicating that optic cup development had been recapitulated *in vitro*, and importantly, in the absence of any external signaling sources, such as lens, demonstrating the capacity for self-organization.

They next used multi-photon microscopy to explore the mechanisms behind this process of self-assembly in 3D. They found that after the ES cell-derived retinal precursors differentiated into pigmented epithelial and neuronal layers, the tissue underwent a four step morphological rearrangement on its way to assuming the optic cup structure. When they examined cytoskeletal behaviors in this process, they noted that myosin activity dropped in the region of the epithelium that bend inward to form the cup, giving the flexibility needed to form a pocket driven by expansion of the epithelium through cell division.



Four-step morphological transformation in formation of optic cup *in vitro* revealed by two-photon microscopy over three-period.

Computer simulation of the mechanics behind this revealed that three principal forces can explain the optic cup-forming event. First, the a region of the epithelium must lose rigidity, allowing it to buckle inward, after which cells at the hinge points (defined by the border between presumptive pigment epithelium and neuronal regions) must undergo apical constriction, giving them a wedge-like shape. Once these conditions are met, expansion of the tissue surface by cell division results further involution of the cup, all of which are very much in line with the experimental findings.

As a final test of the *in vitro* structure's ability to mirror its embryonic counterpart, Eiraku excised the neuronal layer from the ES cell-derived optic cup and allowed it to develop in 3D cell culture under conditions optimized for spurring neuronal maturation. He found that the retinal neurons underwent active mitosis and ultimately organized into a six-layer stratified and synapse-forming neuronal structure closely resembling that of the post-natal retina.

“What we've been able to do in this study is resolve a nearly century-old problem in embryology, by showing that retinal precursors have the inherent ability to give rise to the complex structure of the optic cup,” says Sasai. “It's exciting to think that we are now well on the way to becoming able to generate not only differentiated cell types, but organized tissues from ES and iPS cells, which may open new avenues toward applications in regenerative

RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

medicine.” Potential applications include regenerative medicine approaches to the treatment of retinal degenerative disorders, such as retinitis pigmentosa.