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Mechanical effects of the surface ectoderm on optic vesicle morphogenesis in the chick embryo

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ABSTRACT

Precise shaping of the eye is crucial for proper vision. Here, we use experiments on chick embryos along with computational models to examine the mechanical factors involved in the formation of the optic vesicles (OVs), which grow outward from the forebrain of the early embryo. First, mechanical dissections were used to remove the surface ectoderm (SE), a membrane that contacts the outer surfaces of the OVs. Principal components analysis of OV shapes suggests that the SE exerts asymmetric loads that cause the OVs to flatten and shear caudally during the earliest stages of eye development and later to bend in the caudal and dorsal directions. These deformations cause the initially spherical OVs to become pear-shaped. Exposure to the myosin II inhibitor blebbistatin reduced these effects, suggesting that cytoskeletal contraction controls OV shape by regulating tension in the SE. To test the physical plausibility of these interpretations, we developed 2-D finite-element models for frontal and transverse cross-sections of the forebrain, including frictionless contact between the SE and OVs. With geometric data used to specify differential growth in the OVs, these models were used to simulate each experiment (control, SE removed, no contraction). For each case, the predicted shape of the OV agrees reasonably well with experiments. The results of this study indicate that differential growth in the OV and external pressure exerted by the SE are sufficient to cause the global changes in OV shape observed during the earliest stages of eye development.

1. Introduction

In the vertebrate embryo, the primitive eyes initially develop as bilateral bulges that grow outward from the part of the prosencephalon (forebrain) that later becomes the ventral diencephalon. As these optic vesicles (OVs) elongate, they come into contact with the surrounding surface ectoderm (SE), and both layers then invaginate to create the optic cup (prospective retina) and lens vesicle (Martinez-Morales and Wittbrodt, 2009). The initial shaping of the OVs sets the stage for these later events.

This paper deals with the mechanics of OV morphogenesis in the chick embryo before the onset of invagination. At Hamburger–Hamilton (HH) stage 9 (30 h of a 21-day incubation period) (Hamburger and Hamilton, 1951), the OVs are relatively spherical (Fig. 1A). By HH13 (50 h), they become pear shaped and bend toward the caudal and dorsal sides of the embryo, similar to the human OV at a comparable stage (Fig. 1B, C). The open connection to the forebrain forms the optic stalk, which later becomes the optic nerve.

Recent studies in fish have shown that cells are added to the OVs during evagination through both cell division and migration from the prosencephalon, with migration playing the greater role (Kwan et al., 2012; Rembold et al., 2006). Although further study is needed, some evidence suggests that cells move similarly in chick OVs (Kwan et al., 2012). Regardless of the specific mechanism, it appears that addition of new cells to the OVs drives evagination, rather than, for example, active changes in cell shape. In addition, Hilfer et al. (1981) speculated that OV shape is influenced by external constraints on its expansion, but this idea apparently has heretofore not been confirmed.

Here, we examine the hypothesis that OV morphogenesis is driven by differential growth constrained by contact with the SE. First, we use mechanical and chemical perturbations to determine how the SE affects OV shape. Then, we develop computational models to simulate the growing OV with and without the SE. The results indicate that a combination of differential growth in the OV and external pressure exerted by the SE is sufficient to cause the global changes in OV shape observed during early eye development.

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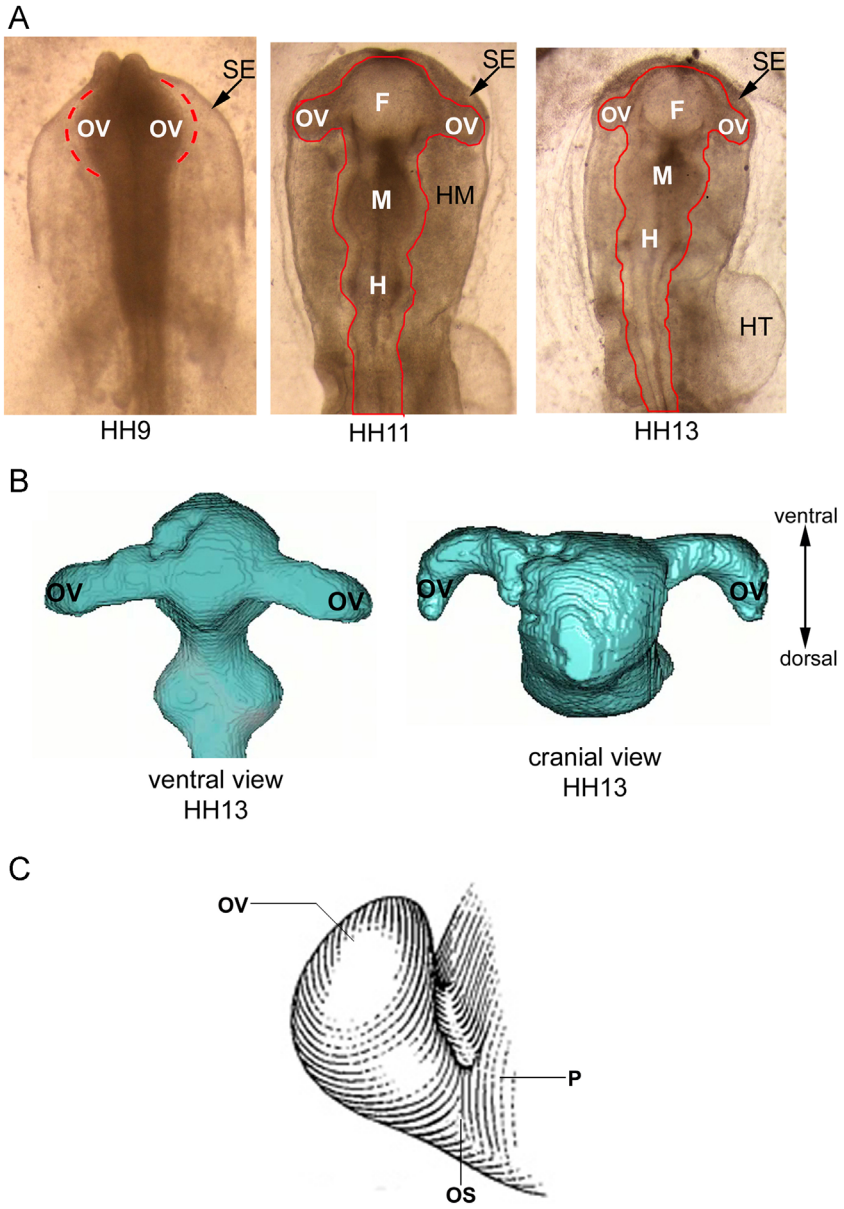

Fig. 1. Eye development in early chicken embryo. (A) Embryos at stages HH9, HH11, and HH13. Major subdivisions of the brain include three vesicles: forebrain (F), midbrain (M), and hindbrain (H). The optic vesicles (OV) grow as protrusions from the forebrain. The brain is surrounded by head mesenchyme (HM) and surface ectoderm (SE). HT=heart tube (dorsal view). (B) 3-D reconstruction of anterior part of HH13 brain lumen obtained from OCT images (ventral and cranial views) (courtesy Ben Filas). Note prominent dorsal bending of OVs in the cranial view. (C) Schematic diagram of human optic vesicle (OV) at stage 13. Reprinted from O'Rahilly and Müller (1987) with permission. OS=optic stalk; P=prosencephalon (forebrain).

2. Methods

2.1. Embryo preparation

Fertilized white Leghorn chicken eggs were incubated in a humidified, forced draft incubator at 37 °C for 33–52 h to yield embryos between HH stages 9 and 13 (Hamburger and Hamilton, 1951). Whole embryos were removed from the eggs using a filter paper method (Voronov and Taber, 2002). To preserve the stresses normally present in the tissue, the embryo and underlying vitelline membrane were kept intact. Each embryo was then placed in a 35 mm culture dish, completely submerged under a thin layer of liquid culture media, and incubated at 37 °C in 95% O_2 and 5% CO_2. This method prevents artifacts caused by fluid surface tension, which alter the mechanical stresses in the embryo (Voronov and Taber, 2002).

2.2. Perturbations

To determine the effects of the SE on OV shape, the SE was dissected from the OV in the region of contact. At early stages (HH9–10), there is not yet significant adherence between the OV and SE, and we were able to perform the dissection using microscissors and thin glass needles after first removing the vitelline membrane for access. As a control, embryos cultured with only the vitelline membrane removed appeared to develop normally. To loosen the relatively strong adherence at later stages (HH13), we first applied 2% Nile Blue sulphate. After a few seconds, the surface ectoderm blistered and could be easily removed (Araujo et al., 1998; Hyer et al., 2003). To inhibit diffusion of Nile Blue during topical application, the embryo was temporarily placed in albumen before being transferred to culture media for subsequent incubation.

In selected experiments, cytoskeletal contraction was suppressed by culturing embryos in the dark in media containing blebbistatin (bleb, 50 μM), which is a nonmuscle myosin II inhibitor.

2.3. Optical coherence tomography (OCT)

Cross-sectional images of living embryos were acquired using a Thorlabs (Newton, NJ) OCT system coupled to a Nikon FN1 microscope. Image stacks were acquired at approximately 10 μm resolution in a 3×3 mm scanning window. Subsequent image analysis (including image cropping, contrast optimization, and