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Fate and plasticity of the endoderm in the early chick embryo

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Abstract

In vertebrates, the endoderm is established during gastrulation and gradually becomes regionalized into domains destined for different organs. Here, we present precise fate maps of the gastrulation stage chick endoderm, using a method designed to label cells specifically in the lower layer. We show that the first population of endodermal cells to enter the lower layer contributes only to the midgut and hindgut; the next cells to ingress contribute to the dorsal foregut and followed finally by the presumptive ventral foregut endoderm. Grafting experiments show that some migrating endodermal cells, including the presumptive ventral foregut, ingress from Hensen's node, not directly into the lower layer but rather after migrating some distance within the middle layer. Cell transplantation reveals that cells in the middle layer are already committed to mesoderm or endoderm, whereas cells in the primitive streak are plastic. Based on these results, we present a revised fate map of the locations and movements of prospective definitive endoderm cells during gastrulation.

Keywords: Endoderm; Fate map; Gastrulation; Cell movement; Chick embryo; Primitive streak; Hensen's node

Introduction

In vertebrates, the definitive endoderm, which gives rise to the epithelium of the digestive tract, arises from the epiblast during gastrulation. The endoderm starts to become regionalized along its anteroposterior and dorsoventral axes after gastrulation and finally subdivides to give rise to morphologically and functionally diversified regions and to the organs of the digestive and respiratory systems. Although there are many studies of the molecular mechanisms involved in the establishment of the endoderm during gastrulation (Stainier, 2002; Tam et al., 2003) and of the differentiation of certain digestive organs (Yasugi, 1994; Wells and Melton, 1999; Duncan, 2000; Grapin-Botton and Melton, 2000; Yasugi, 2000; Fukuda and Yasugi, 2002), little is known about when or how the endoderm segregates from the other germ layers and starts to become regionalized. To start to address these issues, fate maps of early stages showing both the location of endodermal progenitor cells and the origin of these cells that contribute to the various regions of the gut are essential.

Fate maps of the endoderm of the chick embryo during gastrulation have already been constructed by many authors using carbon particles (Bellairs, 1953a,b, 1955, 1957), ^{3}H-thymidine labeled grafts (Rosenquist, 1966, 1970a,b, 1971a,b, 1972), quail–chick transplantation (Fontaine and Le Douarin, 1977) and fluorescent dyes (Kirby et al., 2003; Lawson and Schoenwolf, 2003). All of these studies showed that the definitive endoderm forms during gastrulation from cells in the anterior primitive streak or Hensen's node, which ingress into the lower layer and replace the hypoblast, forcing the latter into an extraembryonic position. These fate maps also suggested that presumptive ventral gut endoderm ingresses into the lower layer earlier than dorsal endoderm (Rosenquist, 1971a). Nevertheless, these maps were made at very low resolution, and, in most cases, the middle layer was also labeled by these methods, which precluded precise distinction of endoderm and mesoderm cells.

Here, we present a detailed fate maps of the endoderm of the primitive streak stage (Hamburger and Hamilton, 1951; HH 2–5) chick embryo by a newly developed labeling method: very small focal injections of DiI placed exclusively in the lower layer. This enabled us to find hitherto undescribed behaviors of

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prospective endoderm cells during gastrulation. First, we reveal that the endodermal cells that appear first in the lower layer will contribute to the mid- and hindgut, later ingressing cells contribute to the dorsal foregut, followed finally by presumptive ventral foregut cells. Second, cell labeling and grafting experiments reveal that many migrating endodermal cells, including the prospective ventral foregut, ingress from the anterior primitive streak not directly into the lower layer but only after lateral migration in the middle layer, which was previously thought to contribute only to the mesoderm. Finally, we use grafting experiments to show that mesendoderm cells acquire their mesoderm or endoderm identity during gastrulation. These results reveal a more complex pattern of movements of endodermal cells than previously thought and provide a base to examine the molecular mechanisms responsible for endoderm specification.

Materials and methods

Method for focal labeling of lower layer cells

In this study, 499 embryos were labeled, of which 324 survived. Of these, 93 embryos had been appropriately labeled and were used for analysis.

To construct a detailed fate map of the lower layer of the chick embryo and to determine the exact timing of incorporation of endodermal cells into the lower layer during gastrulation, we devised a strategy to label very small groups of cells restricted to the lower layer. During gastrulation, the ventralmost layer of the embryo is very thin and fragile, and established methods of DiI labeling (pressure injection of a dye solution) tend to spill into the adjacent middle layer. After exploring several alternatives, we found that placing a "microcrystal" of DiI (see below) on the lower layer for 1 h before removing it carefully allowed us to label a very small group of cells exclusively within the lower layer (Fig. 1A).

Fertilized hens' (White Leghorn) eggs were incubated at 38°C for 12–24 h to obtain embryos from HH stages 2 to 5. Embryos were explanted in Pannett–Compton saline (Pannett and Compton, 1924) using a modified version of the New culture method (Stern and Ireland, 1981). Microcrystals of the carbocyanine dye DiI (1,1-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate) (DiI-C_{18}; Molecular Probes) were prepared as follows: DiI was first dissolved at 0.5% (w/v) in absolute ethanol and the solution diluted 1:1 in 50% sucrose in distilled water. A droplet of this was deposited into a large volume of Pannett–Compton saline, which generated a precipitate of very small DiI crystals (each approximately 5–30 μm in diameter). After 30 min, DiI crystals of appropriate size, some 10–15 μm in diameter, were selected for labeling.

An individual DiI crystal was placed directly onto the lower layer carefully to avoid injury. One hour later, the DiI crystal was carefully removed. Following marking with DiI, embryos were incubated at 38°C in a humid atmosphere until stage 11. Images of the labeled embryos were taken immediately after labeling and subsequently at stages 5 and 11, using a MZ FLIII fluorescence stereomicroscope and Image Manager (Leica). After incubation, some embryos were processed histologically to confirm the localization of labeled cells. For this, embryos were fixed in PBS containing 0.25% glutaraldehyde and 4% paraformaldehyde for 1 h then the fluorescence was photooxidized with 3-3′ diaminobenzidine (DAB) in 0.1 M Tris–Cl (pH 7.5) as previously described (Izpisúa-Belmonte et al., 1993). The embryos were then embedded in paraffin, serially sectioned at 10 μm, mounted on glass slides and dewaxed in xylene before being mounted in Entellan NEW (Merck).

Transplantation experiments

Cells in the middle layer just lateral to Hensen's node or lateral to the mid-primitive streak at stages 3^{+}–4 were labeled by applying a solution of DiI (0.5% DiI in ethanol, diluted 1:10 in 0.3M sucrose) using air pressure from a micropipet. A small group of these labeled cells (approximately 20 cells) was then excised and grafted homo- or heterotopically and homo- or heterochronically into host chick embryos in modified New culture. The host embryos were allowed to heal at room temperature for 30 min and then photographed. They were then cultured at 38°C, and the positions of DiI-labeled cells examined every 2–4 h. At the end of the incubation period (various times following the graft), embryos were fixed overnight in PBS containing 4% paraformaldehyde, embedded in paraffin and sectioned at 10 μm and examined by bright-field and fluorescence microscopy.

In situ hybridization

Embryos were fixed with 4% paraformaldehyde overnight, replaced with 30% sucrose in PBS at 4°C for 5 h and embedded in OCT compound (Sakura Finetechnical Co.). In situ hybridization with digoxigenin-labeled probes was performed on 12 μm frozen section as described by Ishii et al. (1998), after recording the DiI fluorescence photographically. *cSox2*, *cSox3* (Uwanogho et al., 1995), *CdxA* (Ishii et al., 1997), *HFH8* (Clevidence et al., 1994) and *cPax9* (Muller et al., 1996) were used as probes for in situ hybridization.

Results

Fate map of the lower layer at HH stages 2–3^{+}

Previous studies (Bellairs, 1953a,b, 1955, 1957; Vakaet, 1962, 1970, 1984; Nicolet, 1965, 1967, 1970; Rosenquist, 1966, 1970a,b, 1971a,b, 1972; Fontaine and Le Douarin, 1977; Selleck and Stern, 1991; Psychoyos and Stern, 1996; Kirby et al., 2003; Lawson and Schoenwolf, 2001, 2003) have established that the definitive (gut) endoderm arises from the epiblast via the anterior primitive streak prior to HH stage 4. In

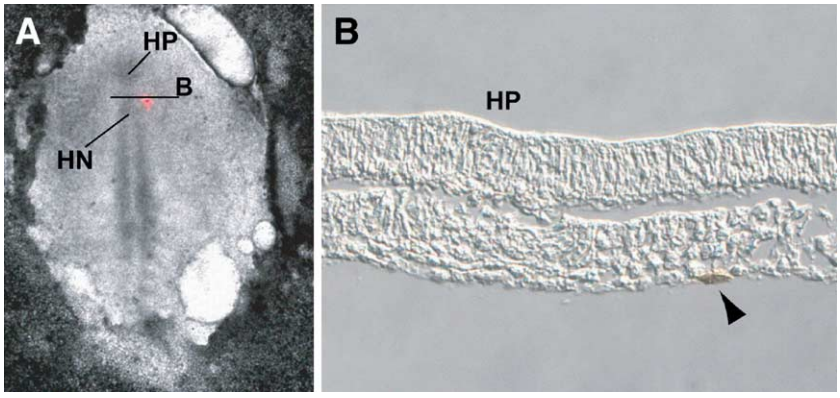

Fig. 1. Specific labeling of the lower layer with DiI in the embryo. (A) Embryo labeled at stage 5. (B) Transverse section through the embryo shown in panel A, at the level indicated by the transverse line. Photooxidized DiI was found exclusively in the lower layer. HN, Hensen's node; HP, head process.