Available online at www.sciencedirect.com
SCIENCE DIRECT
Developmental Biology 294 (2006) 432–444
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Global cell sorting is mediated by local cell–cell interactions in the *C. elegans* embryo

Marcus Bischoff, Ralf Schnabel *

Technische Universität Braunschweig Carolo Wilhelmina, Institut für Genetik, Spielmannstr. 7, D-38106 Braunschweig, Germany

Received for publication 2 September 2005, revised 23 February 2006, accepted 7 March 2006
Available online 13 April 2006

Abstract

The *Caenorhabditis elegans* embryo achieves pattern formation by sorting cells into coherent regions before morphogenesis is initiated. The sorting of cells is coupled to their fate. Cells move extensively relative to each other to reach their correct position in the body plan. Analyzing the mechanism of cell sorting in in vitro culture experiments using 4D microscopy, we show that all AB-derived cells sort only according to their local neighbors, and that all cells are able to communicate with each other. The directions of cell movement do not depend on a cellular polarity but only on local cell–cell interactions; in experimental situations, this allows even the reversal of the polarity of whole regions of the embryo. The work defines a new mechanism of pattern formation we call "cell focusing".

Keywords: Cell–cell interactions; Cell movements; Pattern formation; Cell sorting; 4D microscopy; Cell polarity

Introduction

During embryogenesis, cells have to find their correct positions as they build patterns. Sorting of cells contributes to this patterning. Cell sorting processes have been the subject of intense research since Townes and Holtfreter first described aggregation experiments with amphibian neurula cells in 1955. Since then, many different models explaining cell sorting have been proposed. The most common hypothesis, the differential adhesion hypothesis (Steinberg, 1963), proposes that cells sort only because of their specific adhesive properties. This mechanism was, indeed, observed in vitro and in vivo (Cortes et al., 2003; Duguay et al., 2003; Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998; Kostetskii et al., 2001). Another mechanism to guide cell sorting is based on active signaling events between cells as shown, for example, for the Ephrin/Eph-receptor system (reviewed in Xu et al., 2000). All analyses performed so far refer to sorting processes which were either limited to a specific part of the body or included only a few different cell types in cell culture experiments. Therefore, the process analyzed here represents the first example of cell sorting in the entire embryo.

We have described a global cell sorting process in the *Caenorhabditis elegans* embryo which guides cells to their terminal position at the premorphogenetic stage (Fig. 1A; Schnabel et al., 1997, 2006). Cell movements take the main part in this patterning process while the mitoses do rather little (Schnabel et al., 2006). Movements start after the initial specification of founder cells and bring the cells to their terminal positions at the premorphogenetic stage. At this stage, the descendants of the 12-cell stage embryo form coherent regions which do not mix (Schnabel et al., 1997). Alteration of the cell fates of the 8-AB-derived blastomeres present at the 12-cell stage results in a positioning of descendants according to their new fate, suggesting that the fate of a cell determines its terminal position in the embryo (Fig. 1B; Schnabel et al., 2006).

We proposed the "cell focusing" hypothesis (Schnabel et al., 2006) to explain the extensive cell sorting. According to this hypothesis, cells autonomously generate a positional value on their surface. As a result of comparing these values, cells move relative to each other until they find their "correct"

* Corresponding author. Fax: +49 531 391 5765.
E-mail address: r.schnabel@tu-bs.de (R. Schnabel).

0012-1606/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.03.005

position as defined by their neighbors at the premorphogenetic stage (Fig. 1C).

The "cell focusing" hypothesis raises testable predictions. Firstly, sorting should occur only locally since cells are provided with positional information by only their neighbors. Secondly, cells should be able to sort out independently of their position in the embryo and independently of the fate of neighboring cells. Previous experiments did not solve the question whether cells use their polarity to move in a certain direction or whether cells obtain directional information only from their local environment (Schnabel et al., 2006). In the latter case, cells might become trapped in a locally correct but globally wrong place. We refer to this situation as a "local minimum" (Fig. 1D). In contrast, cells which use a general polarity to sort should overcome this trapping by using this additional information (i.e., the polarity) to identify a right neighbor lying on the wrong side and to move accordingly (Fig. 1E). Therefore, the formation of "local minima" is inconsistent with an involvement of a general cell polarity in the cell sorting process.

The phenomenon of cellular polarity is studied extensively in *C. elegans* (reviewed in Gotta and Ahringer, 2001; Labbe and Goldstein, 2002; Lyczak et al., 2002; Pellettieri and Seydoux, 2002). Cells generally divide in anterior–posterior (a–p) direction and cell fates are specified according to the cleavage direction (Sulston et al., 1983). However, it is not known to which extent this cellular polarity contributes to pattern formation.

We removed cells from the eggshell and combined blastomeres in vitro. These manipulations alter the cell fates and the neighborhoods of cells—this challenges cells to sort in environments they normally never face. Analyzing the manipulated embryos by 4D microscopy, we show that cells sort locally "correctly" according to their fate, only relying on the local neighborhood. We show that the direction of cell movement does not depend on a cellular polarity but only on local cell–cell interactions in the embryo.

Materials and methods

Nematode strains and culture

Methods for culturing and handling of worms have been described elsewhere (Brenner, 1974). The following mutant alleles and strains were used: N2 Bristol (Brenner, 1974) and *glp-1 (e2144)* LG III (Priess et al., 1987).

In vitro culture of embryos and blastomeres

Preparation of embryos was carried out in a humidity chamber with 99% relative humidity at 25°C. Eggshell and vitelline membrane were removed according to Edgar (1995). Embryos and blastomeres were handled and dissociated using a drawn-out capillary needle (Biomedical Instruments, Zöllnitz, Germany) (Edgar, 1995). P_2 blastomeres were obtained by sequentially dividing AB and P_1 and then EMS and P_2. Cells were cultured in embryonic growth medium (EGM) supplemented with egg yolk (Edgar, 1995). To culture embryos under the 4D microscope, two different techniques were used. Cells were either put in a small hole in a 1% agarose pad on a microscope slide made by removing Sephadex beads (G50 super fine, Amersham Pharmacia) with an eyelash or cells were placed on a microscope slide equipped with spacers out of two layers of cling film. Microscope slides and cover slips were coated with 3.6 mg/ml Poly(2-Hydroxyethylmethacrylate) (Sigma) in 95% ethanol to prevent adhesion of cells to surfaces. Cover slips (24 × 60 mm, 1 mm) were sealed with pure white Vaseline to avoid evaporation.

Micromanipulation of embryos

P_1 was removed by sucking it with the mouth pipette through a hole in the eggshell, made by a thin glass needle (Gendreau et al., 1994). The manipulation was performed in a drop of EGM covered with mineral oil to avoid evaporation under an inverted microscope.

4D microscopy

The methods for 4D microscopy were described previously (Hutter and Schnabel, 1994; Schnabel et al., 1997). Modifications of the 4D microscope system are described in (Schnabel et al., 2006). Embryos were recorded at 25°C.

Lineage analysis

The 4D recordings were analyzed using the database SIMI©Biocell (Schnabel et al., 1997; http://www.simi.com). By following every cell in the recording, the 3D coordinates of the cells can be assigned to the cell lineage. Thus, data of cell descent, cell position, cell cleavage, and cell morphology (cell fate) are collected. These data can be used to generate 3D representations of all nuclear positions at any given time point of development and, thus, 3D movies. In this study, cell sorting is visualized by such movies as well as by showing the "starting point" of our analysis (the 12-cell stage with 8-AB blastomeres) and the "end point" of this process (the premorphogenetic stage with 256-AB blastomeres). Although we analyzed most embryos up to the premorphogenetic stage, cell positions of earlier generations are shown in some figures since 3D representations of the premorphogenetic stage tend to be confusing due to the large number of cells.

Results

General polarity of the lineage

All conclusions of the manuscript are based on lineage analyses with the 4D microscope system. This tool enables the identification of the fates of the descendants of the 8-AB blastomeres present at the 12-cell stage by prominent features of the corresponding lineages (Hutter and Schnabel, 1994; Schnabel et al., 1997). Such an analysis is exemplified here, using the ABala- and ABarp-derived fates which are the most common fates in this study. ABala is the only AB-derived lineage producing four cell deaths in characteristic positions after the ninth cleavage. In contrast, ABarp produces only one early cell death in ABarpaaapp and 22 major hypodermal cells which can be distinguished by their size and morphology from the precursors of other cell types (Sulston et al., 1983). These features can be used to determine cell fates in cultured embryonic fragments which normally cease development after the first round of cell deaths and after the differentiation of the major hypodermis. In normal embryos with eggshells, the assignment of the a–p polarity after a cleavage—an important step in reconstructing the cell lineage—is never a problem since in a normal embryonic context the anterior daughter cell also executes the anterior cell fate. However, during the analysis of embryonic fragments, for example, of individual AB blastomeres, it is unknown whether the fragment has a "polarity" and,