Mechanisms of Development xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Development

journal homepage: www.elsevier.com/locate/mod

New insights from a high-resolution look at gastrulation in the sea urchin, *Lytechinus variegatus*

Megan L. Martik, David R. McClay *

Department of Biology, Duke University, Durham, NC 27708, USA
University Program in Genetics and Genomics, Duke University, Durham, NC 27708, USA

ARTICLE INFO

Article history:
Received 3 January 2017
Received in revised form 27 May 2017
Accepted 28 June 2017
Available online xxxx

Keywords:
Gastrulation
Sea urchin
Archenteron

ABSTRACT

Background: Gastrulation is a complex orchestration of movements by cells that are specified early in development. Until now, classical convergent extension was considered to be the main contributor to sea urchin archenteron extension, and the relative contributions of cell divisions were unknown. Active migration of cells along the axis of extension was also not considered as a major factor in invagination.

Results: Cell transplantations plus live imaging were used to examine endoderm cell morphogenesis during gastrulation at high-resolution in the optically clear sea urchin embryo. The invagination sequence was imaged throughout gastrulation. One of the eight macromeres was replaced by a fluorescently labeled macromere at the 32 cell stage. At gastrulation those patches of fluorescent endoderm cell progeny initially about 4 cells wide, released a column of cells about 2 cells wide early in gastrulation and then often this column narrowed to one cell wide by the end of archenteron lengthening. The primary movement of the column of cells was in the direction of elongation of the archenteron with the narrowing (convergence) occurring as one of the two cells moved ahead of its neighbor. As the column narrowed, the labeled endoderm cells generally remained as a contiguous population of cells, rarely separated by intrusion of a lateral unlabeled cell. This longitudinal cell migration mechanism was assessed quantitatively and accounted for almost 90% of the elongation process. Much of the extension was the contribution of Veg2 endoderm with a minor contribution late in gastrulation by Veg1 endoderm cells. We also analyzed the contribution of cell divisions to elongation. Endoderm cells in *Lytechinus variagatus* were determined to go through approximately one cell doubling during gastrulation. That doubling occurs without a net increase in cell mass, but the question remained as to whether oriented divisions might contribute to archenteron elongation. We learned that indeed there was a biased orientation of cell divisions along the plane of archenteron elongation, but when the impact of that bias was analyzed quantitatively, it contributed a maximum 15% to the total elongation of the gut.

Conclusions: The major driver of archenteron elongation in the sea urchin, *Lytechinus variagatus*, is directed movement of Veg2 endoderm cells as a narrowing column along the plane of elongation. The narrowing occurs as cells in the column converge as they migrate, so that the combination of migration and the angular convergence provide the major component of the lengthening. A minor contributor to elongation is oriented cell divisions that contribute to the lengthening but no more than about 15%.

1. Introduction

Gastrulation is a dynamic period in the development of an embryo and involves many different cell movements. Given the importance of early morphogenesis in establishing differences in animal body plans, it would be valuable to understand how those processes work. In the sea urchin, gastrulation is relatively simple, easy to observe, and thought to be the prototype of deuterostome gastrulation, all of which makes it a valuable model for investigating questions of morphogenesis, in particular, gastrulation.

At embryonic 4th cleavage of the sea urchin, an unequal cell division gives rise to four macromeres in the vegetal hemisphere (Fig. 1A). At 5th cleavage, the tier of four macromeres divides meridianally to produce 8 cells. In the experiments below, one of these 8 macromeres was replaced with an identical but fluorescently labeled cell and the progeny imaged by timelapse later during gastrulation. At 6th cleavage, the macromeres divide in an equatorial plane to become the lower Veg2 and upper Veg1 tiers of macromeres. Veg2 progeny give rise to endomesoderm, which later becomes the non-skeletogenic mesoderm plus endoderm of the foregut and midgut. The Veg1 tier gives rise to

* Corresponding author.
E-mail address: dmcclay@duke.edu (D.R. McClay).

http://dx.doi.org/10.1016/j.mod.2017.06.005
0925-4773/

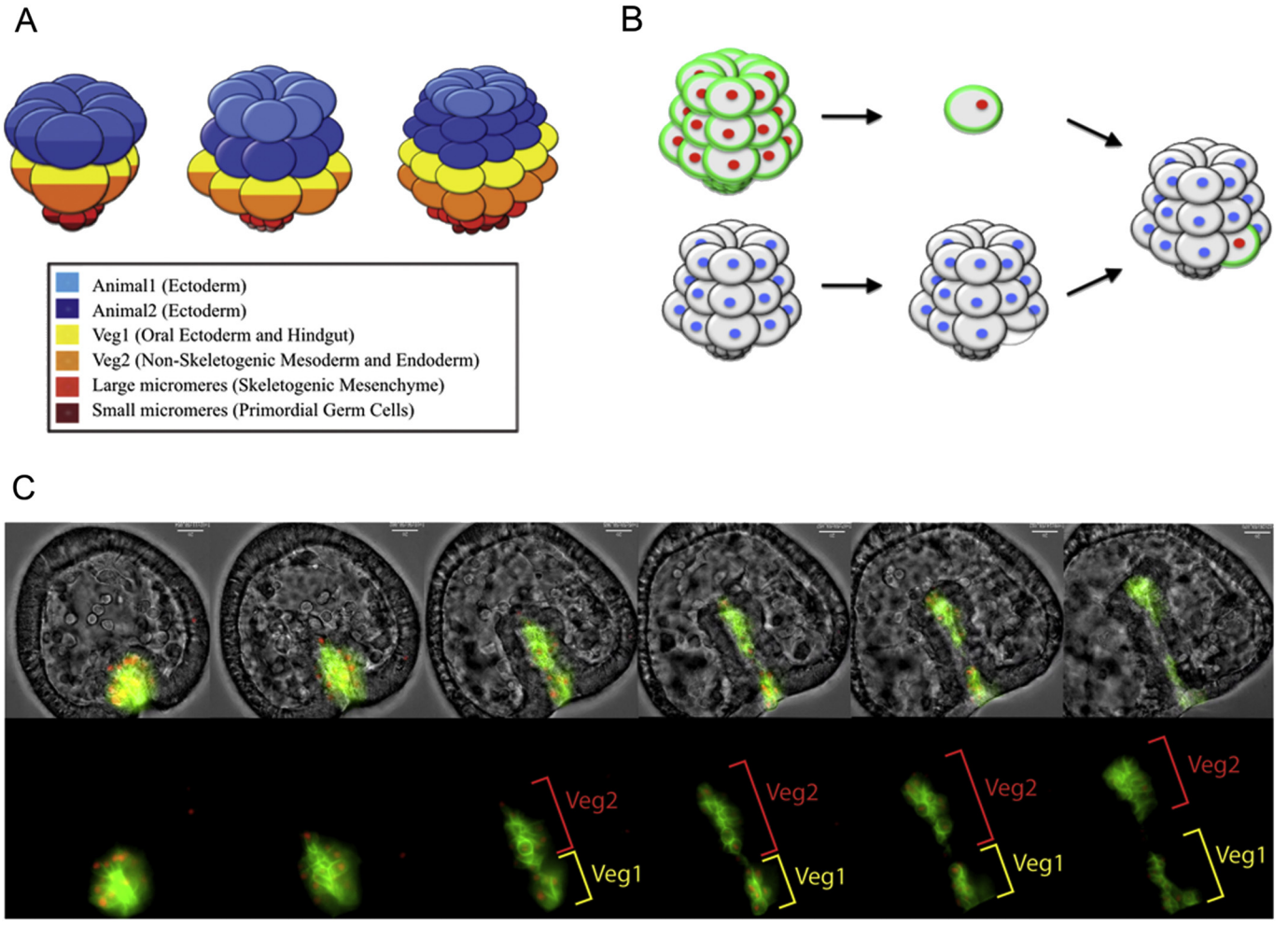

Fig. 1. Transplantation of cells in the gut lineage allows gastrulation to be captured at a higher resolution. (A) Progeny of the Veg tier of cells will comprise the entire gut. (B) Transplantation of a single membrane-GFP/histone2B-RFP labeled Veg macromere to an equivalent location in an unlabeled embryo of the same stage. (C) The transplanted cell produces 16–32 Veg progeny cells (depending on time of doubling) found in a patch at the beginning of gastrulation. Time-lapse visualization of the Veg lineage shows the cells slide by one another parallel to the axis of elongation—with intercalation occurring at an angle as one cell slips past another as both move along the axis of elongation. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

endoderm of the midgut and hindgut as well as contributing to posterior ectoderm (Logan and McClay, 1997). The gastrulation movements analyzed here will describe the movements primarily of the Veg2 endoderm progeny.

At the beginning of gastrulation in *Lytechinus variagatus*, the Veg2 tier of cells is found at the vegetal plate, and the Veg1 tier is found adjacent to the vegetal plate (Logan and McClay, 1997). Gastrulation begins between ninth and tenth cleavage such that the Veg1 and Veg2 lineages constitute between 128 and 256 cells at the beginning of gastrulation. A small number of those cells soon go through an epithelial-mesenchymal transition to become pigment cells, while the remaining Veg1 and Veg2 cells then engage in archenteron invagination (Ettensohn, 1984, 1985; Hardin, 1988, 1989, 1987).

Primary invagination of the archenteron occurs by a series of cell shape changes to force the initial inbending of the flat vegetal plate. Many mechanical and cell biological properties have been observed and mechanistically proposed to account for the inbending of the cell sheet. Veg2 endomesoderm cells elongate along the apico-basal axis to form bottle cells, which results in a thickened vegetal plate (Amemiya, 1989). Other contributions in addition to bottle cells have been suggested by computational approaches using algorithms to predict the forces contributing to the inbending (L. A. Davidson et al., 1995; L. A. Davidson et al., 1999; Kimberly and Hardin, 1998; Lane et al., 1993). The hyaline layer serves as a major mechanical component of the blastula wall and retains stiffness to promote the bending of the vegetal plate (L. A. Davidson et al., 1999). Osmotic pressure differences in the blastocoel, do not generate the major force of invagination (contrary to Rhumbler, 1902), but osmotic pressure could contribute to the process and ease the inward bending by decreasing before the onset of primary invagination (Takata and Kominami, 2001).

Secondary invagination is the main elongation step of the archenteron. It has long been thought that the main driving force of sea urchin gut elongation is by way of mediolateral convergent extension (Ettensohn, 1985; Hardin, 1989). Then, once the archenteron reaches about two thirds its final length a third process becomes a contributor to the extension. Filopodia at the tip of the archenteron extend and attach to the basement membrane lining the blastocoel roof and assist in pulling the archenteron to its final length near the animal pole (Hardin and McClay, 1990). Coincidentally, the Veg1 cells begin to make their way into the gut as a late population of cells contributing to the mass of the archenteron (Logan and McClay, 1997). Thus, much of the initial length of the gut is provided by the Veg2 endoderm morphogenesis with most of the Veg1 cells remaining near the vegetal plate until late in gastrulation. As will be described below, the cells of the Veg2 endoderm lineage contribute much of the archenteron length by cell migration, cell division and convergence-extension. The goal of this analysis was to tease apart the relative contributions of each mechanism.

Please cite this article as: Martik, M.L., McClay, D.R., New insights from a high-resolution look at gastrulation in the sea urchin, *Lytechinus variegatus*, Mechanisms of Development (2017), http://dx.doi.org/10.1016/j.mod.2017.06.005