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ARTICLE *in* DEVELOPMENTAL BIOLOGY · JULY 2006

Impact Factor: 3.55 · DOI: 10.1016/j.ydbio.2006.03.004 · Source: PubMed

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SCIENCE DIRECT
Developmental Biology 294 (2006) 418–431
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Global cell sorting in the *C. elegans* embryo defines a new mechanism for pattern formation

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

Abstract

4D microscopic observations of *Caenorhabditis elegans* development show that the nematode uses an unprecedented strategy for development. The embryo achieves pattern formation by sorting cells, through far-ranging movements, into coherent regions before morphogenesis is initiated. This sorting of cells is coupled to their particular fate. If cell identity is altered by experiment, cells are rerouted to positions appropriate to their new fates even across the whole embryo. This cell behavior defines a new mechanism of pattern formation, a mechanism that is also found in other animals. We call this new mechanism "cell focusing". When the fate of cells is changed, they move to new positions which also affect the shape of the body. Thus, this process is also important for morphogenesis.

Keywords: Cell migration; Cell guidance; Pattern formation; 4D microscopy; Bioinformatics

Introduction

In the early days of developmental biology, organisms were classified according to two principally different modes (strategies) of development. In "indeterminate" or "regulative" development, as defined for the vertebrates, the fate of a cell is determined accordingly to its position in the embryo. With the exception of the gastrulation movements, no global sorting of cells was supposed to occur during the establishment of the initial body plan. But today, we know that in the second phase of vertebrate development, the body plan depends greatly on cell movements, for example, of neural crest cells (Le Douarin, 2004). The regulative mode of development was seen as a contrast to the "determinate" or "mosaic" development of such animals as nematodes, such as *Caenorhabditis elegans*, or ascidians. It was thought that a stereotypic cleavage pattern, according to which cell fates are assigned, builds the body plan in these animals. The existence of such a general strategy of development was apparently confirmed by Sulston and co-workers (1983) who analyzed the embryogenesis of *C. elegans* by direct observation. They concluded that the development of this nematode is "invariable" and that, with a few exceptions as well as the gastrulation movements, the positions and fates of cells are determined by the cleavage pattern ("cells are born where they are needed").

Until the 1980s, it was presumed that different mechanisms underlie these different modes of development. But today, we know that the "same" molecular mechanisms are used to specify "autonomous" or "non-autonomous" processes in all animals, and that the molecular machinery already existed near the beginning of evolution (Kusserow et al., 2005). This insight shifted the main interest of developmental biology towards molecular mechanisms. Today, development is mostly seen as a chain of molecular events, processes being defined by the genes involved rather than by the general embryonic context. Thus,

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doi:10.1016/j.ydbio.2006.03.004

apparently completely different ways to make a mouse, a fruit fly, or a nematode are often seen as a variation of the use of the "same" (molecules) and not as the execution of very different general strategies (biocybernetic programs) to achieve pattern formation.

Using 4D microscopy and bioinformatics, we describe here in *C. elegans* a general embryonic strategy for pattern formation which was thought not to exist (Armstrong, 1989). After specifying the identity of early blastomeres, all cells are sorted by long-range movements, according to their fate, into specific regions. This sorting process may define a new general mechanism of pattern formation since we find similar movements also in other animals. The sorting process may also define new molecular mechanisms for pattern formation, since none of the genes known to guide cell migrations in the late development of animals, for example, during the patterning of the nervous system or the migration of epidermal cells, appear to be involved in this sorting. We call this new mechanism "cell focusing".

Background

Fate determination in the early embryo

In *C. elegans*, the first differentiation of tissues in the AB lineage occurs after the 9th cleavage round of the embryo. Earlier, blastomere fates are diversified by a series of binary switches depending on the *glp-1* (*Notch*) and Wnt pathways (Schnabel and Priess, 1997). Thus, the cell sorting and the concomitant cell movements studied occur when some cells still have the potential to give rise to both ectoderm and mesoderm. For example, the ABplpapp cell at the 64-AB cell stage later produces an epithelial cell, two neurons, two rectal cells (one of which is a blast cell), the excretory cell and a cell death (Sulston et al., 1983). At this developmental stage, sorting is well under way. Therefore, the sorting is not a sorting of specific tissues but of temporary cell identities.

The eight different fates of the AB-derived blastomeres present at the 12-cell stage embryo depend on at least five inductions, four of which are mediated by the gene *glp-1*, the *notch* homolog of the worm (Schnabel and Priess, 1997). Therefore, mutants in this gene or its ligand *apx-1* and/or manipulations with a laser microbeam allow us to alter the fates of the eight AB-derived blastomeres of the 12-cell stage embryo and, thus, the composition of cell fates in the embryo. The altered fates of the eight blastomeres were initially defined by 4D microscopic and immunochemical analyses of the differentiation patterns of the eight AB-derived blastomeres. For example, when the GLP-1 activity is strongly reduced in the *glp-1* mutant *e2144*, not eight but only two different AB-derived fates (ABala and ABarp) are executed four times each in the embryo. As a consequence, the most anterior AB-derived cell fate (ABala) is also executed in the posterior of the embryo, whereas the more posterior ABarp fate is executed anteriorly (Figs. 1C and 2B). The inductions and, thus, the function of *glp-1* are completed before the cell movements are initiated (Hutter and Schnabel, 1994, 1995a,b; Schnabel and Priess, 1997). We restrict our analysis to the AB lineage, although P_1-derived cells also move large distances.

Positioning of cells during development

The invention of 4D microscopy has allowed us to reconstruct the development of several normal embryos (Schnabel et al., 1997). Comparative analysis of these embryos suggested that cell positions vary considerably at the 50- to 100-cell stages, and that cells sort afterwards into a more conserved premorphogenetic stage (380 to 400 cells, 256 derived from AB) from which the L1 larva develops during morphogenesis. It became obvious that descendants of the 8-AB blastomeres present in the 12-cell embryo form discrete regions at the premorphogenetic stage (Figs. 1A, 2B; Movie S1A, Supplementary material). During development, some regions elongate over almost the entire length of the embryo. A "manual" quantification of cell movements showed that most is not due to the displacement of cells by the mitoses themselves but is achieved by movements between the cleavages (Schnabel et al., 1997). The paths of movements differ among embryos, suggesting that these movements are directed by signals from the environment (Schnabel et al., 1997). In this and an accompanying paper (Bischoff and Schnabel, 2006), we investigate the nature of this guiding system.

Materials and methods

See also the accompanying manuscript (Bischoff and Schnabel, 2006) for Materials and methods.

Worm culture and strains

Methods for culturing and handling of worms have been described by Brenner (1974). The following mutant strains and alleles were used:

N2 Bristol (Brenner, 1974)
glp-1 (e2144) LG III (Priess et al., 1987)
apx-1 (t2063)/DnT1 LGs IV and V (RS unpublished).

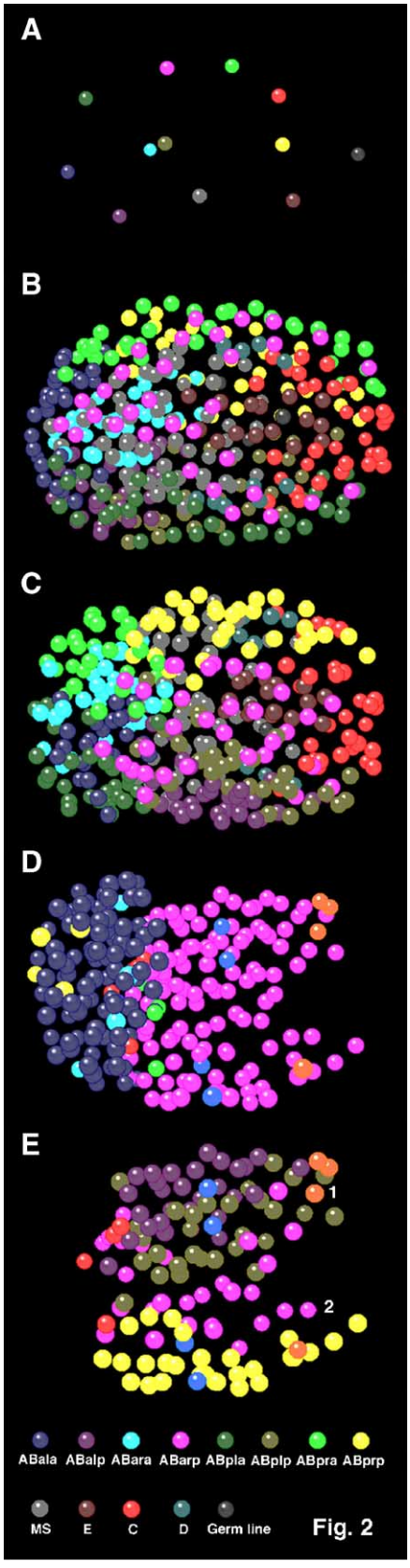
Microscopy and laser ablation

The methods for 4D microscopy and the manipulation of embryos by laser ablation were described previously (Hutter and Schnabel, 1994; Schnabel et al., 1997). The 4D microscope was meanwhile modified. A Zeiss-Axioplan-Imaging-2 microscope with an internal focus drive was used to move the temperature-controlled stage to record the z-series (25–32 focal levels, increment 1 μm). Pictures from a Hamamatsu Newvicon camera are digitized with an Inspecta-3 frame grabber (Mikroton, Germany) and compressed tenfold with a wavelet function (Luratec, Germany). The microscope is controlled with a PC using software programmed in C++. Embryos were recorded at 25°C.

Lineage analysis

4D movies were analyzed using the database SIMI©Biocell (Schnabel et al., 1997). During analysis with SIMI©Biocell, cells in the developing embryo are marked by the observer with the mouse pointer in the middle of the nucleus. The database stores the coordinate of the marked cell and its name. The collected data of cell fate, cell positions, and cell cleavages can be used to generate 3D representations of all nuclear positions at any given time point of development. Cell fate is indicated by the color of the spheres. Cell fates of the 8-AB

descendants present at the 12-cell stage were assigned using the lineage-specific patterns of the programmed cell death after the 9th cleavage round of the embryos. In addition, very characteristic cell fates like the major hypodermis were used to discriminate between lineages (data not shown; see Hutter and Schnabel (1994 and 1995a,b) for fate transformations). The analysis of cell fates is also described in the accompanying paper (Bischoff and Schnabel, 2006).



Bioinformatics

The methods and terms are explained briefly in Fig. 3. The parameters were calculated with programs written in C++ or Delphi using the cell coordinates collected with SIMI©Biocell. The methods are described carefully in the Supplementary material.

Results and discussion

The database SIMI©Biocell allows us to investigate the cell sorting and the cell movements in the early *C. elegans* embryo. We reanalyzed the time lapse recordings of three wild-type embryos described earlier (#1, #2, #5 (Schnabel et al., 1997)) and also analyzed two new mutant *glp-1* embryos by marking all cells every 30 or 35 s. Thus, cell cleavages and cell positions can now be recorded precisely. Our estimates show that the "lineaging error" with which the position of a nucleus is determined is less than 2.5% of the embryo length (EL). The error which occurs when determining the distances of cell movements should be less than 10% EL (see Fig. S1 and "Bioinformatics" in the Supplementary material). The resulting sets of 4D coordinates enable us to analyze the developmental events quantitatively by bioinformatics and also to visualize development in 3D representations. The dynamics and extent of cell movements can be seen in 3D movies (Movies S1, S2, S3, S4, Supplementary material).

As an example, the development of the normal embryo #1 is shown in the Movies S1A, S1B, and S1C (Supplementary

Fig. 1. Cell movements in normal and *glp-1* embryos. The figure shows 3D representations of the positions of the nuclei produced with SIMI©Biocell. Anterior is to the left. The color code for the cell fates is shown at the bottom. (A, B) Normal embryo (#1; Schnabel et al., 1997). (A) 12-cell stage. (B) Dorsal view of all cells, including the P1-derived ones, at the premorphogenetic stage (383 cells). The descendants of the blastomeres present at the 12-cell stage do not mix but form discrete and coherent regions. (C–E) Embryo #1 from a *glp-1 (e2144)* homozygous mother at the "premorphogenetic" stage (359 cells). The 12-cell stage of this embryo is very similar to the wild-type embryo (Figs. 2A and B). The ABala, ABara, ABpla, and ABpra lineages execute an ABala and the ABalp, ABarp, ABplp, and ABprp cells execute an ABarp fate. (C) Dorsal view of all cells. The color code refers to the descent of a cell (bottom; for a fate-dependent color coding see (D)). Cells with an ABala fate remain or move anterior whereas cells with an ABarp fate stretch out along the a–p axis in the posterior of the embryo. (D) Ventral view, only the AB-derived cells are shown. The nuclei of the embryo from panel C were restained according to their fate and not descent (ABala dark blue, ABarp pink); cells sort according to their fate. In addition, cells with the most anterior (ABxxxaaaaa), a central (ABxxxapppp), and the most posterior lineage (ABxxxppppp) were stained yellow, light blue, and green in the regions with an ABala fate and for those with an ABarp fate red, blue, and orange, respectively. Cells of the same fate sort into approximately the same a–p positions of the embryo. (E) Only the regions with an ABarp fate (pink) from panel D are shown. The regions were again coded with the original colors for their lineage descent. Additionally, spheres are marked with the color code used in panel C to indicate the lineage position cells are derived from. As one can see cells from different lineage descent intermingle. Interestingly, the ABarp region (pink) splits and the most posterior left cell (V6R, ABarpppppp, (1)) joins the homologous cells from ABalp (violet) and ABplp (dark yellow), whereas the most posterior right cell (V6L, ABarppapppp (2)) joins the homologous cell derived from ABprp (yellow).

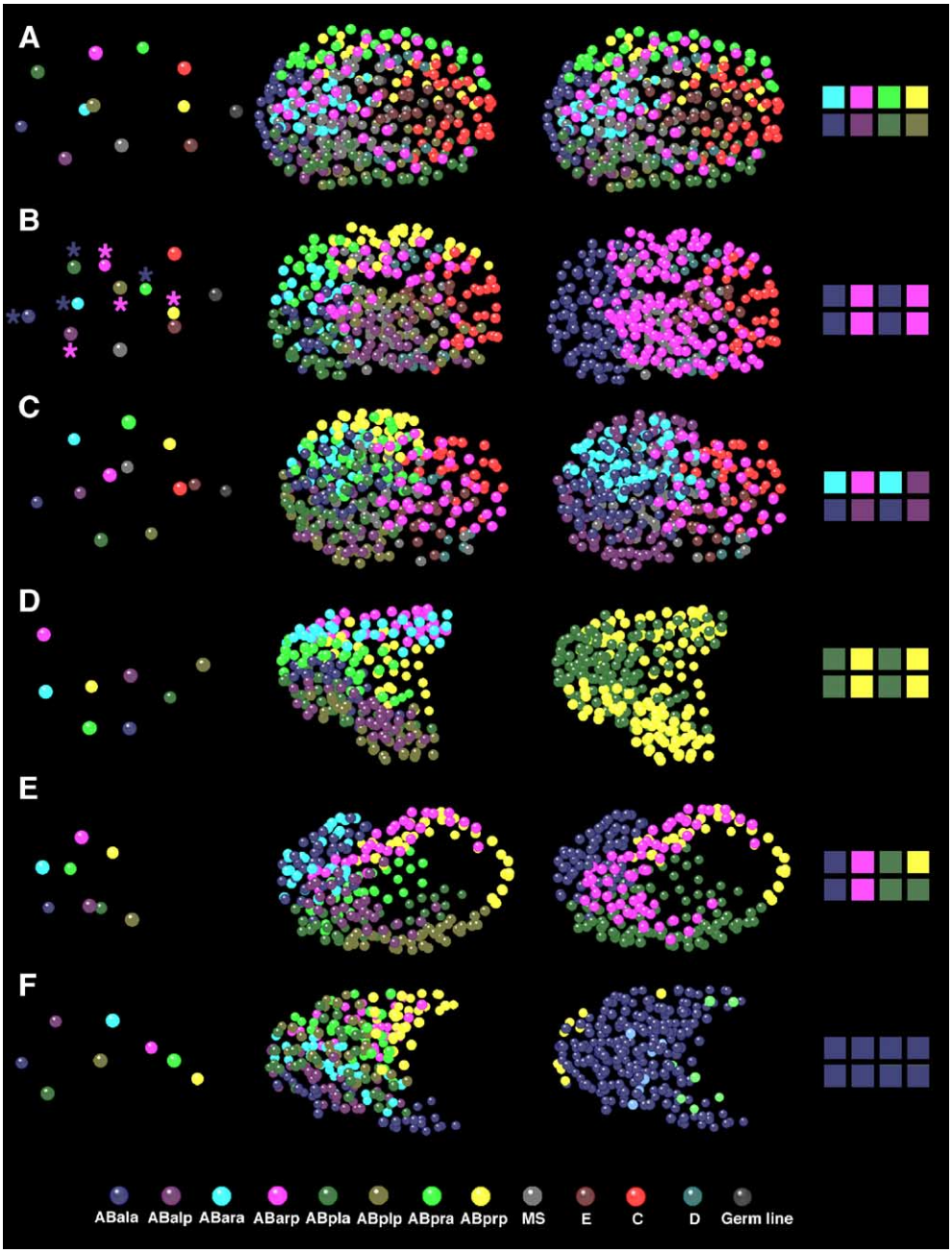


Fig. 2. Sorting of regions according to fate. The figure shows 3D representations of the positions of nuclei produced with SIMI©Biocell. The left column shows the 8-AB ("12-cell") stages of the embryos so that the positions of the cells founding the regions in the embryo can be seen. When P1 was ablated (D–F), the descendants of this cell are missing (MS, E, C, P3). By comparing these representations with the rectangular schemes in the last column, which indicate the fates of the AB-derived cells in the different embryos, one can deduce the occurrence of the different fates at the 12-cell stage. An example is shown in panel B: the colored asterisks besides the spheres indicate the new fates. The second and third columns show embryos at the premorphogenetic stage. Panels A–C and E show dorsal views. In panels D and F, no axes but the a–p axis can be defined anymore, since the cell fates are more or less rotational symmetric. Spheres were color coded according to descent (left and central column) and the (new) fate (right column), respectively. (A) Normal development exemplified by embryo #1, all cells shown. (B) *glp-1 (e2144)* embryo, all cells shown. (C) *apx-1 (t2063)* embryo, all cells shown. (D) In this embryo ("push"), P1 was ablated weakly in the middle of its cell cycle, and then ABa and ABp were pushed with a needle to both contact the P1 blastomere for 10 min. Afterwards, the embryo was mounted under the 4D microscope. This manipulation causes the execution of only ABp-derived fates (Hutter and Schnabel, 1994). Only the AB-derived cells are shown. (E) A wild-type embryo in which the P1 blastomere was ablated (Hutter and Schnabel, 1995b). Only the AB-derived cells develop and are shown. (F) *glp-1 (e2144)* embryo in which P1 was ablated early in cell cycle. All cells execute an ABala-derived fate (Hutter and Schnabel, 1995b). Only the AB-derived cells develop and are shown. To show again that cells arrange their position according to fate three cells from each ABala lineage were highlighted with the same color code used in Fig. 1D. Only one region (ABprp) remains in the posterior, perhaps due to obstruction by other cells.

material). Although cells already move around earlier, the directed movements start after the 50-cell stage (120 min) and proceed through the proliferation phase until the premorphogenetic stage when 400 cells is reached (245 min). Cells move freely relative to each other and are generally not moved through pushing by neighbors. This is shown unambiguously in Movie S1B (Supplementary material), which highlights the movement of ABplapa and its posterior descendants: It can be observed that two groups of ABpla-derived cells which lose

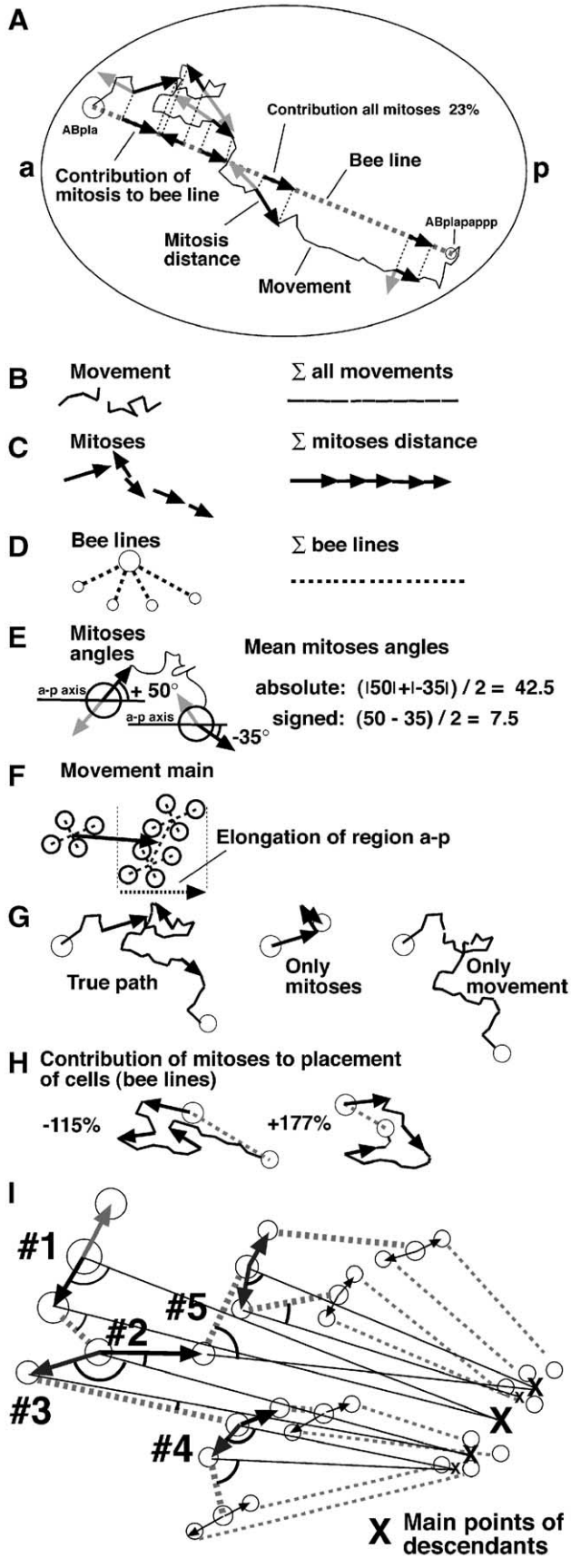


Fig. 3. Graphic explanation of the terms and calculation procedures used in the quantitative bioinformatical analyses. Additional information about the bioinformatical analyses can be found in the Supplementary material (Chapter "Bioinformatics")."(A) A realistic scenario of the mitoses and movements of the cells producing ABplapappp between the 12-cell and the premorphogenetic stage of embryo #1. This cell will later produce two cell deaths and two neurons. It is also highlighted in Movie S1B (Supplementary material). The positions of the first three mitoses were slightly spread apart to avoid crowding of lines. All distances shown here in two dimensions were calculated in three dimensions. The mitoses are indicated by arrows. The grey arrows show the displacement of the sister cells not followed further. Due to the spatial constraints in the embryo, cells may be deflected from a straight line during cleavage. (B) "Movement" corresponds to the cell movements in between the mitoses. (C) "Sum mitoses distances" adds all distances cells are displaced by the cleavage of their mother. (D) In "Sum bee lines", all direct distances between the founder cells at the 12-cell stage and the cells at the premorphogenetic stage were added up. (E) Calculation of "Mean mitosis angles". Here, two parameters are of interest. Firstly, the absolute deviation from the a–p axis, which reflects the mitosis angle only and does not take into account whether the division occurs in dorsal (+) or ventral (−) direction ("absolute"). Secondly, the division angle in which its sign and, thus, the dorsal/ventral direction of the division is considered ("signed"). The latter provides information about the direction in which the mitoses point in average. See also Bioinformatics (Supplementary material). In these calculations, it was assumed that the cleavage angle of the posterior daughter reflects the angle by which both daughters were produced during the cleavage. (F) "Movement main point" shows how far the center of gravity of a group of cell (regions) moves during development. This reflects the displacement of the whole region from the position of its founder cell at the 12-cell stage. The elongation of a region indicates its approximate shape if the cell number is the same. (G) Calculation of the placement of cells when either only mitoses or only cell movements are considered, which is shown in Fig. 5. In the case shown here, the first two mitoses contribute negatively to the final placement of cells. (H) Examples, how mitoses may contribute to more than 100% to the final placement of cells. In both cases, cell movements compensate a strong displacement of cells from their final target positions by mitoses which preferentially occurs in short, compact regions like that formed by ABara. (I) Calculation of the "aiming" of mitoses or cell movements towards the final positions of cells. Hatched lines show the "final path" (as bee line) a cell moved between it was born and then divides again. The thin lines connect the cell to the final target which has to be reached. All cells but the mother of the final cell at the premorphogenetic stage give rise to several descendants. Therefore, we defined the aims for these cells as the main point of the descendants, which will still be produced. The angle of the bee line of movement after the last division producing the final cell at the premorphogenetic stage will be zero, irrespective of the means – directed or random movement – the cell is placed. Therefore, we do not show the angles corresponding to these cells. Cell #1 still produces 8 cells and is, therefore, connected to the main point of all descendants. The angle by which its daughter #2 is born is 98° off this line. Thus, the cleavage does not point into the direction, where the descendants are placed later on. The net direction of this cell's movement is only 30° off the target. When cell #2 cleaves, its daughter #3 is even transported against the target direction (160°). However, this is corrected by the subsequent movement which is only 3° off the target. Cell #5 is born by a cleavage of #2 which occurs almost in the direction of the target position (15°), however, the subsequent movement is perpendicular to the target position.

contact are united again later in development. 4D microscopy furthermore allows us to appreciate the gastrulation movements of the AB-derived cells. These – compared to the other movements of the AB-derived cells "minor" movements – are part of the general process of pattern formation and can vary from embryo to embryo. The Movie S1C (Supplementary material) shows the gastrulation movements in an anterior view of the normal embryo #1 and follows the gastrulation movements.

Potential mechanisms of cell movements

During cell sorting, the cells move relative to each other. Cells are not obviously polarized or elongate significantly during movement. We generally do not observe an alteration of cell shape, for example, we see no leading edge or significant protrusions in the direction of movement. However, short filopodia can be observed in normal embryos with and without an eggshell. In an embryo cultured without eggshell, where cells are sometimes further apart, we recorded a scene in which a cell contacts two different neighbors and then extends a small lamellopodia-like structure towards one of the neighbors to establish a contact by moving towards it. This scene could be interpreted as an example for cell sorting by cell movements (Fig. S2, Supplementary material). A similar mode of movement – i.e., without any obvious polarization – was reported for tumor cells in Matrigel invasion essays (Sahai and Marshall, 2003).

Cell fate determines the address

Analyzing mutant *glp-1 (e2144)* embryos with the first version of the 4D microscope – still without SIMI©Biocell (Hutter and Schnabel, 1994) – we observed that cells derived from a blastomere whose fate was transformed from ABalp to ABarp not only executed the differentiation pattern normally characteristic of the ABarp blastomere but also that many cells moved from the anterior (where normally ABalp descendants reside) to the posterior of the embryo (where ABarp descendants are located). The significance of this finding, however, escaped our attention until we characterized the normal development using the 4D microscope system and asked how cells are guided during their long-range movements through the embryo. One possibility was that cells move according to their fate (Schnabel et al., 1997) and if so that would lead to a repositioning of cells after fate transformation.

A complete 4D analysis of two *glp-1 (e2144)* embryos now shows that, indeed, cells move according to their fate. All descendants of ABpra, which have an ABala fate in these embryos, move to the anterior. Those of ABpla, which also execute an ABala fate, remain anterior although descendants of the ABpla and ABpra blastomeres normally move posterior. In contrast, all regions with ABarp fate either move to and elongate in the posterior of the embryo (those regions derived from ABalp and ABarp) or, when the corresponding founding blastomere is already located posteriorly, just elongate (those regions derived from ABplp and ABprp; Figs. 1C–E and 2B). Thus, at the premorphogenetic stage, four regions with an ABala fate and an ABarp fate each assemble at opposite ends of the embryo, behaving like regions of the same fate in a wild-type embryo.

There is, however, a difference to the wild-type embryo. The ABala-derived cells normally form a U-shaped region, where the left arm is derived from ABalap and the right arm from ABalaa. In the *glp-1* embryo, cells arrange in an anterior–posterior (a–p) direction (Fig. 1D), and the U shape is lost. In a wild-type embryo where the ABalp and ABara blastomeres, which contact the ABala descendants on either side of the U-shaped region, were ablated with a laser microbeam, the ABalaa- and ABalap-derived cells still exchange their left–right (l–r) position, as in normal development. However, they do not form the U-shaped structure anymore, and the ABala region also elongates in a–p direction (data not shown). This suggests that the descendants of ABalp and ABara direct the ABala descendants into their final left–right positions. In the *glp-1* embryo, ABalp is transformed into ABarp and ABara into ABala, and, thus, the cells which normally guide the ABala descendants are no longer present.

How is the substantial reorganization of the *glp-1* embryo achieved? Observing Movies S2A and S2B (embryo *glp-1* #1; Supplementary material), one can see that, just like in the wild-type, the embryo is reorganized by active movements of cells relative to each other and not through a "pushing" of cells by neighbors. Accordingly, Fig. S3 and Movie S2C show an example how individual cells which have lost contact with their relatives are able to navigate independently in the embryonic field (for a corresponding observation in a normal embryo, see Movie S1B). Analyzing the positioning of single cells carefully, we found that cells with the same fate reach about the same position on the a–p axis although they originated from different positions in the 12-cell stage embryo (Figs. 1C, D and 2B, Movie S3, Supplementary material). The descendants of early blastomeres which acquired the same fate in the *glp-1* embryo intermingle extensively during this alignment.

Distance maps

The 3D representations do not permit us to deduce the exact alignment of cells. Therefore, we used a new method, a "distance map", to show the cell arrangement in embryos (Bignone, 2001). These maps display all distances among all cells in an embryo in a matrix using a color code to represent the distances in percent embryo length. This allows a visual evaluation of data by comparing patterns of colors. The distances among all cells define the positions of cells in an embryo unambiguously—only a chiral structure will have the same distance map. Thus, the positional information is not lost using this method. The map of the *glp-1* embryo #1 has a striking similarity to a map which predicts the distances of all cells with the assumption that all cells would occupy exactly the same positions as cells with the same fates in normal development (Fig. 4). Thus, all cells are very close to the positions which correspond to their (new) fates in normal development. However, the map is slightly blurred since the positioning may not be perfect and two cells of the same fate can

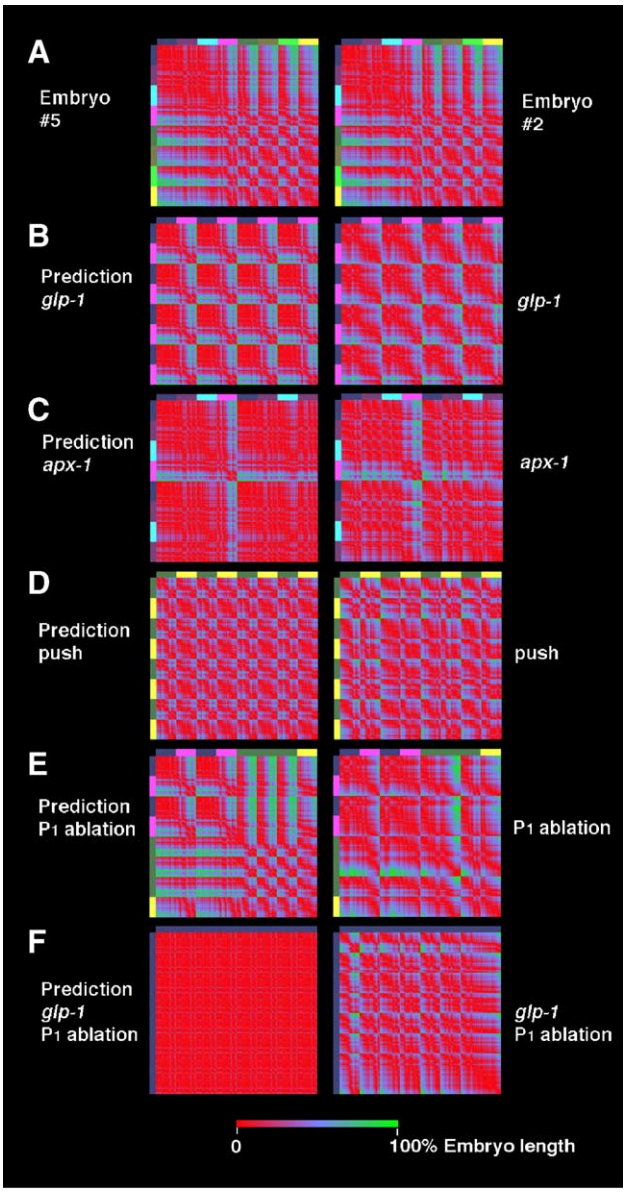


Fig. 4. Quantitative evaluation of cell positioning using distance maps. The panels (matrices) visualize the distances of all AB-derived cells at the premorphogenetic stage in wild-type, mutant, and/or manipulated embryos with respect to the a–p axis of the embryos. Cell fates are indicated along both axes of the panel using bars, which code the cell fates with the same colors used in the 3D representations shown in Fig. 1. Each colored bar along the two axes corresponds to 32 cells, thus, each axis comprises the 256 cells of the AB lineage at the premorphogenetic stage. The color code used to display the distances between all pairs of cells is shown at the bottom. Panel A compares the normal embryos #5 and #2. In panels C–F, the left column shows the predictions of distance maps which assume that cells with the indicated fate in the mutant and/or manipulated embryos move to exactly the same positions as cells with the same fate in the wild-type embryo #5. The predictions were produced by copying the 32 × 32 pixel panels representing the cell distances of the corresponding pairs of founder cells in embryo #5 and pasting them in a new matrix according to the altered fate pattern in a mutant and/or manipulated embryo. The right column shows the real distances in the corresponding embryos. A comparison of prediction and real data allows to assess to which extent cell sorting actually produces a pattern that resembles the prediction.

in any case not take exactly the same position in a real embryo. The distances that underlie the distance maps are also the basis of a quantification of the similarity of embryos.

Similarity values

To our knowledge, there is no established method to quantify the morphogenetic behavior of whole embryos on a cellular level. Differences of structures, for example, in the folding of proteins, are quantified using the root-mean-square method (RMS method; Cohen and Sternberg, 1980). This algorithm calculates a "dissimilarity" value by comparing all distances among all elements of two structures (see Fig. S1, Supplementary material, for explanation). However, due to the spatial restriction in the embryo, this procedure results in a non-linear representation of the quantitative differences of the positions of cells in two embryos. Since a non-linear representation of a dissimilarity is counterintuitive, we rescaled the RMS values for the distance maps shown in Fig. 4 which only consider the a–p axis to describe the similarity (*S*) in a linear function. Complete identity between two embryos (RMS = 0) corresponds to 100% and complete dissimilarity (random positioning of the 256 cells, RMS = 0.3) to 0% similarity.

Using this algorithm, the three wild-type embryos described earlier have similarity values between 98% and 99% (see also Fig. S1, Supplementary material, for RMS values). However, this is only true for the later embryonic stages. In 1997, Schnabel and co-workers, using the 3D representations of the nuclear positions, found that the 50- and 100-cell stages of wild-type embryos varied significantly in their configurations but were unable to quantify these differences. The newly introduced similarity algorithm now confirms their notion—normal embryos are clearly different at the early stages. This dissimilarity (variability) decreases during the later cell movements which sort the cells into their terminal positions at the premorphogenetic stage (Fig. S1, Supplementary material).

The *S* value for the *glp-1* embryo is 57% when compared to the wild-type embryo #5 and 82% when compared to the prediction that cells take the positions according to their new fates in the mutant.

Interestingly, the RMS algorithm can also be used to analyze the phenotype of a mutant embryo after lineaging up to the premorphogenetic stage, without assigning any cell fate. The principle is to use the fact that cells move "according to their fate". By comparing the positions of cells (regions) in the mutant embryo with those of all cells (regions) in a normal embryo, the lowest RMS value will indicate the (new) identity of the founding cell forming a region (AH and RS unpublished). This method is exemplified in Fig. S1 (Supplementary material) using the *glp-1* embryo.

"It always works"

The above observations and quantifications suggest that the fate of a cell (region) determines its position in the embryo. We also observe that, when cellular (regional) identities are duplicated in the embryo, cells of the same identity interact to

form one large region. This is in contrast to a wild-type embryo where cells of the eight AB descendants of the 12-cell stage embryo never mix (Schnabel et al., 1997). The striking sorting capacity observed in the *glp-1* embryo suggests that there is positional information (a "coordinate system") in the embryo that guides cells to their appropriate position (see below). However, our observations could still be the result of a special situation in *glp-1* embryos. Therefore, we investigated whether or not other abnormal combinations of cell fates can be resolved like this, so that transformed cells sort to the positions which correspond to their fate in normal development. We analyzed four more situations in mutant and mechanically manipulated embryos where the arrangement of the fates of early blastomeres was significantly altered from wild type by cell fate transformations.

In the first experiment, an *apx-1* embryo, there are only ABa-derived fates present (like in the *glp-1* embryo), but the complexity of the embryo is increased by the presence of more fates. The induction of ABp fates is missing (Mello et al., 1994), whereas the primary induction of the l–r asymmetry still occurs. Therefore, the ABa-derived fates are normal but the ABp-derived blastomeres now execute anterior fates. The 12-cell stage embryo contains two blastomeres with an ABala fate, three with an ABalp fate, two with an ABara fate, and one with an ABarp fate (Fig. 2C). Thus, normally anterior positioned fates are present in the posterior of the 12-cell stage embryo. Again, all cells migrate to the positions which are characteristic of their fates in normal development. The descendants of blastomeres with an anterior fate, which are born in a posterior position, move anteriorly. The descendants of ABprp, which execute an ABalp and therefore a "left" fate on the right side of the embryo, fail to correct their displacement to the right side of the embryo. The distance map of this embryo shows a surprising similarity to the predicted map (S = 88%).

To analyze a situation in which only posterior cell fates occur (ABpra and ABprp), we completely lineaged an embryo already recorded by Hutter and Schnabel in 1995b. In this embryo (named "push"), ABa was induced to execute an ABp fate by forcing a contact of ABa with the weakly ablated P1 blastomere by pushing it back. In this embryo, cells with posterior cell fate, e.g., those derived from ABara which now execute an ABpra fate, are born in the very anterior of the embryo and do not remain in the position of their founding blastomeres. Instead, the ABpra region extends throughout the whole embryo, like the ABpra region in a normal embryo. The regions derived from blastomeres executing an ABprp fate position themselves on the a–p axis relative to those executing an ABpra fate, as in normal development. The corresponding maps show a good correlation between the predicted and the real distance pattern (S = 80%; Figs. 2D and 4D).

A third combination of cell fates was achieved by ablating the P1 blastomere which partly impairs the induction of the posterior fates at the 8-AB cell stage. Since P1 is the grandmother of MS, the induction of the l–r asymmetry also fails (Hutter and Schnabel, 1995b). In the embryo shown in Fig. 2E, the ABa-derived fates are the same as in *glp-1* embryos, and ABp produces ABpra three times and the ABplp fate once. The anterior of the embryo is well sorted, and the distances of cells are almost identical to those in the *glp-1* embryo (Figs. 4B and E). However, the remaining large P1 blastomere appears to hamper the posterior cell movements (Fig. 2E); this is also reflected in shorter distances in the distance map than expected (Fig. 4E). Nevertheless, the similarity between the prediction and the real embryo is high (S = 77%). The fact that sorting still occurs, despite 40% of the embryo being occupied by ablated blastomeres, suggests that the underlying mechanism must be very stable, even after substantial alteration of neighborhood relations.

Finally, an embryo representing an extreme situation – all blastomeres execute an ABala fate – was analyzed (Fig. 2F). Such an embryo can be created by ablating the P1 blastomere of a *glp-1 (e2144)* embryo early in its cell cycle. This interrupts all five inductions diversifying the AB lineage (Hutter and Schnabel, 1994, 1995b). A 4D analysis of the embryo shows that seven out of the eight AB descendants form a large "super" region, extending from the anterior of the embryo over the sides of the ablated P1 blastomere, which is located posteriorly. Cells migrate from anterior to posterior and vice versa in this "super" region and align according to their a–p fate. This phenomenon has already been described for the *glp-1* embryo. Since the cells are not repelled by the posterior ABarp-derived cells, as in the *glp-1* embryo, the regions stretch out along the whole embryo length. Only the descendants of the ABprp blastomere do not participate in this large scale sorting; they appear to be stuck in a posterior position (Fig. 2F). Although extensive cell sorting occurs in this embryo, the final pattern is very different from that found in normal embryos. This is also reflected in the corresponding distance maps; they show extremely different patterns (S = 44%; Fig. 4F). This S value falls into the range of the S values (27 ± 27%) which are measured when all embryos shown are compared with a randomized embryo.

In summary, cells generally migrate correctly according to their fate even when their neighborhood is altered intensively. Before we address the question how cells are guided during the sorting process, we first investigate in more detail how cells behave during the sorting process in normal and manipulated situations.

The identity of a region determines its shape

The alteration of a region's fate is accompanied by a remodeling of its shape. In a *glp-1* embryo, a normally long region (e.g., ABpla) not only moves to the anterior of the embryo when its identity is changed to that of ABala, but it also shortens to a length similar to that of the ABala region (Figs. 1B and C). In the *apx-1* embryo, the same occurs when long regions (ABpra and ABprp) change their fates to ABara and ABalp, respectively (Figs. 2A and C). In the "push" embryo, the opposite happens. The normally short regions ABala, ABara, and ABalp now stretch out to reach through almost the whole embryo as the regions with the corresponding fates ABpra and ABarp normally do (Figs. 2A and D). This suggests that the shape of a region is established by the sorting pattern of cells; and these are coupled to a region's fate.

It has been speculated that the regions of the *C. elegans* embryo are the equivalents of the compartments found in other animals (Garcia-Bellido et al., 1973; Lawrence and Struhl, 1996; Schnabel et al., 1997). A general definition of a "compartment" includes the formation of "clonal boundaries" caused by the lack of intermingling of cells. Above, we have described that cells from regions with different identity never intermingle, not even in combinations that are missing in normal development. Thus, "clonal boundaries" are always respected. However, if regions with identical fate are created in multiple copies, cells intermingle by cell movements—and cells of identical fate migrate to similar positions along the a–p axis. Thus, the regions behave similarly to compartments. Whether this behavior is reflected in regional identities or is merely caused by cell specific positional identities remains open.

Quantification of the cell sorting process

Contribution of mitoses and cell movements

It was the general notion that, apart from gastrulation movements, cell movements are of minor importance and cell cleavages place the cells where they are needed in the *C. elegans* embryo (Sulston et al., 1983). As already mentioned, the first analysis of normal embryogenesis with a 4D microscope showed that cells may move considerably between cleavages (Schnabel et al., 1997). Here, we now quantify the behavior of the AB-derived cells in wild-type embryos between the 12-cell and the premorphogenetic stage using a computer program. The terms and corresponding methods for the calculations are explained in Fig. 3. The most important results are summarized briefly below—for a more detailed account please consult Tables S1 and S2 (Supplementary material).

The sum of all displacements of AB-derived cells by mitoses amounts to only 18% (27 ± 1 times embryo EL) of the sum of all general movements of all cells between mitoses (150 ± 18 EL). Cells do not move in straight lines (bee lines) between the 12-cell stage and their terminal position at the premorphogenetic stage but more than 60% of the movements are detours from the direct paths. The relative contributions of mitoses and cell movements for a selection of individual cells are listed in Table S2 (Supplementary material). The detours may be caused by mitoses and/or searching for the right path. Additionally, other cells may hinder directed movement. The directions of the spindles deviate, on average, by 35° from the a–p axis and between 23° and 57° in the different regions. Although the AB-derived regions have different final shapes, this is not caused by a specific alteration of cleavage directions since the spindles are oscillating around the a–p axis; the average deviation from this axis is close to 0° (Table S1, Supplementary material).

We visualize the relative contributions of mitoses and movements in 3D representations shown in Fig. 5. We determined the absolute contributions of mitoses to the placement of cells by discarding all cell movements between mitoses and adding up the distances cells were displaced by mitoses. Using the reversed procedure, we calculated the coordinates cells would have reached if only movements had placed them in the embryo (Fig. 3G). Although, of course, mitoses generally locate anterior cells in front of posterior cells, the movements play a major role in positioning the cells. For example, in the ABara- and ABprp-derived regions, movements substantially reorganize the pattern of cell positions which would be formed by mitoses only (Fig. 5). The relative contributions of the two processes were calculated by projecting the actual paths of cells on the bee lines connecting the "first mother cell" in the 12-cell embryo with the position of the cell at the premorphogenetic stage (Figs. 3A and H). The contribution of mitoses to the final placement of cells ranges from −26% to 338% of the bee lines in the examples shown in Fig. 5. Thus, mitoses can place cells in all directions far away from their final position. The average contribution of the cleavages to the placement of cells is 22% to 30% in the three normal embryos analyzed. These observations show that the final pattern is only achieved through sorting by cell movements since mitoses do not position cells in their final positions.

Movement of regions

We were surprised to find that each of the eight regions contributes about 12% to the overall cell movements in the wild-type embryo, although some regions spread out over the whole embryo while others are centered around the position that the founder cell occupied at the 12-cell stage embryo (Figs. 1A and B). These movements may be caused by the processes which contribute to the detours. The different shapes of regions can be seen and quantified nicely by calculating the "a–p elongation" factor (Fig. 3F). For example, an average ABala-derived region stretches out 16% EL, the ABpra-derived region 73% EL along the a–p axis. Between the 12-cell and the premorphogenetic stage, the "main points" of the regions (Fig. 3G) move significantly (up to 45% EL) and differently from embryo to embryo (e.g., ABplp between 8% and 32% EL). Thus, the embryo is significantly reorganized during development by movements of the regions relative to each other.

Cell behavior in mutants

A quantitative analysis of cell behavior in the mutant embryos (see also Table S1, Supplementary material, for a detailed account) shows that the far-ranging movements during the repositioning of cells neither increase the general movement of all cells (128 EL in *glp-1*, 155 EL in *apx-1* compared to an average of 150 EL for the three wild-type embryos) nor that in specific regions. The bee lines do not differ significantly from the wild type either. Concerning the mitoses, note that the angles generally deviate more from the a–p axis than in the normal embryos. Probably, this is induced by the crowding of cells in the anterior part of the embryos (Table S1, Supplementary material). Since cells move three times more than necessary to reach their final position (as they also do in the wild-type embryo), no intensified movement is required to reroute cells even halfway through the embryo (Movie S2A, Supplementary material).

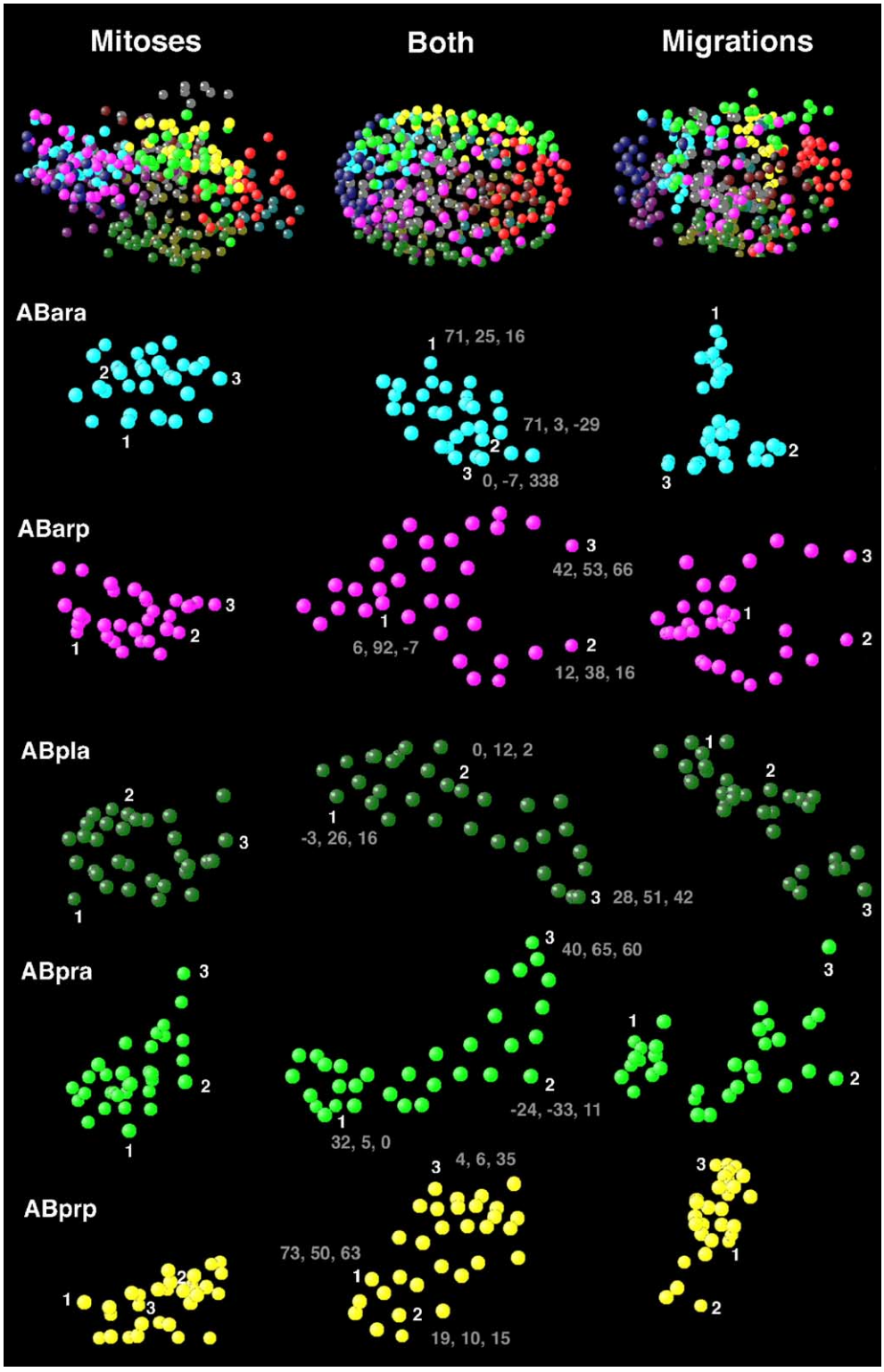


Fig. 5. Contributions of mitoses and movements to pattern formation. The top row shows 3D representations of the AB-derived cells of embryo #2 (dorsal views). The left column shows the pattern of nuclear positions when only mitoses are considered, the center column the "normal" pattern with mitoses and cell movements, and the right column the cell positions when only cell movements are considered (Fig. 3G). Each region is displayed in such an orientation that it is always positioned in the upper most position of the 3D representation. The white numbers (1, 2, 3) identify specific cells which allows to see the relative contribution of mitoses and movements to the final position when the three columns are compared. Cell identities and the corresponding values for mitoses, movements and bee lines are listed in Table S2 (Supplementary material). The grey numbers (e.g., 71, 25, 16 for cell 1 in ABara) indicate the contribution of mitoses (in percent of the bee line; Figs. 3A and H) to the paths these cells and their precursors moved from the 12-cell stage until the premorphogenetic stage in the three normal embryos (#1, #2, #5). For example, the values 0, −7, and 338 listed at cell 3 of ABara means that the contributions of the mitoses for this cell are 0% in embryo #1, −7% in embryo #2, and 338% in embryo #5. To understand how negative values and a contribution of more than 100% can be explained, see Fig. 3H.

Guidance of cells

After presenting evidence that pattern formation in *C. elegans* is achieved by fate-dependent cell sorting and not by a determinate pattern of cell cleavages, we now address the question how cells could be guided during sorting. Three basic mechanisms have been described so far: gradients, guide posts, and local cell–cell interactions (Seeger and Beattie, 1999).

Gradients and guide posts

To test whether gradients or guide posts supply positional information within the embryo, we laser ablated individually the ABa, ABp, EMS, and P_2 blastomeres in the 4-cell embryo. This should remove potential sources of such signals. 4D microscopic analyses showed that the remaining regions behave as in the normal embryo. For example, if ABa is ablated, the anteriormost descendant of ABpla still moves past the ablated blastomere in the anterior of the embryo. After ablation of ABp, the posteriormost descendant of ABarp also passes the ablated blastomere to position itself in the posterior of the embryo. If P_1 is ablated, the posteriormost AB descendants also move past the ablated cell (Fig. 2E and data not shown; Hutter and Schnabel, 1995b). This suggests that cells are neither guided by graded signals, whose source would be located within the embryo, nor by discrete guide posts. A potential problem of the laser ablation experiments is that the ablated blastomeres could still supply positional information. However, we believe that this is rather unlikely since cells of embryonic fragments derived from isolated AB blastomeres still show "correct" sorting (see accompanying manuscript Bischoff and Schnabel, 2006). The phenomenon that cells pass by ablated cells suggests that they are not the source of directional cues, since cells should stop in front of the signal.

To test whether any positional information is located on the inside of the eggshell, we removed the eggshell from early embryos and cultured them. Even under these circumstances cells migrate long distances to their "terminal" positions (Fig. S1C, Movie S4, Supplementary material), suggesting that the eggshell does not provide positional information.

Local cell–cell interactions

The third possibility that cell movements are directed by attraction and repulsion of specific cells can also be evaluated using the *glp-1* embryo (Figs. 1C–E). The AB descendants of this embryo execute only ABala- and ABarp-derived fates. Descendants of these lineages barely touch during normal embryogenesis and, if they do, this occurs only in the very anterior part of the embryo (Fig. 1B, Movie S1A, Supplementary material). Nevertheless, the descendants of the blastomere ABpra, which execute an ABala fate (Fig. 2B), move out of an environment consisting only of ABarp-derived cell fates to join the other cells executing an ABala fate (Movies S2A, S3, Supplementary material). Thus, cells migrate correctly in an environment they normally never face. This sorting behavior makes it unlikely that a cell recognition system – which needs cell–cell interactions within a distinct subset of cells occurring only in a local environment during normal embryogenesis – is used for the sorting.

Redundancy of guide posts and local cell–cell interactions

To exclude a redundancy between guide posts and attraction and repulsion of cells, we ablated P_1 late in its cell cycle in *glp-1 (e2144)* embryos. This manipulation removes all posterior cells and, thus, potential signal sources from the embryo, but still allows the specification of the ABarp fate (Hutter and Schnabel, 1995b). Furthermore, neighborhoods are substantially altered with ABala and ABarp blastomeres being distributed in a salt and pepper pattern in the earlier embryo. Nevertheless, these embryos still sort the regions as the *glp-1* embryo discussed above (data not shown). We do not see how this finding could be explained by the establishment of non-autonomous long- or short-range acting signal centers. In this experimental situation, there should be multiple signal centers distributed in the embryo if they were derived from ABala and/or ABarp blastomeres. It is also rather improbable that specific local attractive and repulsive cell–cell interactions direct cells in this situation—as the cells sorting in this situation normally do not face each other. Since none of the tested scenarios appears to apply to the cell sorting observed, we propose an alternative mechanism.

A model for guidance by "cell focusing"

The robustness of the cell sorting process suggests that a "universal" guiding system controls the positioning of cells in the second phase of embryogenesis when cell movements start. Our observations suggest that a "coordinate system" is created autonomously by the cells in the embryo itself. Meinhardt proposed a model he called "cell focusing" after electrofocusing used in chemistry where proteins are sorted in a pH ("positional") gradient depending on their isoelectric point. He proposed that cells autonomously generate a positional value on their surface depending on their fate. Our observation that fate alterations of blastomeres modify the "address" or final destination of the migrating descendants is consistent with Meinhardt's hypothesis. Cells read the positional information of their neighbors and try to match their own positional value with that of their neighbors. In the case of a discrepancy, they move relative to their neighbors until the position matches (Fig. 6).

How could a positional value needed for cell focusing be generated?

The sorting (focusing) occurs before tissues are differentiated and should, thus, not be based on tissue identities. It was shown that the terminal cell fates are specified through a binary anterior–posterior specification of cell identities during the

proliferation phase of embryogenesis (Kaletta et al., 1997; Lin et al., 1998). These temporary cell identities comprise all the different cell fates derived from the specific blastomeres which are later present in the "region" formed by this cell. Therefore, these temporary cell identities have also the potential to code for temporary positional values which can be used for the sorting of cells during the specific phase of development.

Does cell focusing occur only along the a–p axis?

Our analysis describes the sorting and movements along the a–p axis. We mentioned that we see no correction of a "wrong" positioning of ABalp on the right side of an *apx-1* embryo. Thus, one can ask whether guiding systems along the l–r and d–v exist. We believe there may be no requirement for guiding systems along these axes. At the 12-stage, the stereotyped early cleavages placed the founding blastomeres approximately in the correct positions according to the l–r and d–v axis in the embryo. When the cells afterwards sort into the right neighborhoods by cell focusing, cells are "indirectly" forced also in the right places according to the other axes (Fig. 5).

However, our analyses are restricted to the AB descendants. Thus, we do not know whether the gastrulation movements of MS and E descendants are driven by the same mechanism. The terminal phase of ventral closure, i.e., the sealing of the ventral cleft left open after the internalization of the MS cells, depends on genes which appear unnecessary for cell focusing (e.g., *vab-1*; see below). However, we consider the shaping of the ABplp and ABprp regions before the premorphogenetic stage, which could be seen as gastrulation movements, as part of the cell focusing process.

Is an inherent polarity essential for cell focusing?

How is the polarity of movement specified? Since the cells of the worm divide generally in an a–p direction and cell fates are specified according this cleavage direction (Sulston et al., 1983), this inherent polarity could be used to resolve wrong contacts. Such a resolution of wrong contacts was observed in the *glp-1* embryo in which all cells with an ABala-derived fate position in front of those with an ABarp-derived fate. Alternatively, the polarity of sorting could be only determined by the local environment (Fig. 6). Our cell focusing model has testable predictions, which are discussed and tested in experiments using an in vitro system (see accompanying manuscript Bischoff and Schnabel, 2006).

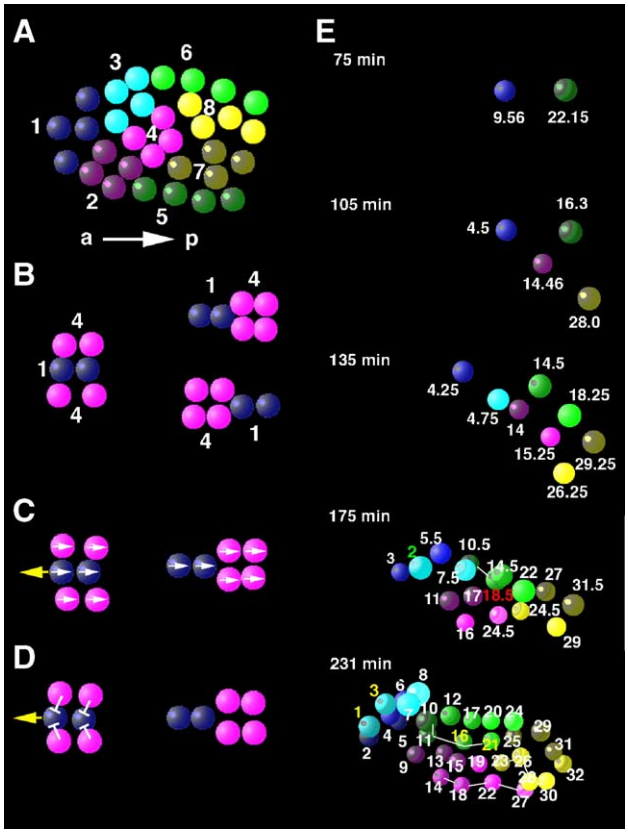


Fig. 6. The cell focusing model. (A) Cells express autonomously a positional value according to their fate which is here indicated by numbers on the level of the regions of the embryo. The polarity of the embryo is indicated by the a–p arrow. (B) If cells with different and incompatible positional values meet, they move relative to each other along the a–p axis of the embryo. In absence of any directional information, the cells with the value "1" (blue) can end up in any position relative to the cells with the value "4" (pink). To assure correct positioning of cells ("1" anterior to "4"), two mechanisms are conceivable. (C) Directional information could be derived from a general polarity inherent to the cells (white arrows) or from the local environment as shown in panel D. In the accompanying manuscript (Bischoff and Schnabel, 2006), it will be shown that cells do not use an internal polarity and sort only according to the local environment. (E) Cell sorting in an AB-derived region. A sorting along the a–p axis can also position cells along the l–r and the d–v axis. The panel shows the development of the ABplp region of embryo #1 from 2 cells (ABplpa (blue) ABplpp (dark green), 75 min) to 32 cells (231 min). When four cells are present ABplpaa is blue, ABplpap violet, ABplppa green and ABplppp dark green. After subsequent divisions, the posterior cells were always colored in a lighter tone. The 32 cells present at 231 min are numbered following strictly the a–p order of cells. If, as the cell focussing model postulates, cells "know" their neighbors – for example, if cell 1 "knows" that it has to touch, the cells 2, 3, and 4 – its position is well defined. The stacking up of cells which is required to extend the region on the l–r and d–v axis is also established by the order of cells set up earlier. The influence of the neighboring regions not shown here (ABalp, ABpra, ABplp, and earlier MSa) may also be crucial. As explained in the main text, our model is based on the temporary identities of cells which are specified in a binary manner. Here, we tested if a simple system of positional values coupled to cell identities could explain the cell sorting observed in this region. To assign a positional value to the mother of two daughter cells, the positional values of the daughters were added and divided by two-starting with the values of the cells at 231 min. Interestingly, these calculated values always correlate with the positions where the cells are along the a–p axis—with the exception of two cells at 175 min. ABprpaapa has a positional value of 2 (shown in green) but is positioned behind the cell with a positional value of 3. It moves in the next 5 min anteriorly to correct this "wrong" position (not shown here). The daughters (1, 2; yellow numbers) then travel further anteriorly to reach their final positions at 231 min. The other cell ABprpaap has the positional value of 18.5 (red number) but is positioned anteriorly of the cell with a value of 14.4. The cells exchange their order in the next 22 min and the daughters of ABprpaap move further posteriorly (16, 21; yellow numbers). We are aware that the cell focusing model is a rather abstract hypothesis which is, however, suited to explain the observed sorting of cells also in mutant embryos where cell identities have been altered (Figs. 1 and 2). The actual sorting system can only be identified by molecular analyses.

How general is the mechanism of cell focusing in animal development?

We have shown that pattern formation in the *C. elegans* embryo is achieved by far-ranging cell movements which "focus" cells into their positions. The following observations suggest that this may be a more general mechanism for pattern formation.

We analyzed the embryogenesis of the gastrotrich *Lepidodermella squammata* (AH and RS, unpublished). In this embryo, which appears to develop with a stereotyped lineage (Sacks, 1955), cells move very far towards their final position (Movie S5, Supplementary material)—as in the nematode. We compared the deviation of the direction of mitoses or movements with the direct path the cell would have to move to its final position between these two animals (Fig. 7, for method see Fig. 3I). Using our new method, we learned that in *C. elegans* as well as in *L. squammata*, neither mitoses nor cell movements are generally directed towards the final positions before the 64-cell stage of the ectodermal cells. Afterwards, movements but not mitoses start to point significantly towards the final positions of the cells. We conclude that both organisms use a cell sorting mechanism for pattern formation.

We used the same method to analyze embryonic development of two highly regulative organisms, the tardigrade *Thuliana stephaniae* (Hejnol and Schnabel, 2005) and the sea urchin *Psammechinus milaris* (RS and AH, unpublished). In these embryos, there is no large difference in the pointing of mitoses or movements towards the terminal positions of cells during the earlier embryogenesis. This is expected for regulative embryos, where cells are specified according to their position (Fig. 7). However, also in *Drosophila*, where cells are generally specified according to their position in early development, sorting according to fate can occur during pupal differentiation. Lawrence et al. (1999) report that clones in the abdomen of Drosophila, whose anterior–posterior identity was manipulated, always move to the position corresponding to their new identity. They state: "for example groups of cells in the middle of the A compartment that are persuaded to differentiate as if they were at the posterior limit of A, move posteriorly. Similarly, clones in the anterior domain of the A compartment that are forced to differentiate as if they were at the anterior limit of A, move anteriorly." This is a similar behavior as we observed, for example, in *glp-1* embryos. Anterior cells with a more posterior fate move posteriorly and vice versa. Thus, cells may also focus, at least in later development, in regulative animals. Such a phenomenon may help determine the shape of organs.

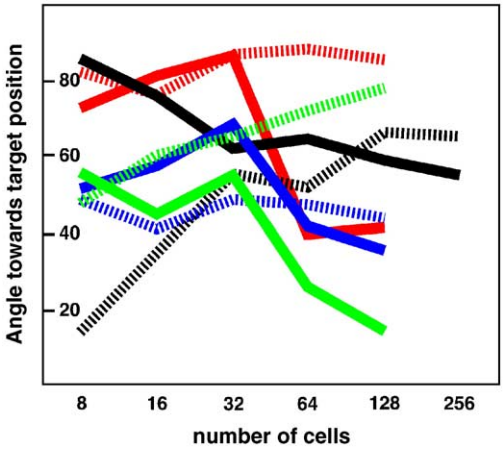


Fig. 7. Relative contributions of mitoses and cell movements to the final placement of cells in different animals. The figure shows the deviations of the cleavage angles (hatched lines) or movement angles (solid lines) at the different embryonic stages from the direction in which cells are finally positioned. The method of calculation is explained in Fig. 3I. (green) *C. elegans*. (red) *L. squammata*. (blue) *T. stephaniae*. (black) *P. miliaris*. In *C. elegans* and *L. squammata*, cell movements point – after the 32-cell stage – significantly towards the final positions of cells, whereas the mitoses appear rather unorganized. This is an indication that cell sorting occurs during embryogenesis. In *C. elegans,* where cleavages generally occur in a–p direction (±35°), the difference of cleavage angles with respect to the final position increases during development. This can be explained by the fact that cells move significantly in lateral directions in later development (Fig. 5). In the tardigrade *T. stephaniae* and the sea urchin *P. miliaris* cleavages and movements after the 32-cell stage deviate equally from the direction in which cells are positioned at the last developmental stage, we analyzed. Thus, cells do not aim towards specific positions during the phase of development we observed. This may be expected in regulative embryos.

Conclusions and prospects

In this study, we show that pattern formation up to the premorphogenetic stage is not achieved by a stereotyped cleavage pattern, as proposed earlier (Boveri, 1910; Stent, 1985; Strassen zur, 1959; Sulston et al., 1983), but by a cell sorting process that is associated with extensive cell movements. Our observations may, thus, define a new embryonic strategy for pattern formation which was thought not to exist in animals (Armstrong, 1989). There is evidence that this strategy may not be unique to *C. elegans*.

In the first phase of development, up to the onset of gastrulation, a stereotyped cleavage program is executed, which maintains certain cell–cell contacts and assures a stereotyped pattern of cell–cell interactions. After this initial phase, cells move extensively to reach their appropriate positions before morphogenesis is initiated. A quantitative comparison of the development of different normal embryos – using an algorithm which compares the cell positions during development – shows that the similarity among embryos increases until the premorphogenetic stage is reached. This patterning process is coupled to the movement of cells. We were surprised to learn that the cells of the different regions all migrate the same distances. This was not anticipated since the regions have very different shapes. While the cells of some regions cluster around the position of their founder cell, other regions extend over the whole length of the embryo. One possible explanation for this phenomenon is that cells are only able to sort while they move to explore the identity of their neighbors. This would also explain why the actual distance a cell moves to reach its terminal position is much larger than the bee lines between the starting and the end point of the movement. Cell divisions do not contribute much to this movement of cells.

Our observations suggest that the mechanism responsible for the sorting of cells is robust. When the identity of a blastomere is altered so that a fate is executed in a region of the embryo

where it normally does not occur, the descendants migrate to the position where cells of this particular fate are located in a normal embryo. The corresponding region also changes its shape according to its new fate. This adaptation of shape appears not to be linked to the direction of mitoses but is rather due to an altered sorting behavior. Cell sorting within a region may be an autonomous feature of the region itself. It has to be based on movements mediated by cell–cell interactions within the region. The ordered arrangement of the regions relative to each other may also be based on cell–cell interactions. Since the embryo appears to be able to sort "correctly" even in environments with different compositions of cell fates, the cells must have a general capability to recognize each other. This is taken into account by the "cell focusing" model.

The molecular nature of the cell sorting system controlling the cell movements, however, remains elusive. To our knowledge, none of the amorphic mutants in candidate signaling systems affect cell sorting—neither mutations in genes like the single Ephrin receptor *vab-1*, nor semaphorin, nor PTP-3/LAR RPTP show a phenotype before the premorphogenetic stage. The earliest function of *vab-1* is during ventral enclosure (Chin-Sang et al., 2002). Using RNAi to inactivate many genes, which one could suspect to be involved in the sorting because of their importance in other systems, did not affect cell movements (e.g., cadherin, integrin, laminin, IG-CAM, fibronectin, G proteins, wunen, Cepak and diverse kinases, WASP, tyrosine kinase receptors, homeobox genes; Ingo Büssing and RS unpublished). Thus, we are currently trying to identify more genes involved in the movement by using a classical genetic approach.

Acknowledgments

We thank Gabi Sowa, Franco Bignone, Heinke Holzkamp, and Dörte Seider for their help with lineaging the many embryos, Cathrin Struck for the technical assistance, and Peter Lawrence, Norbert F. Käufer, and an anonymous referee for the valuable comments on the manuscript, and the members of the Schnabel group for the helpful discussions. The sea urchin *Psammechinus miliaris* was analyzed at the Marine Station List, Helgoland, Germany. The work was initially supported by the Max-Planck-Society and then by the Deutsche Forschungsgemeinschaft.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ydbio.2006.03.004.

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