A Posterior Centre Establishes and Maintains Polarity of the Caenorhabditis elegans Embryo by a Wnt-Dependent Relay Mechanism

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Cellular polarity is a general feature of animal development. However, the mechanisms that establish and maintain polarity in a field of cells or even in the whole embryo remain elusive. Here we provide evidence that in the Caenorhabditis elegans embryo, the descendants of P1, the posterior blastomere of the 2-cell stage, constitute a polarising centre that orients the cell divisions of most of the embryo. This polarisation depends on a MOM-2/Wnt signal originating from the P1 descendants. Furthermore, we show that the MOM-2/Wnt signal is transduced from cell to cell by a relay mechanism. Our findings suggest how polarity is first established and then maintained in a field of cells. According to this model, the relay mechanism constantly orients the polarity of all cells towards the polarising centre, thus organising the whole embryo. This model may also apply to other systems such as Drosophila and vertebrates.

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Introduction

Many developmental processes depend on polarised cells; cell movements and cell divisions are oriented [1–3] and also the specification of cell fates is influenced by polarity [4–6]. The polarity of individual cells has been studied in Drosophila [7,8] and Caenorhabditis elegans [9,10], but the establishment and maintenance of polarity in a field of cells is still mysterious [11,12].

It is a general notion that during development of the C. elegans embryo, cell fate specification [13,14] and division orientation [15] are coordinated strictly in the anterior-posterior (a-p) direction. The first cleavage generates an asymmetric 2-cell stage embryo with an anterior AB and a posterior P1 blastomere [15]. Most of the cells (70%) of the hatching embryo are derived from the AB blastomere. The first quantitative 4D-microscopic analyses of cleavage directions in the C. elegans embryo showed that AB-derived cells do not cleave strictly along the a-p axis but deviate, on average, approximately 36° from the a-p axis until the premorphogenetic stage (256-AB cell stage) of embryogenesis [16]. During early development from the 4-AB to the 64-AB cell stage, the deviation from the a-p axis is even higher (approximately 45° ± 20°; see Figure S1 for examples of cleavages in a normal embryo).

Goldstein reported that in vitro contact with P2, the posterior daughter of P1, orients the mitotic spindle in its anterior sister EMS and that P3, the posterior daughter of P2, does the same to its anterior sister E [17]. Besides the influence of P1 descendants on EMS cleavage orientation, it was also shown that the orientation of the ABar division depends on contact with the C blastomere, the anterior daughter of P2 [18]. Furthermore, the combination of blastomeres in vitro demonstrated that also individual AB-derived cells of the 12-cell stage embryo align their mitotic spindles towards MS and C [19]; however, the directions of the next cleavages were not reported. Spindle orientation, as well as cell fate specification, was shown to depend on Wnt signalling in C. elegans [19–22].

To investigate cellular polarity in AB-derived cells of the C. elegans embryo, we performed blastomere combination experiments in vitro [23,24]. The generated embryonic fragments were analysed by 4D-microscopy, which allowed us to monitor cell positions, mitoses, movements, and division orientation of all cells during development (see Materials and Methods and [16,25]).

Here we show that P2 and its descendants introduce a polar bias into the cleavages of AB-derived cells, which causes a shift of the average division angle with respect to the a-p axis from 62° to 45°. This polarisation depends on a MOM-2/Wnt signal originating from P2 and its descendants. Moreover, we provide evidence that P2 and its descendants constitute a polarising centre, which signals throughout early development. Furthermore, we show that the Wnt signal is transduced from AB descendant to AB descendant by a relay mechanism.
Results/Discussion

Elongation of AB-Derived Embryonic Fragments Depends on the Presence of P2

AB blastomeres isolated from P1 at the 2-cell stage show an abnormal “spiralian-like” cleavage pattern, in which the direction of cleavages rotates approximately 90 ° after each division [26,27]. This is due to the migration pattern of the centrioles, which always move orthogonally out of the plane defined by the last two divisions [28]. The developing embryonic fragments have a spherical shape (Table 1, row A; Figures 1A and 2D). This suggests that AB descendants do not have any intrinsic polarity to orient their divisions in the normal a-p direction [15,16] and that the a-p polarity of these cells is induced after the 2-cell stage. This appears to disagree with Park and Priess, who showed that sister pairs of 16-AB and 32-AB cell stage AB blastomeres display an asymmetric expression of POP-1, although they never touched P2 or its descendants [19]. However, we recently showed that isolated AB blastomeres display a-p asymmetries in cell fates [27]. Thus, the POP-1 asymmetry described by Park and Priess might indicate an asymmetry in cell fates rather than in the polarisation of cleavage direction.

When a P2 blastomere is added to the two daughters of isolated AB blastomeres, the developing AB-derived embryonic fragments elongate and become twice as long as they are wide (Table 1, row B; Figure 1A). The eight regions deriving from the eight AB descendants of the 12-cell stage embryo [25] are also elongated. This finding contrasts with the embryonic fragments without P2, in which the regions are approximately spherical (Figure 1A). In the elongating embryonic fragments, the cells do not divide “spiralian-like,” but orient towards the added P2 blastomere (white box in Figure 1C). This suggests that the observed elongation is due to cleavage orientation.

Elongation of Embryonic Fragments Results from Oriented Divisions

We then tested whether the orientation of cell divisions was the main cause of the elongation. We used SIMI Biocell to record the 3-D coordinates of the mother cells and then those of the daughters 105 s after the initiation of the cleavage furrow, and we calculated the cleavage angles of all cells relative to the a-p axis (see Materials and Methods). A comparison of division angles between isolated AB blastomeres and AB blastomeres cultured with an added P2 blastomere showed a significant difference (p < 0.001, Student’s t-test). In isolated AB blastomeres, average divisions deviated 63 ° ± 20 ° (n = 3 embryonic fragments) from the a-p axis, whereas in embryonic fragments with P2, divisions deviated on average 45 ° ± 22 ° (n = 3) from the a-p axis.

To assess cleavage directions quantitatively, we projected the division angles onto a target screen (Figure 2A) (“Löwe projection”, named after the mathematician Harald Löwe, Braunschweig, who kindly suggested this method). The Löwe value is a measure of the deviation of cleavages from the a-p axis in all three dimensions. An isolated AB-derived fragment demonstrated a Löwe value of 6.3 ± 9.8, which corresponds to the mean angle of 62 ° ± 23 ° with respect to the a-p axis. After addition of P2, the Löwe value decreased to 2.9 ± 4.4 and the angle to 44 ° ± 22 ° (Figure 2D and 2E).

The direction of cleavages correlates very well with the variation of shape (elongation) of the embryonic fragments; the statistical correlation between elongation, the average cleavage angle, and the Löwe value are better than 0.92 (p < 0.005; considering 2,200 cell divisions). Thus, we used the shape of embryonic fragments to assess division orientation in the large number of embryonic fragments analysed (120 fragments in 30 experiments; Table 1). For many of the experiments, the Löwe projections show allow a more comprehensive comparison.

To assess the contribution of cell movements between cleavages, we removed these movements from our dataset using an algorithm described in [16]. In this way, only the contribution of mitoses is left and can be seen in the 3-D representations of the developed fragments (Figure 1). Even after this procedure, embryonic fragments including P2 were approximately two times longer than those derived from only AB blastomeres (Figure 1A). Thus, the shape of the embryonic fragments depends almost entirely on the cleavage directions of cells. Our measurements show that the addition of P2 to an AB blastomere causes a significant alteration of cleavage orientation of AB-derived cells of the embryonic fragment which, in turn, leads to an elongation.

The analyses also indicate that the alteration of cleavage direction is not limited to the AB descendants that touch P2, but occurs in all AB descendants (white box in Figure 1C). P2 divides division orientation from a “spiralian-like” pattern observed in isolated AB blastomeres (corresponding to an average angle of approximately 60 °; i.e., the mean of three cleavages with angles of 0 °, 90 °, and 90 ° with respect to the a-p axis) to 45 ° ± 22 °. This result is very similar to the 45 ° ± 20 ° (n = 3) observed for the cleavages in early development of normal embryos (Figure 2B and 2C).

At first sight, our results differ from studies in C. elegans that have shown that isolated AB-derived blastomeres of the 12-cell stage embryo [19] and the EMS blastomere [17] divide directly towards an added P2 blastomere (0 °) in their first division [22]. However, in these studies, the behaviour of cells in subsequent divisions was not discussed. When we analysed division orientation in normal embryos between the 4-AB and the 64-AB cell stage (the first two divisions of AB occurring perpendicular to the a-p axis), we observed that AB-derived cells divide rarely 0 ° but on average 45 ° ± 20 ° with respect to the a-p axis (e.g., Figure S1 and Figure 2B and 2C). Also, in vivo (in contrast to in vitro [17]), EMS does not divide directly towards P2. In the five normal embryos we analysed, the divisions occurred on average 35 ° ± 6 ° off the a-p axis. Thus, our observations in embryonic fragments are consistent with normal embryonic development.

In normal development, an average cleavage angle of 45 ° may reflect the optimal compromise between minimising the need for compensatory cell movements to less than one embryo length per cell [16] while still allowing a stringent a-p assignment of cell fates [15]. Strict a-p cleavages would displace cells 40 times the embryo length in eight cleavages (see Protocol S1 for further discussion).

Cleavage Angles Are Independent of Cell Fate

Because the addition of P2 induces an ABp fate in the daughters of AB [29], and because ABp-derived regions are longer than those derived from ABa [25], the elongation of embryonic fragments could merely reflect the presence of ABp-derived fates; i.e., the bias in division orientation might
**Table 1. Elongation of Embryonic Fragments**

| Experiment | Design | EI (mean ± SD) | n | ANOVA Interpretation |
|------------|--------|----------------|---|-----------------------|
| A AB [N2]  |        | 1.2 ± 0.3
d | 15|                       |
| B AB + P2 [N2] |        | 2.4 ± 0.2 | 3 | A** (-)                |
| C AB + P2 [glp-1] |        | 2.2 ± 0.7 | 3 | A** (-)                |
| AB [glp-1] + P2 [N2] |        | 1.8 ± 0.3 | 4 | A*, B* (-)             |
| AB [N2] + P2 [apx-1] |        | 2.3 ± 0.4 | 2 | A** (-)                |
| D AB [N2] + P2 [mom-2 (t2072)] |        | 1.5 ± 0.1 | 4 | B** (-)                |
| AB [N2] + P2 [mom-2 (t2180)] |        | 1.4 ± 0.3 | 2 | B** (-)                |
| AB [N2] + P2 [mom-2 (or9)] |        | 1.0 ± 0.2 | 3 | B** (-)                |
| AB [N2] + P2 [mom-2 (or9)2] |        | 1.3 ± 0.3 | 9 | B** (-)                |
| E AB [mom-5 (or57)] + P2 [N2] |        | 0.8 ± 0.2 | 4 | B** (-)                |
| AB [N2] + P2 [mom-5 (zu193)] |        | 1.1 ± 0.4 | 3 | B** (-)                |
| AB [N2] + P2 [mom-5 (zu193)] |        | 2.1 ± 0.4 | 3 | A** (-)                |
| F AB + P2 [N2] (90°) |        | 1.9    | 1 | A*, B* (-)             |
| AB + P2 [glp-1] (90°) |        | 1.7    | 1 |                        |
| AB + P2 [N2] (45°) |        | 1.8    | 1 |                        |
| AB + P2 [glp-1] (45°) |        | 2.2    | 1 |                        |
| G 4 ABxx + P2 [N2] |        | 2.2 ± 0.4 | 3 | A** (-)                |
| H AB + P2 + AB [N2] |        | 2.1 ± 0.5 | 3 (6)* A** (-)         |
| I AB + EMS [N2] |        | 1.7 ± 0.6 | 3 | A*, B* (-)             |
| J AB + C [N2] |        | 1.8 ± 0.4 | 3 | A*, B* (-)             |
| AB + P2 [N2] |        | 2.6 ± 0.4 | 3 | A** (-)                |
| AB + A Bx [N2] |        | 1.1 ± 0.3 | 2 | B** (-)                |
| K AB + AB [N2] (both AB) |        | 1.0 ± 0.2
d | 6 |                        |
| K1 AB + AB [N2] (first AB blastomere) |        | 0.9 ± 0.3 | 6 | B** (-)                |
| K2 AB + AB [N2] (second AB blastomere) |        | 0.9 ± 0.3 | 6 | B** (-)                |
| L AB + AB + P2 [glp-1] (both AB) |        | 2.3 ± 0.5
d | 5 | A**, B*, K1** (-)      |
| AB + AB + P2 [glp-1] (AB, not touched by P2) |        | 1.8 ± 0.7 | 5 | A**, B*, K1** (-)      |
| AB + AB + P2 [glp-1] (AB, touched by P2) |        | 2.2 ± 0.2 | 5 | A**, K2** (-)          |
| M AB + glp-1 |        | 0.6 ± <0.1 | 3 |                       |
| N1 AB + AB + P2 [glp-1] |        | 1.3 ± 0.1 | 5 | M** (-)                |
| N2 AB + AB [N2] + P2 [apx-1] |        | 1.4 ± 0.1 | 5 | M** (-)                |
| N3 AB + AB + P2 [wt]+ |        | 1.3 ± 0.1 | 10 | M** (-)                |
| O AB [N2] + AB [mom-2 (or9)] + P2 [apx-1] |        | 1.0 ± 0.3 | 9 | M*, N1–3**             |
| P AB [N2] + AB [mom-5 (zu193)] + P2 [apx-1] |        | 1.2 ± 0.2 | 5 | M** (-)                |
| Q AB – P2 [N2] (elongated) |        | 1.9 ± 0.2 | 5 | A**, B* (-)             |
| R AB – P2 [N2] (weakly elongated) |        | 1.4 ± 0.1 | 6 | B** (-)                |
| S AB [N2] – P2 [mom-2 (or9)] |        | 1.2 ± 0.2 | 4 | B**, Q** (-)           |

EI is the elongation index of fragments calculated by dividing the length by the width of the fragments in the 3-D representations (Figure 52). If P2 was not present, the +p axis was placed orthogonal to the first AB spindle so that it intercepts the contact point where P2 was normally added. The genotype of blastomeres is indicated in square brackets to the right of the experiment. A scheme of the performed experiment can be found in the Design column (AB, white; P2, black; ablated blastomeres are crossed out). The shape of the embryonic fragments compared to the nonelongated control experiment is shown in the Interpretation column (-elongated; -nonelongated). In a few experiments in which embryonic fragments elongate, the EIs do not only differ significantly from the negative but also from the positive control (indicated by brackets in the Interpretation column). This is due to a larger variance between the single experiments rather than to a lack of elongation. The two AB-derived parts per embryonic fragment were considered separately in the statistical test.

Summary of all three alleles used.

Summary of glp-1 and apx-1 experiments with “wild-type” (wt) phenotype.

Data from [27].

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Figure 1. P₂ Polarises the AB Descendants Depending on MOM-2/Wnt

The left column of panels (A) and (B) shows the combination of the early blastomeres used in the experiments. Blastomere genotypes and fates (as determined by lineaging) are indicated. mom-2 blastomeres are marked with a red margin. The middle column shows the positions of the nuclei at the end of the eighth generation of cells (64 AB descendants) using the 3-D representation feature of SIMI Biocell. Positions were determined by following the development of all cells. AB-derived regions, founded at the 8-AB cell stage (fifth generation) [25], were colour coded independently of their fate to show the topology of each region. P₁ descendants are colour coded according to their fate. The right column shows 3-D representations of the same generation that illustrate how embryonic fragments look if the contribution of cell movements is removed using an algorithm described in [16] so that only the contribution of mitoses is left (“mitoses only”). In (C) 3-D representations of the sixth generation of cells (16 AB descendants) are shown. To indicate the orientation of the cleavages of their mother cell, the two sisters are connected with a white bar shortly after mitosis.

(A) Development of isolated AB blastomeres (the two ABx daughters are shown on the left) compared to AB blastomeres to which a P₂ blastomere was added.
only be due to the execution of specific cell fates. We therefore prevented the ABp induction by using different combinations of blastomeres in which either the responsible receptor GLP-1/Notch or its ligand APX-1/Delta were mutant [29], and we then analysed the elongation of these embryonic fragments. Below, we refer to blastomeres from embryos whose mothers were homozygous for a mutation, for example in glp-1, as glp-1 blastomeres. When glp-1 AB and P2 blastomeres are combined, cells mainly divide away from P2 and the embryonic fragments elongate (Table 1, row C; Figures 1A and 2F). The same is true when other blastomere combinations are used that also prevent the induction of ABp (Table 1, row C). Thus, the induction of ABp-derived fates by P2 is not the reason for the elongation of the fragments. Based on these results, we instead suggest that P2 and/or its descendants influence division orientation by being the source of a polarising signal.

Orientation of Cleavages Depends on a Wnt Signal Originating from P2

Earlier studies have shown that MOM-2/Wnt is involved in spindle orientation in EMS and in individual AB descendants [19–22]. To test if the polarisation of embryonic fragments depends on the MOM-2/Wnt ligand, we generated chimeric combinations by combining wild-type AB and mom-2 P2 blastomeres. Three different mutant alleles interfere with the elongation of the fragments (Table 1, row D). Fragments in which P2 was derived from mom-2(or9) do not elongate at all, because the divisions of the AB-derived blastomeres and their descendants were not aligned towards P2 (Figures 1B, 1C, and 2G). These results suggest that MOM-2/Wnt is the relevant signal given out by P2 or its descendants. The polarisation also depends on the MOM-5/FRizzled receptor [20,21] in AB but not in P2 (Table 1, row E). That MOM-2 and MOM-5 organise division orientation can also be seen in mutant embryos. Cell divisions in AB descendants between the 4- and 64-AB cell stage deviate from the a-p axis by $55^\circ \pm 19^\circ$ in mom-2(or9) embryos and by $55^\circ \pm 20^\circ$ in mom-5(su193) embryos; this is significantly different from wild-type embryos ($40^\circ \pm 20^\circ$ mean deviation from the a-p axis, embryos #2 and IB+RS [16]; Student's t-tests: $p < 0.001$ [$n = 2$ each]).

A Posterior Polarisating Centre

By analysing the polarising signal of P2 and its descendants in detail, we found that these cells display properties of a polarising centre. Regardless of whether P2 is added in a $45^\circ$ or a $90^\circ$ angle (as compared with the normal experimental conformation), P2 always defines the posterior pole of the fragment and induces an elongation (Table 1, row F). Also, embryonic fragments consisting of P2 and the granddaughters of AB (instead of the daughters of AB) elongate, suggesting that polarisation does not depend on the establishment of a polarising signal in the embryonic fragments. This is consistent with our previous results [29].

The following experiments also argue that P2 and its descendants constitute a polarising centre. When two mutually perpendicular P2 blastomeres were added to four AB daughters, two different patterns developed from the same starting configuration (Figure 3). Because in both experiments glp-1(e2144) blastomeres were used, any influence of different cell fates can be excluded. In one fragment, the descendants of the two P2 blastomeres remained in their initial positions, resulting in an L-shaped structure with two a-p axes. In the other fragment, the descendants of one P2 remained in their original position for 75 min, until after the P2 division, and then moved and joined the descendants of the second P2. This resulted in an elongated structure with only one a-p axis. This finding indicates that the AB-derived blastomeres respond continuously to a polarising signal; they even reorganise their polar behaviour when the P2 descendants move. This experiment again demonstrates that the descendants of P2 provide the polarity signal, because not P2 itself but only its descendants reached the posterior part of the fragment.
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The centre of the mother cell through the centre of the daughter cell. The each dividing cell is placed on the a-p axis and a vector is projected from the point where this vector hits a target screen is marked by a dot (the distance between mother cell and screen along the a-p axis is normalised to 1). The red semi-circles represent particular division angles (as indicated). Embryos were analysed from four to 64 AB-derived cells. For statistical analysis, the mean distance of the dots from the target centre and the standard deviation are calculated. This “Löwe value” and its standard deviation give a reasonable measure of the general direction of cell cleavages. This is exemplified by showing nine cleavages of a “polarised” normal embryo (blue) and a “nonpolarised” isolated AB blastomere (green). The mean is indicated by a circle and the standard deviation by a dotted circle. Note that the representation of cleavage angles is not linear, because the distance from the centre to a dot corresponds to the tangent of the cleavage angle to the a-p axis. Thus, angles close to 90° cannot be shown on the target screen (here, this is the case for two cleavages of the “nonpolarised” isolated AB blastomere). It becomes obvious that for the normal embryo, in which cells divide mainly along the a-p axis, most dots are located in close proximity to the centre and the corresponding Löwe value is low. In contrast, the dots are widely scattered for the isolated AB blastomere, in which cells do not mainly divide in the a-p direction, and the Löwe value is high.

(B–G) “Löwe projections” of two normal embryos and the embryonic fragments shown in Figure 1A and 1B. The red circles correspond to the red semicircles in (A). In the lower left corner, Löwe values and mean division angles are shown.

(B and C) Normal embryos #1 and #2 from [16,25]. Cells mainly divide in an a-p direction. The Löwe value and especially its standard deviation are low. If a third normal embryo (not shown) is also considered, normal embryos have Löwe values between 1.3 and 1.6 with standard deviations ranging from 3.0 to 4.2. The mean cleavage angles vary between 45° and 50°, the standard deviations between 20° and 23°.

(D) Isolated AB blastomere. Compared to normal embryos (B and C) and AB blastomeres where a P2 blastomere was added (E), the division angles scatter all over the target area; i.e., cells do not mainly divide in the a-p direction. This is reflected in the higher Löwe value and especially the high standard deviation. Five points are off the target screen due to 90° divisions.

(E) AB blastomere where P2 was added. Cells mainly divide in the a-p direction, and the Löwe value and its standard deviation decrease noticeably compared to the isolated AB blastomere in (D).

(F) AB blastomere where P2 was added in a glp-1(e2144) background. Again, cells mainly divide in the a-p direction.

(G) Addition of a mom-2(39) P2 blastomere to a wild-type AB blastomere. Cells do not mainly divide in the a-p direction. The Löwe value is higher than in embryonic fragments dividing mainly in the a-p direction and its standard deviation resembles the standard deviation of the isolated AB blastomere.

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the embryonic fragment and caused its elongation. An experimental displacement of cells, recorded under the 4D-microscope while development is progressing appears to be impossible to us at this time. Thus, the coincidentally occurring movement of the P2 descendants observed here was a unique chance to examine the consequences of the displacement of signalling cells during development.

The Polarising Signal Reaches Cells Not Touching P2

Some of the experimental results imply that cells that are not directly touched by P2 or its descendants are also affected by the polarising signal. To test this, we combined two pairs of daughters of AB blastomeres with one P2 blastomere so that only one pair of AB daughters touched P2. To ensure that P2 did not induce the ABp fate in the touched AB-derived blastomeres, glp-1 blastomeres were used. The descendants of both AB blastomeres form elongated structures, in contrast to the controls without P2 (Table 1, rows K and L). Thus, the polarising signal is transported to AB-derived blastomeres not touching P2.

How Is the Polarising Signal Transported?

It is not clear how signalling molecules are transported [30]. A secreted molecule could be transmitted actively, it could...
spread passively by diffusion, or it could be passed on from cell to cell by relay. In a relay mechanism, it would be produced newly in each cell in response to the signal from the neighbouring cell [31,32] (Figure 4A). To discriminate among these possibilities, we put a \textit{mom-2} AB blastomere between a wild-type AB and an \textit{apx-1} P2 blastomere. If the signal were transduced via a relay mechanism, the \textit{mom-2} blastomere would inhibit further signalling, because it could not produce a MOM-2/Wnt dependent signal. In contrast, an actively or passively transported signal should still reach the AB descendants (Figure 4B). In these experiments, \textit{apx-1} or \textit{glp-1} blastomeres were again used to obtain only ABa-derived fates. To prevent cells of the different genotypes from intermingling at a later stage, we laser irradiated the two \textit{mom-2} AB daughters after 10 min to prevent further divisions. The cells were not killed (Figure 4D and 4E). Control experiments showed that laser ablation did not hinder the transmission of the polarity signal (Table 1, rows M and N; Figure 4F and 4G). We found that the wild-type AB-derived parts of the fragments, which never touched P2, did not elongate when a \textit{mom-2} AB blastomere was placed between a wild-type AB and an \textit{apx-1} P2 blastomere (Table 1, row O; Figure 4H). Moreover, the directions of mitoses deviated strongly from the axis defined by P2, indicating that the cells were not polarised. The Löwe projections of the cleavage angles for this experiment and the controls are shown in Figure 4F–4H. These experiments suggest that the polarising signal is relayed from cell to cell in \textit{C. elegans}. The MOM-2/Wnt signal is not far-ranging but must be produced anew as each neighbouring cell receives the signal; this is not possible in the \textit{mom-2} mutant blastomere. Placing a \textit{mom-5} AB blastomere between a wild-type AB and an \textit{apx-1} P2 blastomere also impairs the elongation of the wild-type part of the embryonic fragment (Table 1, row P). Embryonic fragments elongate less as compared with wild-type controls; however, the effect is not as extensive as in experiments using \textit{mom-2} blastomeres. These findings confirm that MOM-5 is needed in AB blastomeres to receive the MOM-2/Wnt signal.

### Polarised AB-Derived Blastomeres Can Polarise Nonpolarised AB-Derived Blastomeres

We then sought further evidence that polarised AB descendants are indeed able to produce a polarising signal.

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**Figure 3.** P2 and Its Descendants Organise Polarity Continuously

For details, see legend to Figure 1. Two mutually perpendicular P2 blastomeres were added to the daughters of AB in a \textit{glp-1(e2144)} background. Two different outcomes of the experiment are shown in (A) and (B).

(A) All the P2 descendants stay in their original position; an L-shaped pattern with two a-p axes is formed.

(B) The descendants of one P2 blastomere shifted posteriorly and joined the other P2 descendants (red arrow). This displacement of the P2 descendants began in the shown fifth generation. It resulted in an elongated structure with only one a-p axis as can be seen in the eighth generation.

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A. Diagram showing active or passive transmission of the signal (e.g., diffusion) and relay mechanism.

B. Diagram showing possible active or passive transmission of the signal (e.g., diffusion) and transduction of signal via a relay mechanism.

C. Diagram highlighting the interaction between different cell types.

D. Image of *glp-1(e2144)* showing axial polarity.

E. Image of *glp-1(e2144)* showing axial polarity.

F. Diagram showing 6th and 8th generation with axial polarity.

G. Diagram showing 6th and 8th generation with axial polarity.

H. Diagram showing 6th and 8th generation with axial polarity.

Legend for part H:
- C: Control
- D: Destination
- Germline
- Irradiated
Figure 4. The Polarisating Signal Is Transferred from AB Blastomere to AB Blastomere by a Relay Mechanism

For details see legend to Figure 1. mom-2 blastomeres are marked by a red outline.
(A–C) Schemes showing the designs of experiments.
(D and E) DIC images of the fragment analysed in (G). Bars, 10 μm. (D) shows fragment after laser ablation of the two central blastomeres. Irradiated cells are marked with asterisks. (E) shows terminal stage of the fragment.
(F–H) Experiments where the central AB descendants were irradiated. The leftmost column shows the designs of the experiments. In the next column, division angles of the 16-AB cell stages are indicated by a white bar connecting sister cells shortly after mitosis. Further to the right, the 3-D representations of the 64-AB cell stages are shown. The column most to the right shows the Loewe projections of the division angles. (F) and (G) show two of the control experiments using wild-type, glp-1, or apx-1 blastomeres (Table 1, rows M and N).
(F) Control experiment without P2. No elongation of the nonablated part of the AB-derived embryonic fragment occurred.
(G) Control experiment with P2, glp-1 background to prevent induction of ABp fates. The mitosis directions of the fifth division, which produces the sixth generation of cells, in the nonablated part of the AB-derived embryonic fragment indicate a polarisation of the blastomeres. The fragment elongates. This experiment shows that laser ablation does not hinder a potentially diffusing or transported signal.
(H) Two mom-2 AB-derived daughters between P2 and wild-type AB-derived cells prevent elongation (Table 1, row O). Mitoses are not directed towards P2.
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To further corroborate the finding that AB-derived blastomeres can be polarised by transient contact of P2, we tested the influence of P2 on single AB blastomeres. The behaviour of single AB-derived blastomeres is not different from two combined AB-derived blastomeres; in both, cells do not divide mainly in an a-p direction and the fragments have rounded shapes (Figure 1A, 2D, and 5C; Table 1, rows A and K).

First, we analysed isolated AB blastomeres that touched wild-type P2 transiently (Figure 5D). In five of 12 experiments, the AB-derived fragment elongated (Table 1, row Q); in six fragments, it elongated weakly (Table 1, row R), and in one fragment, no elongation was observed. Second, we analysed AB blastomeres that transiently touched mom-2 mutant P2 blastomeres, and we found that in all four cases, the resulting embryonic fragments did not elongate (Figure 5E; Table 1, row S). These experiments confirm the finding above that a transient signal of P2 can polarise AB-derived blastomeres. Furthermore, the latter experiment again suggests that MOM-2 is the polarising signal. It also shows that an induction of ABp fate by P2 is not the reason for an elongation of the embryonic fragment, because the induction of ABp occurs also in mom-2(aw9) mutant embryos (unpublished data).

Establishment and Maintenance of Polarity in the C. elegans Embryo

Our investigations argue that the a-p polarity of AB descendants in C. elegans is not an autonomous feature established early in embryonic development, but rather that this polarity is induced in AB descendants by P2 for the first time at the 4-cell stage. We show that the division orientations of most of the AB descendants are biased towards P2 and its descendants (Figures 2 and 5) and that AB-derived cells continuously orient their divisions towards it, even when these signalling cells move during development (Figure 5). This suggests that P2 and its descendants constitute a polarising centre in the posterior of the embryo. We further show that MOM-2/Wnt—which has already been implicated in certain aspects of polarity of the early C. elegans embryo [19–22]—is the polarising signal (Figure 1 and 2; Table 1, row D). Moreover, we demonstrate that the MOM-2/Wnt signal is transduced from AB blastomere to AB blastomere by a salutatory relay mechanism (Figure 4).

We restricted our experiments to the AB-derived part of the embryo. An interesting question is how polarity is established and maintained in the remainder of the embryo derived from P1. To address this question, we compared the cleavage angles in wild-type and mom-2(aw9) embryos starting at the 8-cell stage embryo for the next four cleavage rounds. The P1-derived cells from MS, E, C, and D cleave with an average angle of 35° ± 21° in the wild-type embryos as compared to 57° ± 21° in the mom-2 embryos. Thus, a lack of MOM-2 affects the cleavage orientations in the P1-derived cells as significantly as in the AB-derived cells (Student’s t-tests: p < 0.001 [n = 2 each]). It may therefore be that the cleavages of all cells of the embryo are oriented by the same mechanism. Then the polarising activity of MS and C [19] would not be an autonomous feature of these blastomeres but would reflect a polarisation they acquired directly from the P2 daughter, P3 (in the case of C), or indirectly through a relay mechanism (in the case of MS).

Taken together, our findings might explain how polarity in the C. elegans embryo is maintained during ongoing development; after the identity of P2 has been specified by the par genes [33]—based on the initial polarisation of the embryo during fertilisation [34]—P2 might induce polarity in its neighbours ABp and EMS via a MOM-2/Wnt signal through direct contact [19,22]. Afterwards, polarity of all AB descendants might be continuously maintained by the posterior polarising centre whereas the salutatory relay mechanism would ensure that the polarity signal reached all AB-derived cells.

Because there is emerging evidence that the MOM-2/Wnt signalling pathway in the C. elegans embryo shows some similarity to the planar cell polarity pathway [19,22,35], our
Figure 5. Polarised AB Descendants Transfer Their Polarity
(A) Design of the experiment shown in (B).
(B-E) Left column: 3-D representations of the 64-AB cell stages of various experiments. The eight AB-derived regions are colour-coded independently of their fate to show the topology of each region. Middle column: "Löwe projections" (see Figure 2A for explanation). Right column: 3-D representations from which the contribution of cell movements was removed ("mitoses only"; see legend to Figure 1). In all experiments presented, these "mitoses only" representations resemble the original embryonic fragment, which suggests that the shape of the embryonic fragments is mainly due to cleavage orientation.
(B) Combination of an isolated AB blastomere (ABa) and a blastomere which touched P₂ transiently (ABp). Four of the eight AB-derived regions of the ABp blastomere (right side of embryonic fragment; blue, violet, dark yellow, and light yellow) elongate comparable to regions in the experiment where
findings may provide a paradigm of how polarity can be organized in a field of cells in other organisms, such as planar cell polarity in *Drosophila* [11,12] or convergent extension [6] and gastrulation [3] in vertebrates.

**Materials and Methods**

**Strains and genetics.** Worms were grown at 25 °C as described [36]. The following strains and mutations were used: N2 Bristol [36]; LG I: mom-7(su193) [20]; mom-5(or57) [21]; unc-13(e1091) [37]; dpy-5(e1). LG II: glp-1(e2144) [38]; LG V: mom-2(e180) (unpublished data); mom-2[21]; unc-24(e138); dpy-11(e224); hls-9(e1487); apx-1[2063] [16]; apx-1(hd87) (unpublished data). Unless noted otherwise, mutations were previously described in [39].

**In vitro culture of blastomeres.** The in vitro culture of embryos was described previously [27]. Embryos were cultured in embryonic growth medium supplemented with egg yolk [23]. Unless noted otherwise, a P2 blastomere was added to two ABx blastomeres (derived from one AB blastomere divided once) in a way that both ABx blastomeres were touched by it. Two ABx blastomeres were used to ensure that cells of the same embryonic stage were added to P2. 4-D microscopy. Methods for 4D-microscopy were described previously [25]. Modifications of the 4D-microscope system are described in [16]. Embryos were recorded at 25 °C.

**Lineage analysis.** The 4D-recordings were analysed using the database SIMI Biocell (SIMI Reality Motion Systems, Unterschleissheim, Germany; http://www.simicom.com) [16,25]. Cells were followed and their 3-D coordinates were saved on average every 2 min. Cell cleavages were assessed by marking the position of the mother cell immediately before the cleavage furrow ingresses and the position of its two daughters three frames (105 s) later. By following every cell in the recording, the complete cell lineage of an embryo or an embryonic fragment is built. We only show 3-D representations of the eighth generation (64 AB descendants), because the elongation index is calculated by dividing the length of the fragment (red line) by the width of the fragment (green line) in the 3-D representation. The elongation index is defined by P2 and the AB-derived blastomere, which is placed farthest from P2. This axis is always assessed at the time point at which the measurement is taken (at the 64-AB cell stage), because P2 and its descendants move during development (due to their and their neighbours’ divisions) and cells orient continuously towards P2 and its descendants.

**Quantitative Analyses**

The elongation index is calculated by dividing the length of the fragment (red line) by the width of the fragment (green line) in the 3-D representation. The 4-D representations at the 64-AB cell stage (eighth generation) were analyzed. All angles were measured from an anterior to a lateral position during development (bent arrow).

**Development of Embryonic Fragments in which Two P2 Blastomeres Were Added to AB-Derived Blastomeres**

(A) P2 overcomes the polarizing activity of EMS descendants. The regions are oriented only towards P2 (Table 1, row I). The EMS descendants move from an anterior to a lateral position during development (bent arrow). (B) Two P2 blastomeres, which were added to the two AB daughters from opposite sites, induce two elongated structures with opposing polarity (white arrows). If the number of cells is increased by using two AB blastomeres, then again two elongated structures form (white arrows). However, the cells which are further away from the P2 blastomeres loop out to form one common axis (red arrow) which indicates, as discussed in the main text, that cells permanently interact to define the a-p axis.

For details see legend to Figure 1.

**Supporting Information**

**Figure S1. Mitoses May Deviate Significantly from the a-p Axis in Normal Embryos**

3-D representation of a 87-cell stage embryo (64 AB descendants, embryo B+ RS; [16]). Anterior is to the left. Colours refer to cell fate as indicated at the bottom. The cell movements (white bars) and mitoses (arrow) of the precursors of four cells (ABarppaap, ABalpapp, ABPraapp, and ABPrppppp) from the 8-cell stage (four AB descendants) onwards are projected into the 3-D representation. Green arrows show anterior divisions; red arrows show divisions which are opposed to the overall movement of the cell. Mitoses are not strictly oriented in the a-p direction but may deviate strongly from the a-p axis as shown in these examples: ABarppaap 45° ± 23°, ABalpapp 50° ± 26°, ABPraapp 61° ± 25°. The average mitosis angle of all AB descendants in this embryo is 41° ± 20° with respect to the a-p axis from the 8-AB to the 64-AB cell stage.

**Figure S2. Development of Embryonic Fragments in which Two P2 Blastomeres Were Added to AB-Derived Blastomeres**

(A) P2 overcomes the polarising activity of EMS descendants. The regions are oriented only towards P2 (Table 1, row I). The EMS descendants move from an anterior to a lateral position during development (bent arrow).

(B) Two P2 blastomeres, which were added to the two AB daughters from opposite sites, induce two elongated structures with opposing polarity (white arrows). If the number of cells is increased by using two AB blastomeres, then again two elongated structures form (white arrows). However, the cells which are further away from the P2 blastomeres loop out to form one common axis (red arrow) which indicates, as discussed in the main text, that cells permanently interact to define the a-p axis.

For details see legend to Figure 1.

**Figure S3. Development of Embryonic Fragments in which Two P2 Blastomeres Were Added to AB-Derived Blastomeres**

(A) P2 overcomes the polarising activity of EMS descendants. The regions are oriented only towards P2 (Table 1, row I). The EMS descendants move from an anterior to a lateral position during development (bent arrow).

(B) Two P2 blastomeres, which were added to the two AB daughters from opposite sites, induce two elongated structures with opposing polarity (white arrows). If the number of cells is increased by using two AB blastomeres, then again two elongated structures form (white arrows). However, the cells which are further away from the P2 blastomeres loop out to form one common axis (red arrow) which indicates, as discussed in the main text, that cells permanently interact to define the a-p axis.

For details see legend to Figure 1.

**Protocol S1. Supplemental Discussion**

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**Author contributions.** MB and RS conceived and designed the experiments. MB performed the experiments. MB and RS analyzed the data. MB and RS wrote the paper.

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