

Evidence from reversal of handedness in *C. elegans* embryos for early cell interactions determining cell fates

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MANY animals with overall bilateral symmetry also exhibit some left-right asymmetries with generally invariant handedness. Therefore, the left-right embryonic axis must have a consistent polarity, whose origins and subsequent effects on development are not understood (reviewed in ref. 1). *Caenorhabditis elegans* exhibits such left-right asymmetries at all developmental stages. The embryonic cell lineage is asymmetric as well: although the animal is generally bilaterally symmetric, many of its contralaterally analogous cells arise from different lineages on the two sides of the embryo^{2,3}. I accomplished reversal of embryonic handedness by micromanipulation at the 6-cell stage, which resulted in mirror-image but otherwise normal development into healthy, fertile animals with all the usual left-right asymmetries reversed. This result demonstrates that in the 6-cell embryo the pair of anterior (AB) blastomeres on the right is equivalent to the pair on the left, and that the extensive differences in fates between lineally homologous derivatives of these cells on the two sides of the animal must be dictated by cell interactions, most of which are likely to occur early in embryogenesis.

The body plan of the *C. elegans* adult hermaphrodite (Fig. 1) is typical for nematodes. There are a few left-right (l-r) asymmetries, but the majority of tissues and cells are arranged with bilateral symmetry⁴⁻⁶. The embryo, by contrast, shows marked l-r asymmetry, becoming only gradually more symmetric as embryogenesis progresses. Handedness seems to be invariant: *C. elegans* embryos with reversed handedness have never been reported.

Bilateral asymmetry first becomes evident between the 4- and 6-cell stages. Left-right polarity cannot be fixed until after dorsal-ventral polarity is established between the 2- and 3-cell stages⁷, because switching the positions of the two AB cells in the 3-cell embryo by micromanipulation reversed the dorsal-ventral axis but did not lead to handedness reversal. The 4-cell embryo is planar (Fig. 2A, B) and apparently bilaterally symmetric. In the next round of cell division, skewing of the l-r cleavages of ABa and ABp results in positioning of the al and pl daughters somewhat anterior to ar and pr, respectively (Figs 2C, 3d). From this stage onward, there are substantial differences between the two sides of the embryo, not only in the positions of lineal homologues but also in the cell lineages by which they produce progeny cells of similar fates on either side of the animal². This is particularly true of the ABa daughters, which give rise to much of the anterior nervous system as well as muscles of the pharynx: ABal and ABar, although generating similar sets of contralateral analogues, do so by quite different lineage patterns^{2,3}.

This piecemeal generation of symmetry by an asymmetric lineage³ could be accomplished in either of two ways: by cell-autonomous (lineal) programming that has simply evolved differently for AB descendants on the two sides of the animal, or alternatively by cell interactions that dictate similar cell fates

in contralaterally equivalent positions. These alternatives should be distinguishable experimentally if the relative anterior-posterior positions of the cell pairs ABal-pl and ABar-pr at the 6-cell stage (Fig. 2C) could be reversed, by moving ABal-pl back and ABar-pr forward, thereby changing the handedness of the embryo. If the l-r differences in cell fates are lineally programmed, then grossly abnormal development should result as the left and right lineages produce their respective patterns of cell fates in the wrong relative anterior-posterior positions. But if l-r differences are dictated by cell interactions, then a mirror-image but otherwise normal lineage might be generated.

Handedness reversal proved possible to accomplish by micromanipulation during cleavage of ABa and ABp as shown in Fig. 3a and b. This procedure reversed the normal skewing of both AB spindles and resulted in ar and pr being anterior to al and pl, respectively, at completion of the cleavage (Fig. 3c).

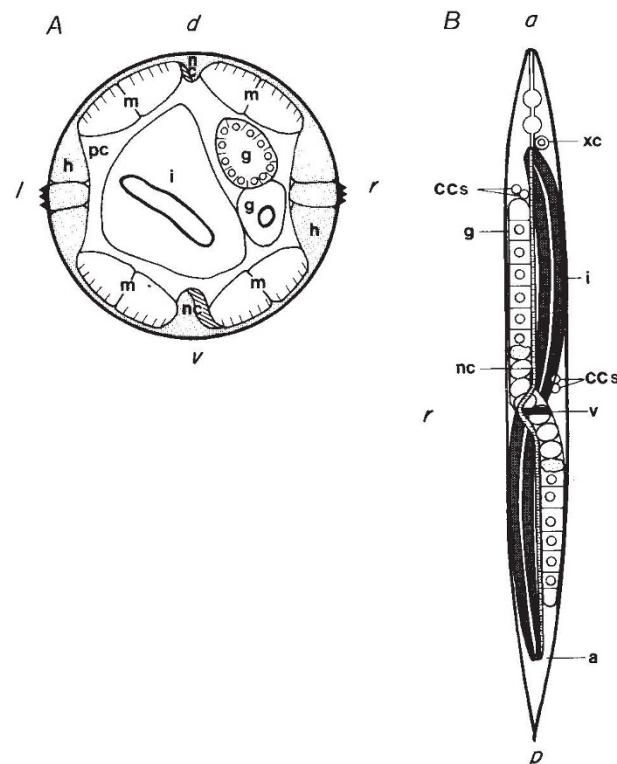


FIG. 1 Bilateral symmetry and l-r asymmetries in the adult *C. elegans* hermaphrodite. A, Cross-section through anterior, between pharynx and uterus, viewed from the rear. B, Ventral view of interior organs. Anterior, posterior, dorsal, ventral, left and right are indicated by a, p, d, v, l and r, respectively. The bilobed gonad (g) and the intestine (i) lie in the pseudocoelom (pc) with the anterior gonad lobe to the right and the posterior lobe to the left of the intestine (A, B), which terminates at the anus (a). The excretory cell nucleus (xc) lies to the left of the midline; the anterior pair of coelomocytes (CCs) is located on the right and the posterior pair on the left of the pseudocoelomic cavity (B). The ventral nerve cord (nc) lies on the midline except that it passes around the vulva (v) on the right side (B). In the ventral nc the cell bodies all lie to the right of the neuronal processes, which run along the left side of the cord; the dorsal nc shows the opposite asymmetry (A)⁵. The gonad and intestine asymmetries can be clearly seen with a dissecting microscope if animals are rolled 90° from their normal orientation (on one side or the other) to ventral- or dorsal-side up (for example, Fig. 3e); the remaining asymmetries were observed under a Zeiss compound microscope equipped with Nomarski optics, in animals mounted¹⁷ ventral-side up. No animals with reversed gonad handedness were seen in examination of more than 2,500 wild-type hermaphrodites reared at 16°. Two hermaphrodites with several reversed asymmetries have been observed, however, by J. Sulston (personal communication) in the course of examining a large number of fixed and stained animals. Animals with reversed asymmetries also have been obtained by the author from embryos treated with chitinase to remove the egg shell and among the progeny of mutagenized populations (unpublished observations).

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The subsequent divisions of the EMS and P₂ cells gave a mirror-image 8-cell embryo (not shown) in which the normal asymmetric positions of non-AB cells (see Fig. 2D) were also reversed, with MS to the left and C to the right of the midline. Reversed embryos underwent subsequent cleavages on schedule and hatched as viable larvae that grew into reversed but fertile adults (Fig. 3e).

The embryonic development of a reversed embryo was observed and recorded for subsequent lineage using a programmable video disc system (J. White, personal communication). Timing of divisions and positioning of specific cells in the reversed embryo were consistent with an enantiomeric but otherwise normal pattern of development, as illustrated by examples from two stages in Fig. 4. In the first-stage (L1) larva that hatched from this embryo, 14 normally asymmetrically placed nuclei⁴ were scored, representing descendants of founder cells AB, MS, C and P₄ (see legend to Fig. 4). All were found in their usual anterior-posterior positions, but on the opposite side of the animal than normally (not shown). The adults that developed from reversed embryos exhibited reversed handedness for all the cells and tissues shown in Fig. 1. These animals were healthy, moved normally and produced normal numbers of self progeny, all of which showed normal l-r asymmetry.

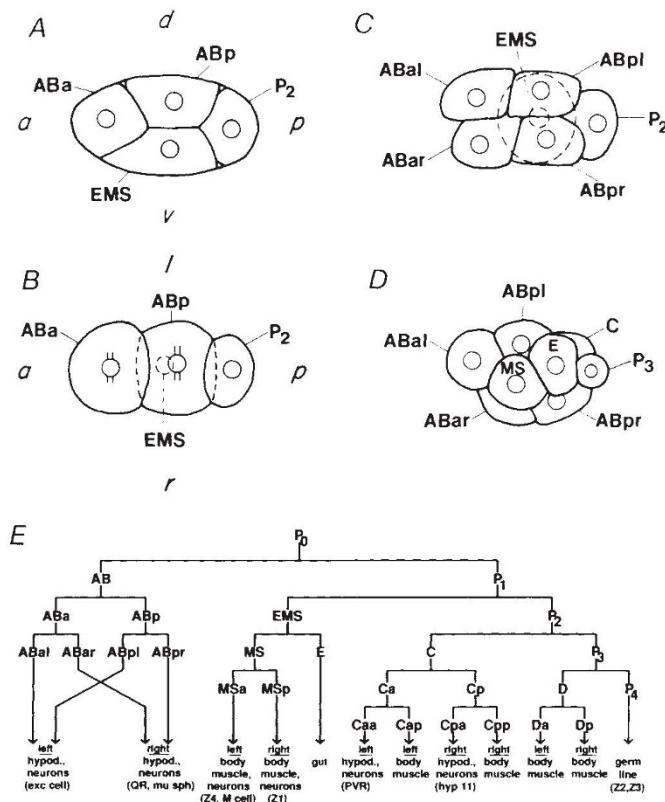


FIG. 2 Cell orientations in 4-cell to 8-cell embryos. **A**, Four-cell embryo, left lateral view (see **E** for explanation of cell nomenclature). **B**, Four-cell embryo, ventral view focused on lower plane. Spindles in ABa and ABp cells are forming perpendicular to the *a-p* axis and will become skewed in an anticlockwise direction as cleavage proceeds. Dashed lines show positions of EMS cell and its nucleus in upper focal plane. **C**, Six-cell embryo, ventral view focused on lower plane. AB cells are arranged asymmetrically as a result of the normal skewing, with ABal and pl daughters somewhat anterior to ABar and pr, respectively. ABal is also somewhat ventral to ABar. **D**, Eight-cell embryo, ventral view. EMS and P₂ have divided, also asymmetrically, so that MS and P₃ are to the right and E and C to the left of the midline. **E**, Lineage diagram of early cleavages showing origins of the six founder cells AB, MS, E, C, D and P₄ and major developmental fates of their progeny. Relative timing of divisions on the vertical axis are not accurately depicted. Progeny of founder cells are named according to their positions following cleavage; for example, ABa is the anterior daughter of AB; ABal is the left daughter of ABa, and so on.

Three significant conclusions can be drawn from these results. (1) Handedness of the embryo at the 6-cell stage determines handedness of subsequent asymmetries throughout development and in the adult. (2) At this stage, ABal must be developmentally equivalent to ABar and ABpl to ABpr; the differences in the fates of these pairs of cells cannot be attributed to intrinsic 'left' and 'right' determinants segregated to the appropriate daughters in the ABa and ABp cleavages. (3) Therefore, the extensive differences in fates of lineal homologues on the two sides of the embryo^{2,3} must be determined by cell interactions, which differ on the left and right because of the asymmetric positioning of AB derivatives relative to each other and to other cells in the embryo.

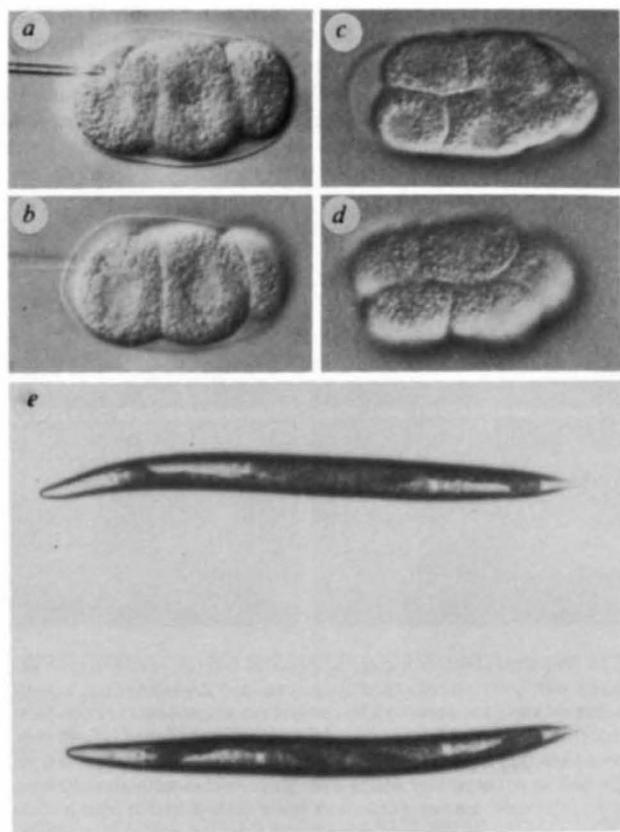


FIG. 3 Handedness reversal: operation and results in embryos and adults. **a**, Nomarski photomicrograph of 4-cell embryo, ventral side up as in Fig. 2B, with microneedle in position for operation (see below). **b**, Same embryo as in **a**, lower focal plane showing ABa nucleus at onset of spindle formation. **c**, Reversed 6-cell embryo following successful operation, focused near lower surface to show cleavage planes optimally. **d**, Normal 6-cell embryo, similar stage and focal plane to **c**. **e**, The reversed (above) and normal (below) adult hermaphrodites that developed from the operated and control embryos shown in **c** and **d**, respectively, viewed ventral-side up. Embryonic handedness reversal, judged by configuration at the 6-cell stage (**c**, **d**), was achieved with a success rate of about 25%, probably depending most critically on timing and orientation of the embryo. All reversed embryos (6/6) developed into reversed but otherwise normal and fertile adults. About a dozen unsuccessfully operated embryos, which appeared as in **d** at the 6-cell stage, were incubated as controls; all developed into fertile adults with normal handedness.

METHODS. *C. elegans* was cultivated and embryos obtained by standard procedures¹⁷. Microneedles were prepared and embryos mounted for micromanipulation according to ref. 7. Embryos were rolled to ventral-side-up at the 4-cell stage, and then downward and rearward pressure was exerted on the left ventral surface of the ABa cell as in **a**, during spindle formation and subsequent cleavage of ABa and ABp. The two adult hermaphrodites (**e**) were lightly anaesthetized in 0.7% phenoxypropanol¹⁷, rolled to ventral-side up on an agar surface, and photographed together using a Wild M400 microscope.

These interactions probably take place early in embryogenesis. The contacts of AB homologues at the 6-cell stage are still equivalent (Fig. 2C), but by the 8-cell stage they are different on the two sides of the embryo (Fig. 2D). In later embryos (>51 cells), laser ablation experiments² provided convincing evidence for cell autonomy of many AB-cell fates. Therefore, most of the determinative interactions are likely to occur between the 8-cell and 51-cell stages.

Conclusion (1) confirms a conjecture put forward nearly a century ago by zur Strassen⁸, who observed that about 2.5% of embryos from the parasitic nematode *Ascaris* normally have reversed I-r asymmetry and postulated that these would give rise to reversed adults. Conclusions (2) and (3) argue against the predominant classical view (see, for example, ref. 9; not shared by all early investigators, such as ref. 10) that nematode embryos are strictly mosaic in their mode of early cell determination. My experiments support the alternative view that *C. elegans* embryos are much more similar to those of vertebrates than previously assumed, in that only some aspects of cell determina-

tion can be attributed to autonomous determinants^{2,11–13}, whereas most others are dictated by intercellular signalling. Earlier evidence on this point came from Schierenberg¹⁴ and in particular Priess and Thomson⁷, who demonstrated equivalence of ABa and ABp at the 4-cell stage and implicated interactions of AB descendants with EMS descendants in the induction of AB-derived pharyngeal muscle cells. Also consistent with this view was the finding¹⁵ that mutations in the *glp-1* gene, which encodes a protein similar to known intercellular signalling components¹⁶, can block induction of AB-derived pharyngeal muscles. The results presented here indicate that extensive cell interactions are required in the early embryo for determination of the many lineal homologues that show I-r differences in developmental fate. □

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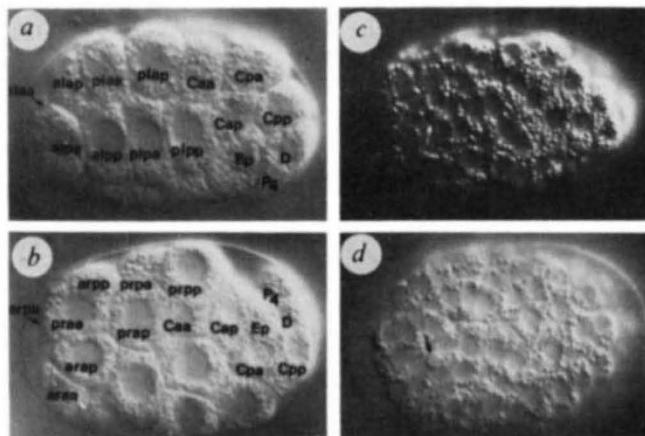


FIG. 4 Nomarski photomicrographs of normal and reversed embryos at two stages, with selected cells labelled. *a, b*, Normal 28-cell embryo, superficial left lateral view, and reversed 28-cell embryo, superficial right lateral view, respectively. Labelled cells with no founder-cell designation are AB descendants. Note that C-cell progeny, normally on the left side in *a*, are on the right side in *b*; conversely, MS cell progeny, which would normally be seen on the right side, are not visible (in a lower focal plane) in both *a* and *b*. *c, d*, Superficial dorsal views of normal and reversed embryos, respectively, at about the 360-cell stage. White arrow in *c* points to ABBarpaapp undergoing programmed cell death (reproduced, with permission, from ref. 2, Fig. 2*d*); the analogous dying cell in *d* was shown by lineage tracing to be ABAlpaaapp (black arrow), which in a normal embryo would give rise to a pharyngeal neuron². Nuclei scored for position in the L1 that hatched from this embryo were as follows. AB descendants: QR (previously Q2; ref. 4), the excretory cell, and the rectal sphincter muscle cell mu sph; MS descendants: Z1 and Z4 in the gonad primordium, the mesoblast M, and the four coelomocytes; C descendants: hyp11 and PVR in the tail; P₄ descendants: Z2 and Z3 in the gonad primordium. The first cleavages of MS and C are predominantly *a-p* in orientation¹⁸, although they also have a I-r component (J. Rothman, personal communication). For both MS and C, the *a* daughters probably give rise to the same cells in reversed embryos (not all lineages were followed) as in normal embryos, though in mirror-image I-r positions (for example, MSa in reversed embryos gives rise to right body muscle and neurons, and also to Z4 and M; compare Fig. 2*E*). The same is true for the *p* daughters of MS and C. Therefore, this experiment cannot distinguish between lineal programming or cell interactions in determining the I-r differences in these lineages.

METHODS. Embryos were mounted as described² and their development was recorded using the 'four-dimension' video recording system (see text), which consists of a Nomarski microscope (Zeiss Axioplan) equipped with a video camera and connected to a computer-controlled optical disc recorder (Sony) and focusing drive motor (Biorad). The system automatically records complete sets of serial optical sections at predetermined intervals and allows subsequent playback of consecutive images from any desired section to facilitate lineage tracing. Photographs were taken from the video monitor.

Requirement for the replication protein SSB in human DNA excision repair

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REPLICATION and repair are essential processes that maintain the continuity of the genetic material. Dissection of simian virus 40 (SV40) DNA replication has resulted in the identification of many eukaryotic replication proteins, but the biochemistry of the multienzyme process of DNA excision repair is less well defined. One protein that is absolutely required for semiconservative replication of SV40 DNA *in vitro* is human single-stranded DNA-binding protein (SSB, also called RF-A and RP-A)^{1–3}. SSB consists of three polypeptides of relative molecular mass 70,000, 34,000 and 13,000, and acts with T antigen and topoisomerases to unwind DNA, allowing the access of other replication proteins. Human SSB can also stimulate the activity of polymerases α and δ , suggesting a further role in elongation during DNA replication^{4–6}. We have now found a role for human SSB in DNA excision repair using a cell-free system that can carry out nucleotide excision repair *in vitro*⁷. Monoclonal antibodies against human SSB caused extensive inhibition of DNA repair in plasmid molecules damaged