Int. J. Dev. Biol. 40: 431-439 (1996)

The use of fluorescent marker dyes for studying intercellular communication in nematode embryos

OLAF BOSSINGER and EINHARD SCHIERENBERG*

Zoologisches Institut der Universität Köln, Köln, Germany

ABSTRACT **As more and more cases of necessary cell-cell interactions are revealed, the classical view of mosaic development in nematodes has to be replaced by a more dynamic picture showing different types of intercellular communication. To investigate the pattern and function of communication pathways between cells, we have developed different techniques to shunt fluorescent marker dyes into embryos and hatched animals and study their distribution *in vivo*. During embryogenesis we find that for a long time all somatic cells form a single dye-coupling compartment while transfer into the germline is restricted already at an early stage. Considerable variations between species with respect to the size of communication channels and the time during which these are functional are observed and can be correlated to differences in the developmental program. A different kind of intercellular communication can be visualized with the help of fluorescent dyes: a transfer of yolk proteins in two phases of the life cycle, in the adult hermaphrodite from the gut into the maturing germ cells, and in the embryo from non-gut cells into the gut primordium. Cell-cell interactions in the nematode embryo can be inhibited with polysulfated hydrocarbon dyes (e.g. Trypan Blue) which bind strongly to the plasma membrane. In summary our data indicate that fluorescent marker dyes can be helpful tools to identify and understand the role of intercellular communication and transfer processes in nematode development.**

KEY WORDS: *pattern formation, soma-germline, gut, micromanipulation, Caenorhabditis elegans*

Introduction

The typical representative of the small free-living soil nematode *Caenorhabditis elegans* is a self-fertilizing hermaphrodite. However, conventional genetics can be performed by mating these with the rare males. Animals and embryos are transparent, so that development can be studied on the cellular level with Nomarski optics (for review see Wood, 1988). *C. elegans* is the only system in which embryonic and postembryonic cell lineages have been described completely (Sulston and Horvitz, 1977; Deppe *et al.*, 1978; Kimble and Hirsh, 1979; Sulston and White, 1980; Sulston *et al.*, 1983). Based on the early studies on parasitic nematodes (Strassen, 1896, 1959; Boveri, 1899, 1910; Stevens, 1909) their embryogenesis has been considered a classical example for mosaic development. Also in *C. elegans* aspects of cell-autonomous early development were found (Laufer *et al.*, 1980; Strome and Wood, 1983; Edgar and McGhee, 1986; Schierenberg, 1988; Junkersdorf and Schierenberg, 1992). However, during recent years increasing evidence has appeared that on top of cell-autonomous decisions various early cell-cell interactions take place in *C. elegans* during early stages of development to modulate the underlying basic program of cell specification (Schnabel, 1995). Mutants have been isolated in which either the correct segregation of cytoplasmic factors or potential steps in the signalling pathway are affected (Kemphues *et al.*, 1988; Mello *et al.*, 1992). We are just beginning to understand what kind of signals are involved and how signal transduction is accomplished in *C. elegans* (Bowerman *et al.*, 1992; 1993; Evans *et al.*, 1994; Mango *et al.*, 1994; Tax *et al.*, 1994).

As a complementary assay to the study of mutants, direct manipulation of blastomeres has proven to be particularly useful to identify cell-cell interactions in the *C. elegans* embryo. Removal and recombination of blastomeres revealed induction of the gut precursor by the neighboring germ line cell (Schierenberg, 1987; Goldstein, 1992; 1995a,b). Switching the position of certain cells let to a concomitant switch in their differentiation program (Priess and Thomson, 1987; Wood, 1991). Thus, those cells must have been originally equivalent in their developmental potential and depend on positional cues to become different from each other. Based on ablations with a laser microbeam and lineage analysis of the remaining blastomeres it has been suggested that sequential induction steps have to take place to establish asymmetries along the embryonic axes (Hutter and Schnabel, 1994, 1995a,b).

Here we describe the use of fluorescent marker dyes in combination with new micromanipulation methods and contrast-

***Address for reprints:** Zoologisches Institut, Universität Köln, Kerpenerstr. 15, 50923 Köln, Germany. FAX: 49.221.4704987. e-mail: eschier@biolan.uni-koeln.de

0214-6282/96/$03.00
© UBC Press
Printed in Spain

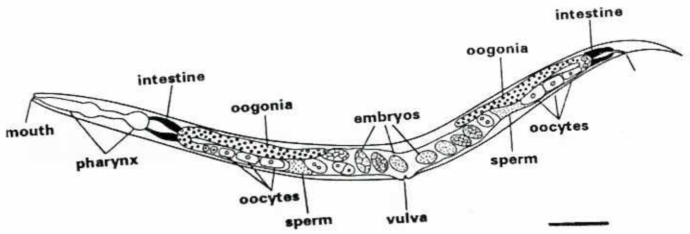

Fig. 1. Adult hermaphrodite of *C. elegans*. *Scheme showing major anatomical features. Bar, 200 µm.*

enhanced microscopy, which allows *in vivo* visualization of intercellular communication pathways, the observation of transfer processes in individual specimens from early embryogenesis onward up to adulthood and the analysis of development after experimental inhibition of cell-cell interactions.

Results

Intercellular communication pathways in nematode embryos

Background

Different pathways of signal transduction can be imagined: (1) release of developmental signals by cells in one region of the embryo and transfer through the intercellular space to distant target sites; (2) a ligand/receptor mechanism between two adjacent membranes; and (3) transfer of signal molecules through communication channels, such as gap junctions (GJ). Studies using ions or dyes of known molecular size and charge demonstrated that GJ allow the intercellular exchange of molecules up to 1,000-3,000 Da, depending on the species studied (Flagg-Newton *et al.*, 1979; Finbow and Pitts, 1981; Berdan, 1987). The importance of gap junctional communication for normal development has been documented in a number of different systems (Green, 1988; Warner, 1992). As little is known about this mode of communication in nematodes, we have begun to perform dye-coupling studies in *C. elegans* and related species. Here we will take into consideration just one of them, *Cephalobus spec.* Its embryogenesis differs considerably from that in *C. elegans* in that it develops several times slower and shows an altered division sequence and spatial arrangement of cells (Skiba and Schierenberg, 1992). We wondered whether these differences in the developmental pattern go along with a modified pattern of cell-cell communication.

Distribution of introduced marker dyes can be followed in vivo in the developing embryo

The nematode embryo is surrounded by a prominent chitinous eggshell and an underlying thin vitelline membrane, which is impermeable for most chemicals. One dye which can pass the vitelline membrane is Neutral Red (NR). Incubation of embryos in a NR solution for a few minutes is sufficient to strongly mark all cells (Fig. 3A). As NR eventually accumulates in the lysosomes of the gut primordium, it can be used as a differentiation marker.

More refined techniques are necessary to obtain stage- and cell-specific *in vivo* staining of the embryo. With the help of a laser microbeam the shell and vitelline membrane can be penetrated at pre-selected time points during embryogenesis to allow the entry of substances into the perivitelline space (Bossinger and Schierenberg, 1992b; Schierenberg and Junkersdorf, 1992). Dyes may either accumulate in the nuclei (Fig. 3B), bind strongly to cell membranes and mark their contours (Fig. 3C), just surround the blastomeres without any apparent binding (Fig. 3D), or pass, like NR, through the cell membranes into the blastomeres (e.g. Acridin Orange, Rhodamin 6G). We found that with the technique of laser-induced penetration substances up to about Mr 90,000 can be shunted into the nematode egg. Larger molecules have to be microinjected which is more difficult but allows labeling of individual cells (Fig. 7B).

When studied under the fluorescence microscope marker dyes may bleach out very rapidly not allowing conventional documentation even with high-speed film. In addition, repeated irradiation – even if only for a few seconds- often damages specimens leading to immediate or later developmental arrest. With video recording as described here, exposure times of 1-2 seconds are sufficient for proper documentation of fluorescent study objects without affecting normal development.

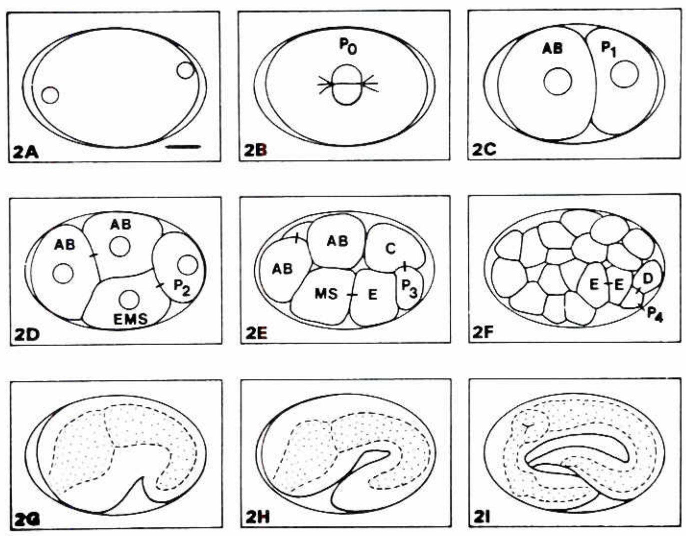

Fig. 2. Embryonic development of *C. elegans*. (A) *Two pronuclei at opposite poles.* **(B)** *Both pronuclei have moved to the center, rotated through 90° and fuse to form the zygote, P_0.* **(C)** *The unequal division of the zygote has generated a larger somatic cell AB (precursor for predominantly nervous system, pharynx, hypodermis) and a germline cell P1.* **(D)** *4-cell stage. AB has divided equally into 2 AB cells; P1 has given rise to a larger somatic cell EMS and a smaller germline cell P2.* **(E)** *8-cell stage. 4 AB cells are present, EMS has generated MS (predominantly body muscle and pharynx) and E (intestine), P2 has divided into C (predominantly hypodermis and body muscles) and a new germline cell P3.* **(F)** *28-cell stage. The last unequal germline division has generated a somatic cell D (body muscles) and the primordial germ cell P4. Gastrulation has started with the immigration of both E cells* **(G)** *About 6 h after* **(A)** *the embryo has essentially completed its embryonic division program (end of the "proliferation phase") and has entered the "morphogenesis phase" with a ventral indentation ("comma stage"; dotted area marks alimentary tract with emerging pharynx and gut).* **(H)** *Advanced morphogenesis stage ("tadpole stage").* **(I)** *About 12 h after* **(A)**. *Rotating vermiform stage prior to hatching ("pretzel stage"). Orientation: anterior, left. Bar, 10 µm (modified after Schlicht and Schierenberg, 1991).*

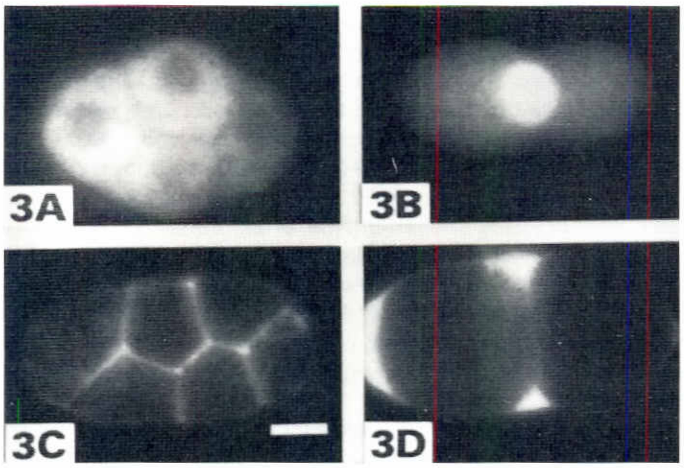

Fig. 3. Different staining properties of marker dyes in the nematode embryo. **(A)** *After incubation with Neutral Red (Mr 289) within a few minutes the lipophilic dye has crossed the eggshell, underlying vitelline membrane, and the cell membrane and marks the cytoplasm of the embryo.* **(B)** *After microinjection of RITC-dextran (Mr 10,000) the dye accumulates in the nucleus.* **(C)** *After laser-induced penetration of the eggshell in a medium containing Trypan Blue (Mr 960) the dye marks cell membranes.* **(D)** *After laser-induced penetration of the eggshell Lucifer Yellow (Mr 457) fills the perivitelline space of an early embryo without entering cells. Epifluorescence, excitation wavelength: 520-560 nm (a,b,c); 436 nm (d). Orientation: anterior, left. Bar, 10 μm.*

Communication channels coupling early blastomeres show different properties

We injected Lucifer Yellow (LY) or RITC Dextran (DE) into early blastomeres of *C. elegans* and *Cephalobus* (Bossinger and Schierenberg, 1992, 1996a). While LY is a widely used marker for the presence of gap junctions (Stewart, 1981), the passage of DE from one cell to another indicates the presence of cytoplasmic bridges or midbodies (Cartwright and Arnold, 1980; Mahajan-Miklos and Cooley, 1994). After an initial delay (Fig. 4B), from the 4-cell stage onwards until the end of the proliferation phase (see Fig. 2) LY quickly diffuses into all somatic blastomeres (Figs. 4C,D, 5D). DE, however, after injection into one blastomere of a 2-cell stage (after completed cytokinesis) always remains restricted to the descendants of this cell (Fig. 4H-J). In contrast to the findings in *C. elegans*, in *Cephalobus* even high molecular weight DE can spread from one somatic cell to another but never enters the germline (Fig. 4K-O). Thus, with respect to dye transfer, from the very beginning germ cells in *Cephalobus* are different from the soma, while in *C. elegans* we observed this phenomenon only at a later time (see below).

The germline forms the first tissue-specific communication compartment

From the 4-cell stage onwards all blastomeres of the early *C. elegans* and *Cephalobus* embryo are dye-coupled (Fig. 5B,D). However, this uniform behavior is lost with the beginning of gastrulation (about 26 cells). In *Cephalobus* the primordial germ cell P4 becomes irreversibly uncoupled from the rest of the embryo at this time (Fig. 5D). In *C. elegans* (with some delay) P4 still incorporates the marker dye and only its daughters are uncoupled from the soma at a much later stage (about 550 cells, Fig. 5B; Bossinger and Schierenberg, 1992a). Nevertheless, in *C. elegans* as well as in *Cephalobus*, the germline forms the first tissue-specific communication compartment, even though at different developmental times.

Somatic communication compartments form at different phases of development in different nematode species

In *C. elegans* all somatic cells of the embryo are still dye-coupled at the beginning of the morphogenesis phase (550 cells;

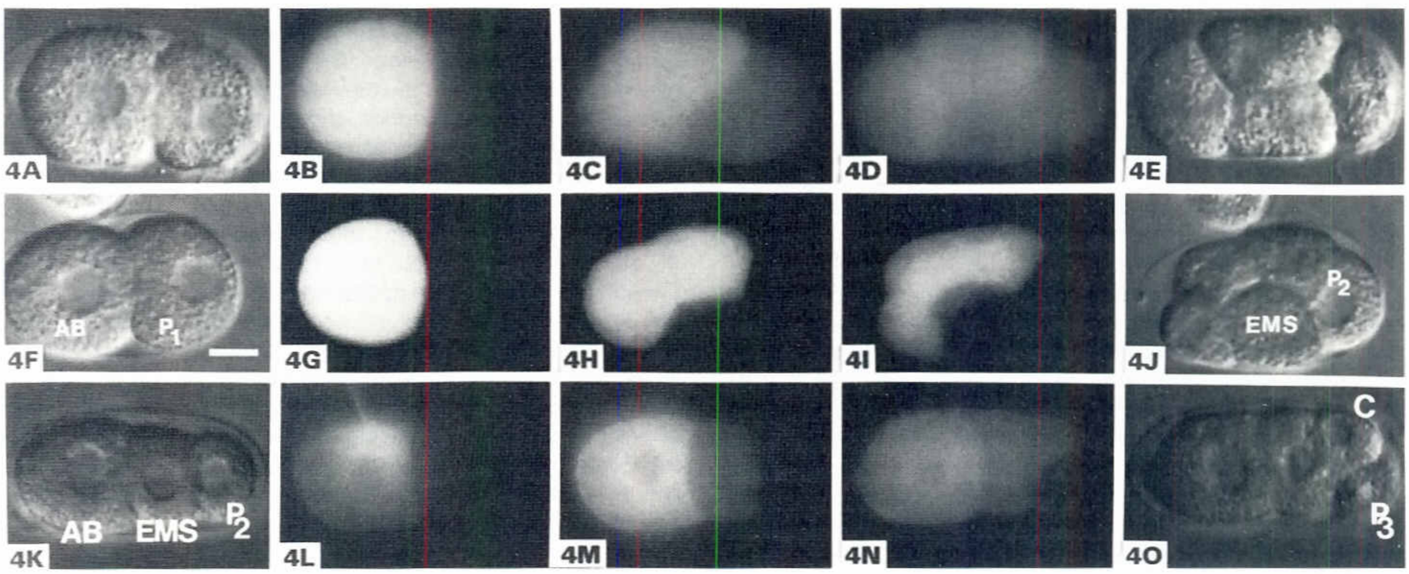

Fig. 4. Pattern of dye-coupling in early embryos of two different nematode species. **(A-E)** *In the C. elegans embryo from the injected AB cell Lucifer Yellow spreads into the other cells.* **(F-J)** *In C. elegans RITC-dextran (Mr 10,000) remains restricted to the injected AB-cell and its descendants.* **(K-O)** *In the Cephalobus embryo RITC-dextran (Mr 70,000; note exclusion from nuclei) spreads from the injected AB cell into EMS but not into P2 and later into C but not into P3 . Left column, Nomarski optics, same stages as B,G,L. Right column, Nomarski optics, same stages as* **(D,I,N)**. *Center columns, epifluorescence. Orientation: anterior, left. Bar, 10 μm.*

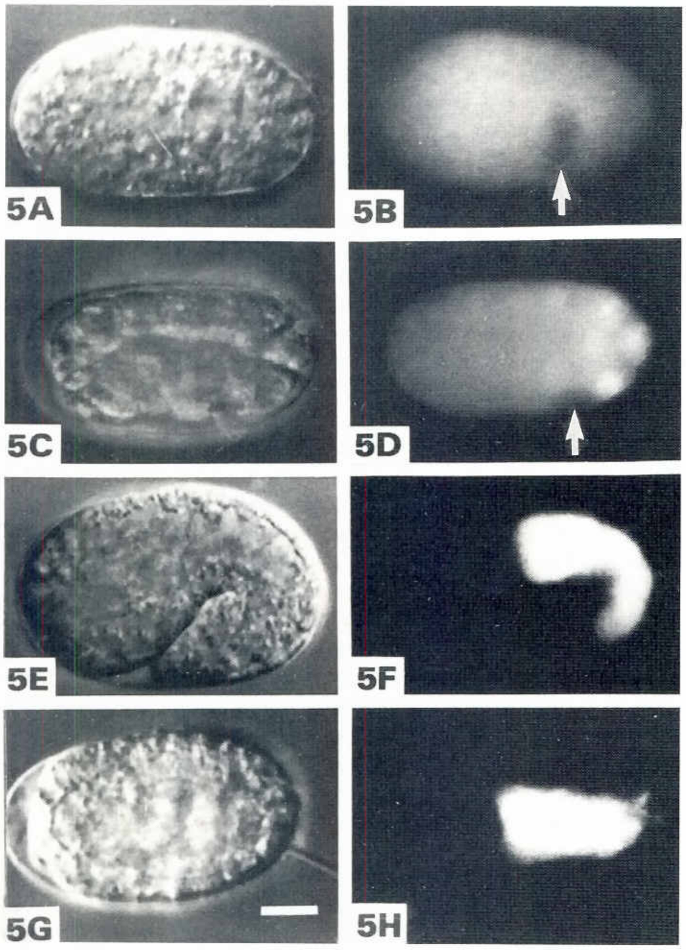

Fig. 5. Establishment of communication compartments in nematode embryos. (A,B) *In C. elegans both daughters of the primordial germ cell P4 get excluded from dye-coupling during the early morphogenesis phase (for developmental stages, see Fig. 2) while all somatic cells still form a single compartment.* **(C,D)** *In Cephalobus P4 (arrow) is already excluded at the beginning of gastrulation.* **(E,F)** *In C. elegans the gut primordium forms a dye-coupling compartment during the advanced morphogenesis phase.* **(G,H)** *In Cephalobus the gut primordium is already uncoupled in the middle of the proliferation phase. Left, Nomarski optics. Right, epifluorescence. Orientation: anterior, left. Bar, 10 µm.*

Fig. 5B). During the following hours we observe the stepwise establishment of different somatic dye-coupling compartments. In the case of the alimentary tract, first a pharynx-gut compartment forms, later the pharynx becomes separated from the gut (Fig. 5F) , and subsequently the pharynx is further subdivided into an anterior and a posterior compartment (Bossinger and Schierenberg, 1992a). As for the germline also somatic dye-coupling compartments appear to be established considerably earlier in the *Cephalobus* embryo, e.g. the gut primordium forms a tissue-specific compartment already around the 200-cell stage (Fig. 5H).

Conclusions

The communication channels we have visualized here represent one of several potential ways of signal transduction (see above). In the early nematode embryo communication channels may not be a means for early inductive cell-cell interactions as at least all somatic cells are equally well dye-coupled. The few well enough studied cases suggest that there receptor/ligand mechanisms acting on the cell surface are involved (Roehl and Kimble, 1993; Evans *et al.*, 1994). Therefore, the communication channels visualized here may serve other functions, such as metabolic coupling (Subak-Sharpe *et al.*, 1966). On the other hand we observed that early blastomeres can acquire the potential to differentially accumulate certain marker dyes which initially had been equally distributed during the first cleavage steps (Bossinger and Schierenberg, 1996a). It remains to be tested whether this may reflect a segregation of cytoplasmic components after cell division.

Injection of suitable antibodies into the early embryo which interfere with proper function of GJ as successfully used in other systems (Serras *et al.*, 1988; Bohrmann and Haas-Assenbaum, 1993) and the analysis of mutants with an altered communication pattern should help to better understand function and structure of communication channels in nematodes.

More straightforward is the interpretation of the (variably) early separation of the germline from the soma in all nematodes we have studied. It has been suggested previously that a separation of germline from soma is necessary to preserve germline potential (King and Beams, 1938; Schierenberg, 1985, 1988; Schlicht and Schierenberg, 1991). Our present data support such a model. Further studies will determine whether the communication channels in *Cephalobus* show more than a superficial similarity to ring channels in the insect gonad, which can be modulated in their diameter (Xue and Cooley, 1993; Robinson *et al.*, 1994).

Early embryonic induction in C. elegans can be inhibited by polysulfated hydrocarbon dyes

During embryogenesis of *C. elegans* cellular interactions are necessary to determine the fate of blastomeres. In one of these, taking place in the 4-cell stage, the germline cell P2 induces longitudinal orientation of the cleavage spindle in the neighboring EMS cell, its unequal division and consequently the establishment of a gut lineage (Schierenberg, 1987; Goldstein, 1992). Another one, the induction of AB-derived pharyngeal muscle cells in the 12-cell stage by MS (Hutter and Schnabel, 1994) appears to involve a surface receptor/ligand interaction (Evans *et al.*, 1994). Application of polysulfated hydrocarbon dyes (Trypan Blue, Evans Blue or Chicago Sky Blue) in the 4- and 12-cell stages following laser-induced penetration of the eggshell inhibits both interactions (Bossinger and Schierenberg, 1996b). In the first case this leads to a transverse division of EMS. In contrast to normal development (see Fig. 2) both daughter cells of EMS behave like MS cells (pharynx and body muscles) and the E-fate (gut) is completely suppressed. In the second case only the pharynx muscle cells coming from MS but not those from AB are generated.

Yolk transfer during nematode development

Background

The gut in nematodes serves several functions including the synthesis of yolk. Here we want to focus on the fate of yolk because this gives a different kind of example for intercellular communication and shows that fluorescent marker dyes can trace the pathway of an essential component during the whole life cycle. In *C. elegans* four major yolk proteins are found, encoded by a single gene family (Sharrock, 1983; Blumenthal *et al.*, 1984). A comparison of proteins synthesized by dissected tissues identified the intestine as the primary site of yolk synthesis. It has been proposed that yolk proteins in *C. elegans* are secreted from the intestine into the body cavity, and taken up from there by the oocytes (Kimble and Sharrock, 1983).

The transfer of yolk from the intestine into to the oocytes can be followed in vivo

In other systems it has been shown that yolk proteins can be fluorescently labeled with Lucifer dyes (Danilchik and Gerhart, 1987; Opresko and Karpf, 1987). We confirmed this for *C. elegans* by studying the pattern of fluorescence in stratified embryos (Fig. 6G,H) and by comparing the staining pattern of LY with that of an antibody against yolk proteins (Fig. 7I,J). After feeding worms with Lucifer Yellow (LY) we followed the pattern of fluorescence *in vivo* focusing on the different phases of transport. First, LY elevates the fluorescence in the intestinal cells (Fig. 6B). Soon afterwards we get a signal from the oocyte membrane (Fig. 6D) and then fluorescence appears in the cytoplasm of the oocytes. With increasing maturity oocytes show stronger fluorescence (Fig. 6F), reflecting the massive incorporation of yolk. Our observations indicate that yolk transfer is a very rapid process taking only a few minutes.

During embryogenesis yolk accumulates in the gut primordium

After feeding worms with Lucifer Yellow (LY) or microinjection into selected blastomeres, the dye remains visible in the cytoplasm all through embryogenesis. If injected for instance in the AB cell (no gut precursor; see, Fig. 2) of a 2-cell stage, LY quickly binds to yolk granules and remains in the descendants of the injected cell (Fig. 7B,D). Towards the end of the proliferation phase, however, the LY-induced fluorescence increases progressively in the differentiating gut primordium, while disappearing correspondingly from the descendants of the injected cell (Fig. 7F). Well before hatching, LY is essentially restricted to the gut (Fig. 7H). A similar pattern is found after immunostaining with an antibody against yolk proteins (Fig. 7I,J). Our observations suggest that an intercellular transfer of yolk from non-gut cells into the differentiating gut primordium takes place during embryogenesis of *C. elegans* rather than a consumption of yolk in all non-gut cells. This view is supported by our observation that the volume of the gut primordium increases considerably during embryogenesis while the embryo itself does not grow (Bossinger and Schierenberg, 1992b). To further characterize the pathway of yolk into the gut primordium, we penetrated the normally impermeable vitelline layer with a laser microbeam in a medium containing LY. If done in early stages, the dye fills the perivitelline space and surrounds the blastomeres but does not enter the cells (Fig. 8B,D). However, at later stages LY is specifically taken up by the cells of the gut primordium (Fig. 8F). The observed uptake is obviously not a general unspecific process. We found no uptake of dextran (as a marker for pinocytosis) but rapid internalization of transferrin molecules (as a marker for receptor-coupled endocytosis). Thus, gut cells appear to express an intense endocytotic activity as part of their tissue-specific differentiation (Bossinger *et al.*, 1996). We found that also this receptor-coupled endocytosis can be blocked by different inhibitors like Trypan Blue and chlorpromazine (Röhrkasten and Ferenz, 1987; Wang *et al.*, 1993).

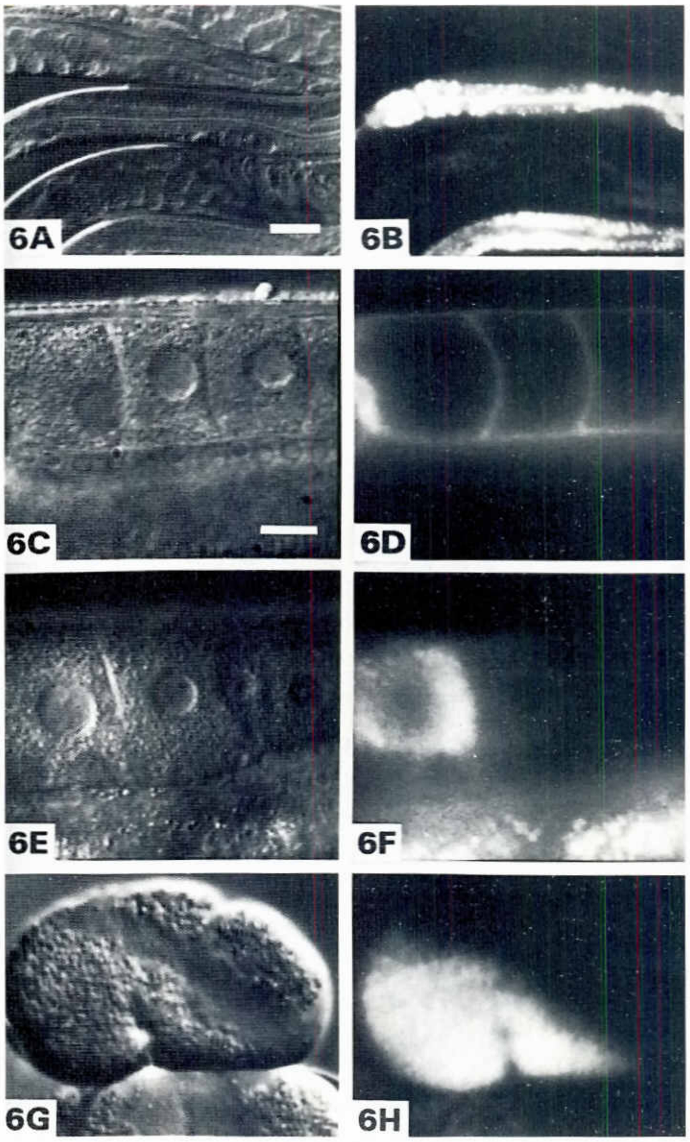

Fig. 6. From the mother to the embryo: the pathway of fluorescently labeled yolk. (A,B) *After feeding worms with Lucifer Yellow (LY VS) the gut expresses a strong fluorescence while control specimens show only weak autofluorescence.* **(C,D)** *LY binds to cell membranes of oocytes.* **(E,F)** *LY is taken up differentially into the cytoplasm of oocytes depending on their degree of maturation. Fluorescence in the lower part marks the gut.* **(G,H)** *After centrifugation (orientation: bottom, centrifugal pole; top, centripetal pole) of an early embryo LY is exclusively associated with the fraction of yolk granules. Left, Normarski optics. Right, epifluorescence. Bars: 100 µm (A,B); 10 µm (C-H).*

Conclusions

Based on our findings reported above we suggest that the inferred transfer of yolk in nematode embryos is accomplished in two steps: (1) exocytosis from non-gut cells into the perivitelline

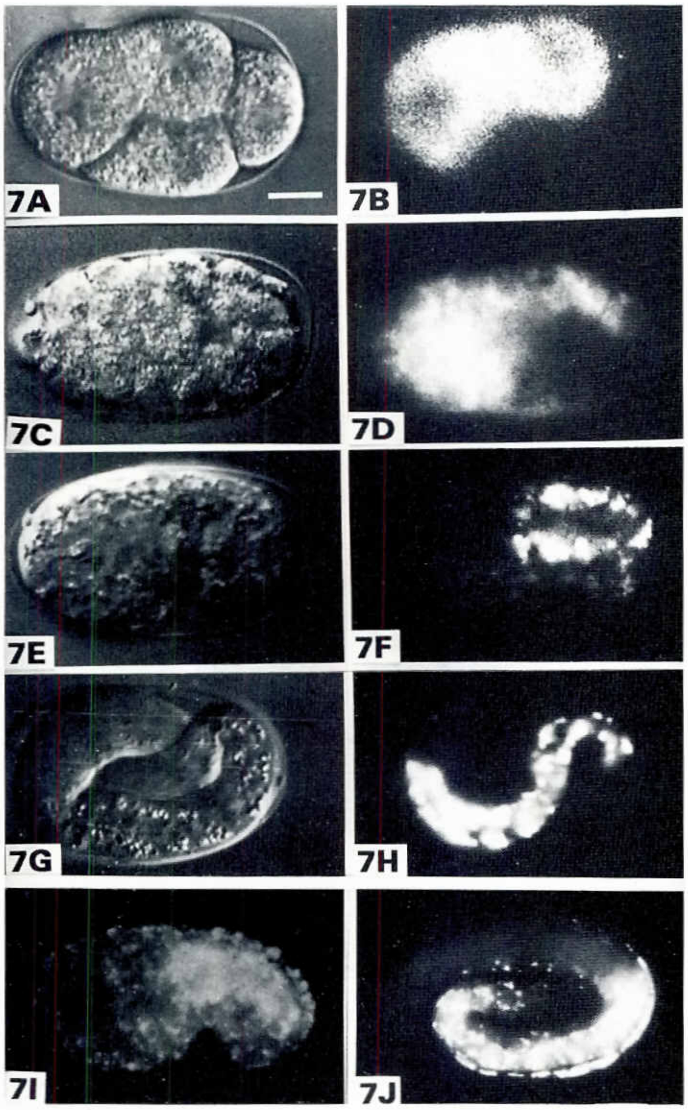

Fig. 7. From non-gut cells into the gut primordium: the way of fluorescently labeled yolk during embryogenesis of *C. elegans*. (A-D) *After injection of Lucifer Yellow into the AB cell of a 2-cell embryo, the dye segregates only to the descendants of the AB cell.* **(E-H)** *During the morphogenesis phase the dye accumulates in the intestine and disappears from the AB descendants.* **(I,J)** *An antibody against yolk proteins marks the gut regions of an early and a late morphogenesis stage.* **(A,C,E,G)** *Nomarski optics.* **(B,D,F,H,I,J)** *Epifluorescence. Orientation: anterior, left. Bar, 10 µm (modified after Bossinger and Schierenberg, 1992a).*

space and (2) endocytosis into the intestine. In various species, yolk is found to be concentrated in the vegetal region of the uncleaved egg (Balinsky, 1975) or is segregated into the large vegetal macromeres which generate the gut (Wilson, 1892; Dorresteijn, 1990). The fact that different mechanisms (prelocalization, segregation, transfer) lead to an accumulation of yolk in the gut primordium points to a general developmental principle. Our observations that a high percentage of embryonic yolk is still present in the intestine of the hatched *C. elegans* juvenile but is lost after starving indicates a function as an energy source for postembryonic survival and development under adverse conditions.

Discussion

We have shown that fluorescent dyes can not only mark various structures in the nematode worm and embryo, but in addition allow us to follow intercellular transfer processes. Our observations together with those demonstrating inductive interactions (see above) indicate that nematodes are much more dynamic systems than it was imagined in the past. Communication pathways are activated and closed during specific phases of development allowing transport in and out of cells. So far, we do not know much about the developmental significance of transfer processes during nematode development and the underlying mechanisms (Spieth *et al.,* 1988). Therefore, we attempt to suppress the function of communication channels with drugs (Ramon and Rivera, 1986) and study subsequent development. The detailed knowledge of the division and differentiation program in *C. elegans* permits detection of even subtle abnormalities. We have also started to examine mutants in which soma-germline differentiation is defective (Strome *et al.,* 1995) and our preliminary results indicate that at least in some of these the typical early dye exclusion of the germline is lost. The intestine of nematodes, though simple in structure, is quite complex in function. It not only digests food, but also appears to take up and release metabolites, and to permanently store waste products (Davis *et al.,* 1982). Thus, this organ may turn out to be the central part in a communication system connecting different cells or tissues. To further study the pathway of substances in and out of the gut we have started to screen for mutants which are not able to properly transfer the fluorescently marked yolk during embryonic or postembryonic development.

We found distinct variations with respect to intercellular communication between different nematode species. A comparative assay correlating this pattern with other parameters like cell lineage and necessary interactions revealed by blastomere isolations and inhibition experiments may help us to better appraise the significance of such differences for development and give additional clues for the delineation of phylogenetic relationships among nematodes.

Materials and Methods

Nematode strains and maintenance

C. elegans (var. Bristol) strain N2, and *Cephalobus spec.* (laboratory designation: ES 501) isolated from soil probes (Skiba and Schierenberg, 1992) were raised on nutrient agar plates with a uracil-deficient strain of *E. coli* (OP 50) as a food source at 16-25°C, essentially as described by Brenner (1974).

Microscopy

C. elegans eggs were dissected out of gravid adults with a scalpel in a drop of distilled water on a microscope slide. Embryos of *Cephalobus* (which are laid before first cleavage) were rinsed directly from agar plates. Early embryos were identified under the dissecting microscope and transferred with a drawn-out glass mouth pipette to a second microscope slide or a coverslip (for microinjection, see below), where they were stuck to a thin polylysine layer (Cole and Schierenberg, 1986). Development of embryos was observed with Nomarski optics and epi-illumination to visualize fluorescent dyes (excitation: 340-380 nm, 436 nm, 520-560 nm; barrier: 415 nm, 490 nm, 580 nm) using a Leitz Diavert microscope equipped with an Olympus 40x APO UV oil-immersion objective.

Centrifugation

One-cell embryos were transferred into a drop of distilled water on a polylysine-coated microscope slide. While slowly settling down they can be oriented with the drawn-out tip of the transfer pipette. Alternatively, embryos were stuck in random orientation and appropriate specimens were selected afterwards. As unprotected microscope slides easily break in the centrifuge, Schlicht and Schierenberg (1991) designed a holder which prevents damage of the slide. The plastic holder sitting in the metal centrifuge beaker was filled with culture medium and then the slide was placed into the slot. Embryos were centrifuged for 15 min at 16000g in a swing-out rotor. The temperature during centrifugation was about 12°C. After centrifugation embryos attached to the microscope slides were covered with a coverslip sealed with Vaseline.

Introduction of marker dyes into embryos or animals

Feeding animals

Specimens were either incubated with the dye on nutrient agar plates overnight or in cell culture medium complemented with the dye and a low concentration of *E. coli* as a food source for a few hours at room temperature. In both cases the dye was taken up orally together with the bacteria. Animals were exposed to the following final concentrations: Rhodamin 6G (10^{-5}%, Serva, Mr 477), Acridin Orange (1×10^{-4}%, Merck, Mr 302), Neutral Red (5×10^{-3}%, Riedel de Haen, Mr 289), Lucifer Yellow VS (1×10^{-2}%, Sigma, Mr 550).

Laser microsurgery

Embryos were attached to a microscope slide as described above and briefly preincubated with a Trypan Blue solution (14 mg/ml, Mr 961, Serva). The dye adheres to the eggshell and allows absorption of the laser microbeam by the normally transparent eggshell. Then the Trypan Blue solution was replaced by cell culture medium containing 1% of Lucifer Yellow VS (Mr 550, Sigma). With a N2-pumped dye laser (Lambda Physics, Göttingen, Germany) coupled to a microscope the eggshell and underlying vitelline membrane were perforated with brief pulses using the laser dye Rhodamine 6G (Laufer and von Ehrenstein, 1981; Bossinger and Schierenberg, 1992b) to allow entry of marker dyes. After 15 minutes, the LY-containing medium was exchanged for the same medium without dye. No LY leaked out from the perivitelline space after that, probably because the vitelline membrane had resealed.

Microinjection

After mounting embryos were first covered with a drop of cell culture medium (Cole and Schierenberg, 1986) and then sealed with a drop of fluorocarbon oil to avoid evaporation while permitting oxygen diffusion. Penetration of embryos was performed with a piezotranslator (PM10, Bachofer, Reutlingen). The dyes were delivered into cells with a microelectrode-amplifier (L/M-1, List-electronic, Darmstadt, Germany). Capillaries with an inner filament (GC120F10, Science Products, Frankfurt, Germany) were pulled on a microelectrode puller (Brown-Flaming P-97A, Sutter Instruments, San Francisco, USA). The microelectrode was backfilled with a 3-5% aqueous solution of the dyes (dye-coupling studies: Lucifer Yellow CH, Mr 457 and RITC-Dextran, Mr 10,000; 70,000; Sigma; yolk labeling: Lucifer Yellow VS, Mr 550; Sigma). A platinum wire was used as reference electrode and placed into the cell culture medium surrounding the embryo. Dye was injected under video observation using a 0,5-2 nA hyper- or hypopolarizing constant current depending on the charge of the dyes. The amount of injected dye was monitored using a remote-controlled shutter between the mercury bulb and the microscope to minimize harmful irradiation of the embryos. As the amount of injected dye is very critical for normal further development, only minimal amounts were injected, such that fluorescence became clearly visible after electronic enhancement (see below).

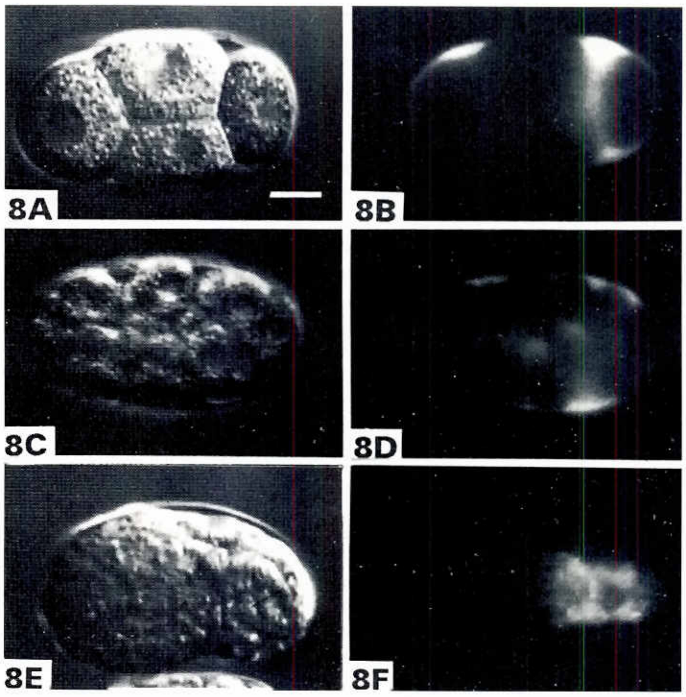

Fig. 8. Stage-dependent uptake of Lucifer Yellow from the perivitelline space into the gut primordium of *C. elegans*. (A-D) *After laser-induced penetration Lucifer Yellow surrounds the cells of the early embryo.* **(E,F)** *During the morphogenesis phase LY disappears from the perivitelline space and accumulates in the gut primordium. Left, Nomarski optics. Right: epifluorescence. Orientation: anterior, left. Bar, 10 μm (modified after Bossinger and Schierenberg, 1992a).*

Immunostaining

Egg preparations were made as described for microscopy and microscope slides frozen in liquid nitrogen. Afterwards the coverslip was removed and the sticking frozen embryos fixed with methanol (10 min) and acetone (20 min). Embryos were incubated for 2 h at room temperature with primary antibodies OICI and PIIA3 against *C. elegans* yolk proteins (kindly provided by S. Strome, Bloomington) and then by an FITC-labeled secondary antibody for at least 2 h, following the procedure given in Wood (1988).

Video recording, electronic enhancement and documentation

Injected embryos were recorded on a time lapse video recorder (Panasonic AG-6720-E) with a video camera (Panasonic WV-BC700 with infrared filter removed). After activation of the electronic "Sensitivity Up" mode of the camera control unit (Panasonic WV-CU204), the appropriate level of sensitivity was selected (accumulation of 2-32 frames). With an image processor (Hamamatsu, Argus-10) the quality was further improved. To document localization of fluorescent dyes, the embryos were exposed to brief fluorescence excitation and recorded simultaneously. Selected images of the recorded specimens were either photographed from the video screen at shutter speed 0.25 sec or printed directly with a video copy processor (Mitsubishi, P66E).

Acknowledgments

We thank Elisabeth Troijtza and Renate Seiler for technical help. The studies reported here were supported by grants of the Deutsche Forschungsgemeinschaft to E.S.

References

BALINSKY, B.I. (1975). *An Introduction to Embryology.* W.B. Saunders, Philadelphia.

BERDAN, R.C. (1987). Intercellular communication in arthropods: biophysical, ultrastructural, and biochemical approaches. In *Cell-to-cell Communication* (Ed. W.C. De Mello). Plenum Press, New York, pp. 299-370.

BLUMENTHAL, T., SQUIRE, M., KIRTLAND, S., CANE, J. DONEGAN, M., SPIETH, J. and SHARROCK, W. (1984). Cloning of a yolk protein gene family from *Caenorhabditis elegans. J. Mol. Biol. 174:* 1-18.

BOHRMANN, J. and HAAS-ASSENBAUM, A. (1992). Gap junctions in ovarian follicles of *Drosophila melanogaster*: inhibition and promotion of dye-coupling between oocyte and follicle cells. *Cell Tissue Res. 273:* 163-173

BOSSINGER, O. and SCHIERENBERG, E. (1992a). Cell-cell communication in the embryo of *Caenorhabditis elegans. Dev. Biol. 151:* 401-409.

BOSSINGER, O. and SCHIERENBERG, E. (1992b). Transfer and tissue-specific accumulation of cytoplasmic components in embryos of *Caenorhabditis elegans* and *Rhabditis dolichura: in vivo* analysis with a low-cost signal enhancement device. *Development 114:* 317-330.

BOSSINGER, O. and SCHIERENBERG, E. (1996a). Cell-cell communication in nematode embryos: Differences between *Cephalobus spec.* and *Caenorhabditis elegans. Dev. Genes Evol.* (In press).

BOSSINGER, O. and SCHIERENBERG, E. (1996b). Early embryonic induction in *C. elegans* can be inhibited with polysulfated hydrocarbon dyes. *Dev. Biol.* (In press).

BOSSINGER, O., WIEGNER, O. and SCHIERENBERG, E. (1995). Embryonic gut differentiation in nematodes: endocytosis of macromolecules and its experimental inhibition. *Roux Arch. Dev. Biol.* (In press).

BOVERI, T. (1899). Die Entwickelung von *Ascaris megalocephala* mit besonderer Rücksicht auf die Kernverhältnisse. In *Festschrift Kupffer*. Fischer, Jena, pp. 383-430.

BOVERI, T. (1910). Die Potenzen der Ascaris Blastomeren bei abgeänderter Furchung. In *Festschrift für R. Hertwig*. Fischer, Jena, pp. 133-214.

BOWERMAN, B., DRAPER, B.W., MELLO, C.C. and PRIESS, J.R. (1993). The maternal gene skn-1 encodes a protein that is distributed unequally in early *C. elegans* embryos. *Cell 74:* 443-452.

BOWERMAN, B., EATON, B. and PRIESS, J. (1992). skn-1, a maternally expressed gene required to specify the fate of ventral blastomeres in the early *C. elegans* embryo. *Cell 68:* 1061-1075.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans. Genetics 77:* 71-94.

CARTWRIGHT J. and ARNOLD J.M. (1980). Intercellular bridges in the embryo of the Atlantic squid *Loligo pealei*. I. Cytoplasmic continuity and tissue differentiation. *J. Embryol. Exp. Morphol. 57:* 3-24.

COLE, T.S. and SCHIERENBERG, E. (1986). Laser microbeam-induced fixation for electronmicroscopy: visualisation of transient developmental features in nematode embryos. *Experientia 42:* 1046-1048.

DANILCHIK, V.M. and GERHART, J.C. (1987). Differentiation of the animal-vegetal axis in *Xenopus laevis* oocytes. I. Polarized intracellular translocation of platelets establishes the yolk gradient. *Dev. Biol. 122:* 101-112.

DAVIS, B.O., ANDERSON, G.L. and DUSENBERY, D.B. (1982). Total luminescence spectroscopy of fluorescent changes during aging in *Caenorhabditis elegans. Biochemistry 21:* 4089-4095.

DEPPE, U., SCHIERENBERG, E., COLE, T., KRIEG, C., SCHMITT, D., YODER, B. and VON EHRENSTEIN, G. (1978). Cell lineages of the embryo of the nematode *Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 75:* 376-380.

DORRESTEIJN, A.W.C. (1990). Quantitative analysis of cellular differentiation during early embryogenesis of *Platynereis dumerilii. Roux Arch. Dev. Biol. 199:* 14-30.

EDGAR, L.G. and McGHEE, J.D. (1986). Embryonic expression of a gut-specific esterase in *Caenorhabditis elegans. Dev. Biol. 114:* 109-118.

EVANS, T.C., CRITTENDEN, S.L., KODOYLANNI, V., and KIMBLE, J. (1994). Translational control of maternal glp-1 mRNA establishes an asymmetry in the *C. elegans* embryo. *Cell 77:* 183-194.

FINBOW, M.E. and PITTS, J.D. (1981). Permeability of junctions between animal cells: transfer of metabolites and a vitamin derived cofactor. *Exp. Cell Res. 131:* 1-13.

FLAGG-NEWTON, J., SIMPSON, I., and LOEWENSTEIN, W.R. (1979). Permeability of cell-cell membrane channels in mammalian cell junctions. *Science 205:* 404-409.

GOLDSTEIN, B. (1992). Induction of gut in *Caenorhabditis elegans* embryos. *Nature 357:* 255-257.

GOLDSTEIN, B. (1995a). An analysis of the response to gut induction in the *C. elegans* embryo. *Development 121:* 1227-1236.

GOLDSTEIN, B. (1995b). Cell contacts orient some cell division axes in the *Caenorhabditis elegans* embryo. *J. Cell Biol. 129:* 1071-1080.

GREEN C.R. (1988) Evidence mounts for the role of gap junctions during development. *BioEssays 8:* 7-10.

HUTTER, H. and SCHNABEL, R. (1994). glp-1 and inductions establishing embryonic axes in *C. elegans. Development 120:* 2051-2064.

HUTTER, H. and SCHNABEL, R. (1995a). Specification of anterior-posterior differences within the AB lineage in the *C. elegans* embryo: a polarising induction. *Development 121:* 1559-1568.

HUTTER, H. and SCHNABEL, R. (1995b). Establishment of left-right asymmetry in the *Caenorhabditis elegans* embryo: a multistep process involving a series of inductive events. *Development 121:* 3417-3424.

JUNKERSDORF, B. and SCHIERENBERG, E. (1992). Embryogenesis of *C. elegans* after elimination of individual blastomeres or induced alteration of the cell division order. *Roux Arch. Dev. Biol. 202:* 17-22.

KEMPHUES, K.J., PRIESS, J.R., MORTON, D.J. and CHENG, N. (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell 52:* 311-320.

KIMBLE, J. and HIRSH, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans. Dev. Biol. 70:* 396-417.

KIMBLE, J. and SHARROCK, W.J. (1983). Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans. Dev. Biol. 96:* 189-196.

KINGS R.L. and BEAMS H.W. (1938) An experimental study of chromatin diminution in *Ascaris. J. Exp. Zool. 77:* 425-443.

LAUFER, J.S. and VON EHRENSTEIN, G. (1981). Nematode development after removal of egg cytoplasm: absence of localized unbound determinants. *Science 23:* 402-405.

LAUFER, J.S., BAZZICALUPO, P. and WOOD, W.B. (1980). Segregation of developmental potential in early embryos of *Caenorhabditis elegans. Cell 19:* 569-577.

MAHAJAN-MIKLOS S., COOLEY L. (1994). Intercellular cytoplasm transport during *Drosophila* oogenesis. *Dev. Biol. 165:* 336-351.

MANGO, S.E., THORPE, C.J., MARTIN, P.R., CHAMBERLAIN, S.H. and BOWERMAN B. (1994). Two maternal genes, apx-1 and pie-1 are required to distinguish the fates of equivalent blastomeres in the early *Caenorhabditis elegans* embryo. *Development 120:* 2305-2315.

MELLO, C.C., DRAPER, B.W., KRAUSE, M., WEINTRAUB, H., and PRIESS, J.R. (1992). The pie-1 and mex-1 genes and the maternal control of blastomere identity in early *C. elegans* embryos. *Cell 70:* 163-176.

OPRESKO, L.K. and KARPF, R.A. (1987). Specific proteolysis regulates fusion between endocytic compartments in *Xenopus* oocytes. *Cell 51:* 557-568.

PRIESS, J.R. and THOMSON, J.N. (1987). Cellular interactions in early *C. elegans* embryos. *Cell 48:* 241-250.

PRIESS, J.R., SCHNABEL, H. and SCHNABEL, R. (1987). The glp-1 locus and cellular interactions in early *C. elegans* embryos. *Cell 51:* 601-611.

RAMON, F. and RIVERA, A. (1986). Gap junctional channel modulation – a physiological viewpoint. *Prog. Biophys. Mol. Biol. 48:* 127-153.

ROBINSON, D.N., CANT, K. and COOLEY, L. (1994). Morphogenesis of *Drosophila* ovarian ring channels. *Development 120:* 2015-2025.

ROEHL, H. and KIMBLE, J. (1993). Control of cell fate in *C. elegans* by a glp-1 peptide consisting primarily of ankyrin repeats. *Nature 364:* 632-635.

RÖHRKASTEN, A. and FERENZ H.J. (1987). Inhibition of yolk formation in locust oocytes by trypan blue and suramin. *Roux Arch. Dev. Biol. 196:* 165-168.

SCHIERENBERG, E. (1985). Cell determination during early embryogenesis of the nematode *Caenorhabditis elegans. Cold Spring Harb. Symp. Quant. Biol. 50:* 59-68.

SCHIERENBERG, E. (1987). Reversal of cellular polarity and early cell-cell interaction in the embryo of *Caenorhabditis elegans. Dev. Biol. 122:* 452-463.

SCHIERENBERG, E. (1988). Localization and segregation of lineage-specific cleavage potential in embryos of *Caenorhabditis elegans. Roux Arch. Dev. Biol. 197:* 282-293.

SCHIERENBERG, E. and JUNKERSDORF, B. (1992). The role of the eggshell and underlying vitelline membrane for normal pattern formation in the early *C. elegans* embryo. *Roux Arch. Dev. Biol. 202:* 10-16.

SCHLICHT, P. and SCHIERENBERG, E. (1991). Establishment of cell lineages in the *C. elegans* embryo after suppression of the first cleavage: support for a concentration-dependent decision mechanism. *Roux Arch. Dev. Biol. 199:* 437-448.

SCHNABEL, R. (1995). Duels without obvious sense: counteracting inductions involved in body wall muscle development in the *Caenorhabditis elegans* embryo. *Development 121:* 2219-2232.

SERRAS, F., BUULTJENS, T.E.J., and FINBOW, M.E. (1988b). Inhibition of dye-coupling in *Patella* (Mollusca) embryos by microinjection of antiserum against *Nephros* (Arthropoda) gap junctions. *Exp. Cell Res. 179:* 282-288.

SHARROCK, W.J. (1983). Yolk proteins of *Caenorhabditis elegans. Dev. Biol. 96:* 182-188.

SKIBA, F. and SCHIERENBERG, E. (1992). Cell lineages, developmental timing and pattern formation in embryos of free-living soil nematodes. *Dev. Biol. 151:* 597-610.

SPIETH, J., MACMORRIS, M., BROVERMAN, S. GREENSPOON, S. and BLUMENTHAL, T. (1988). Regulated expression of a vitellogenin fusion gene in transgenic nematodes. *Dev. Biol. 130:* 285-293.

STEVENS, N.M. (1909). The effect of ultra-violet light upon the developing eggs of *Ascaris megalocephala. W. Roux Arch. Entw.Mech. 27:* 622-639.

STEWART, W.W. (1981). Lucifer dyes – highly fluorescent dyes for biological tracing. *Nature 292:* 17-21.

STRASSEN, O. ZUR (1896). Embryonalentwicklung der *Ascaris megalocephala. Arch. Entw.Mech. 3:* 27-105, 133-190.

STRASSEN, O. ZUR (1959). Neue Beiträge zur Entwicklungsmechanik der Nematoden. *Zoologica 107:* 1-142.

STROME, S. and WOOD, W.B. (1983). Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. *Cell 35:* 15-25.

STROME, S., MARTIN, P., SCHIERENBERG, E. and PAULSEN, J. (1995). Transformation of the germ line into muscle in mes-1 mutant embryos of *C. elegans. Development 121:* 2961-2972.

SUBAK-SHARPE, H., BURKE, R., and PITTS, J. (1966). Metabolic cooperation by cell to cell transfer between genetically marked mammalian cells in tissue culture. *Heredity 21:* 342-343.

SULSTON, J.E. and HORVITZ, H.R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans. Dev. Biol. 56:* 110-156.

SULSTON, J.E. and WHITE, J.G. (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans. Dev. Biol. 78:* 577-597.

SULSTON, J.E., SCHIERENBERG, E., WHITE, J.G., and THOMSON, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans. Dev. Biol. 100:* 64-119.

TAX, F.E., JEARGERS, J.J. and THOMAS, J.H. (1994). Sequence of *C. elegans* lag-2 reveals a cell-signalling domain shared with delta and serrate of *Drosophila. Nature 368:* 150-154.

WANG, L.H., ROTHBERG, K.G. and ANDERSON, R.G.W. (1993). Mis-assembly of clathrin lattices on endosomes reveals a regulatory switch for the coated pit formation. *J. Cell Biol. 123:* 1107-1117.

WARNER, A.E. (1992). Gap junctions in development - a perspective. In *Gap Junctional Communication* (Ed. N.B. Gilula). Seminars in Cell Biology, Vol. 3. Saunders, Philadelphia, pp. 81-91.

WILSON, E.B. (1892). The cell-lineage of *Nereis.* A contribution to the cytogeny of the annelid body. *J. Morphol. 6:* 361-480.

WOOD, W.B. (Ed.) (1988). *The Nematode Caenorhabditis elegans.* Cold Spring Harbor Laboratory, New York.

WOOD, W.B. (1991). Evidence from reversal of handedness in *C. elegans* embryos for early cell interactions determining cell fates. *Nature 349:* 536-538.

XUE, F. and COOLEY, L. (1993). kelch encodes a component of intercellular bridges in *Drosophila* egg chambers. *Cell 72:* 681-693.