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REVIEWS

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Fusomorphogenesis: Cell Fusion in Organ Formation

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ABSTRACT **Cell fusion is a universal process that occurs during fertilization and in the formation of organs such as muscles, placenta, and bones. Very little is known about the molecular and cellular mechanisms of cell fusion during pattern formation. Here we review the dynamic anatomy of all cell fusions during embryonic and postembryonic development in an organism. Nearly all the cell fates and cell lineages are invariant in the nematode *C. elegans* and one third of the cells that are born fuse to form 44 syncytia in a reproducible and stereotyped way. To explain the function of cell fusion in organ formation we propose the fusomorphogenetic model as a simple cellular mechanism to efficiently redistribute membranes using a combination of cell fusion and polarized membrane recycling during morphogenesis. Thus, regulated intercellular and intracellular membrane fusion processes may drive elongation of the embryo as well as postembryonic organ formation in *C. elegans*. Finally, we use the fusomorphogenetic hypothesis to explain the role of cell fusion in the formation of organs like muscles, bones, and placenta in mammals and other species and to speculate on how the intracellular machinery that drive fusomorphogenesis may have evolved.** ***Dev Dyn 2000;218:30–51.*** **© 2000 Wiley-Liss, Inc.**

Key words: organogenesis; membrane fusion; cell migration; invagination; morphogenesis; epithelia; muscles; placenta; bones; pharynx; vulva; uterus; male tail; *let-60*; *lin-39*; Ras; HOM-C/Hox; evolution; *C. elegans*

INTRODUCTION

A fundamental question in developmental biology is how cells undergo regulated shape changes to accomplish pattern formation. Gastrulation and elongation during embryogenesis as well as the formation of organs of different shapes require intracellular and intercellular changes. We have recently proposed that a combination of regulated intracellular and intercellular membrane fusion processes may have a novel function in the generation of structural differentiated cellular states in different organs in multicellular organisms (Podbilewicz, 2000). Here the fusomorphogenetic hypothesis will be revisited and we will apply it to try to explain how different organ primordia in vertebrates and invertebrates use membrane fusion as a morphogenetic force.

In vertebrates, organ formation starts after the three germ layers are established and cellular interactions cause the mid-dorsal ectodermal cells to form the neural tube. Organs often contain cells derived from two or more germ layers. Epithelio-mesenchymal interactions have a central role in the induction of different organs (e.g., kidney, limbs, bones, teeth, sweat glands, mammary glands, lungs, thymus, thyroid, pancreas, placenta, and liver) (Saxen and Sariola, 1987; Cross et al., 1994; Reddi, 1994; Thesleff and Nieminen, 1996; Gilbert, 1997). Cell migrations play widespread functions in the organization and construction of organs (Gilbert, 1997). The formation of multinucleated cells (syncytia) by cell-to-cell fusion play a poorly understood function in the formation of muscles, bones, and placenta (Wakelam, 1988; Stegmann et al., 1989; Blau et al., 1993; Paululat et al., 1995; West et al., 1995; Hernandez et al., 1996; Doberstein et al., 1997; Filvaroff and Derynck, 1998; Nolan et al., 1998).

In the nematode *Caenorhabditis elegans* the sequence of cell divisions that give rise to all the cells in the adult is invariant (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983). One third of all the somatic nuclei in the adult are part of cells containing many nuclei in a common cytoplasm. These syncytia are generated in a reproducible spatial and temporal pattern by the process of cell fusion (Podbilewicz and White, 1994; Podbilewicz, 2000).

This review summarizes our current understanding of the cellular, subcellular, and genetic basis of cell fusion during morphogenesis in *C. elegans*. The extensive genetic, anatomical, physiological and molecular information available in *C. elegans* (Wood, 1988; Riddle et al., 1997; Blaxter, 1998; The *C. elegans* Consortium, 1998) facilitates research to determine the role of the cell fusion process during development. *C. elegans* is

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the only multicellular organism in which cell fusions can be followed *in situ* as they occur in a living animal, thus, it is an ideal system for future studies that will provide mechanistic answers to universal molecular, biochemical, and biophysical questions.

Transient and Permanent Cell Fusions in Metazoans

The process of cell fusion during fertilization and mating in eukaryotic cells has central roles in development (Yanagimachi, 1988; Trueheart and Fink, 1989; Myles, 1993; Faure et al., 1994). This type of cell fusion is transient since nuclear fusion will result in the formation of a diploid cell that in the case of multicellular organisms will undergo rapid cell division cycles to eventually give rise to all the cells of an organism.

Some transient syncytial cells are formed in a cell fusion-independent process. For example, transient syncytial cells have been found in the gonads of plants, nematodes, and mammals where they participate in gametogenesis (Gilbert, 1997; Wolpert et al., 1998). In the early embryonic development of many arthropods, the zygote undergoes nuclear proliferation without cytokinesis forming a transient syncytial cell that eventually cellularizes (e.g., syncytial blastoderm in Drosophila) (Gilbert, 1997; Wolpert et al., 1998). In humans, binucleate megakaryoblasts give rise to polyploid megakaryocytes that shed platelets (Wheater et al., 1979).

In annelid embryos (e.g., leech), endodermal, mesodermal, and ectodermal derived cells fuse to form transient syncytial yolk cells. These syncytial yolk cells later cellularize to form part of the intestine (Isaksen et al., 1999). Thus, the formation of transient syncytia in different organisms can be obtained by fusion-dependent and fusion-independent mechanisms using at least three different strategies: First, by the process of fertilization or mating. Second, by the process of nuclear division without cytokinesis during the syncytial blastoderm stage (e.g., in *Drosophila*) and during gametogenesis (e.g., in humans). Third, by non-autonomous cell fusion followed by cellularization (e.g., in leech). In summary, transient syncytia formation is an efficient way to regulate the generation of a large amount of cellular components.

Cell fusion is also required during development of multicellular organisms for the formation of permanent syncytia in somatic tissues (Stegmann et al., 1989; White, 1992; Podbilewicz and White, 1994; Hernandez et al., 1996). For example, programmed cell fusion is part of the development of myoblasts that form muscle fibers (Knudsen and Horwitz, 1977; Wakelam, 1988; Paululat et al., 1995; Rushton et al., 1995; Doberstein et al., 1997). Monocytes fuse to form osteoclasts involved in bone formation and resorption (Jee and Nolan, 1963; Baron et al., 1986; Takahashi et al., 1994; Filvaroff and Derynck, 1998). Epithelial cytotrophoblasts fuse to form the syncytial trophoblasts in the placenta (Cross et al., 1994; Damsky et al., 1994; West et al., 1995). Mesenchymal cells fuse to form larval skeleton in sea urchin (Hodor and Ettensohn, 1998). And one third of all the somatic cells generated during *Caenorhabditis elegans* development fuse to form syncytia (Podbilewicz and White, 1994; Podbilewicz, 1996).

The pathway resulting in cell fusion during normal organ formation can be divided into the following stages:

(1) Proliferation, cell fate determination, and differentiation;
(2) Cell migration;
(3) Cell-to-cell recognition and adhesion;
(4) Plasma membrane fusion;
(5) Mixing of cytoplasmic contents and rearrangement of the cytoskeleton.

Each of the five stages enumerated above may be subdivided in additional steps. After somatic cells fuse, syncytia are usually irreversibly committed to a certain fate, do not divide and are less motile. Cell fusions have also been observed in many tissues with pathological conditions (Robertson et al., 1993; Sitar et al., 1994). Giant syncytial cells have been found in many cells infected with enveloped viruses and in certain tumors (Sutton and Weiss, 1966; Daniels et al., 1985; Wiley and Skehel, 1987; Sitar et al., 1994; Rey et al., 1995; Hernandez et al., 1996). In general, multinucleated cells formed by cell fusion are not in a proliferative state and this is true for normal tissues, cells expressing viral fusogens and giant cells in tumors (Pierce and Midgley, 1963; Sutton and Weiss, 1966; Huang et al., 1993; Robertson et al., 1993; Sitar et al., 1994). Although numerous cell fusion events have been described in normal and abnormal development, with the exception of enveloped viruses (Wiley and Skehel, 1987; Stegmann et al., 1989; White, 1992; Hernandez et al., 1996), very little is known about the mechanisms of cell fusion and the biological functions of somatic cell fusion are poorly understood.

To study the molecular basis of cell fusion during development other researchers and we are using molecular, biochemical, and genetic strategies in *Caenorhabditis elegans*, a free-living nematode worm (Blaxter, 1998). *C. elegans* is an ideal system to analyze cell fusion during development because in this nematode there is a conserved sequence of embryonic and postembryonic epithelial cell fusions (Podbilewicz and White, 1994) and the complete anatomy at high resolution, the lineages of all cells and the complete genomic sequence have been published (Wood, 1988; Hodgkin et al., 1995; Riddle et al., 1997; The *C. elegans* Sequencing Consortium, 1998)

Survey of all the Cell Fusions During *C. elegans* Development

Most but not all somatic cell fusions in *C. elegans* are homotypic and occur between epithelial cells in the hypodermis, excretory gland cells, vulva, uterus and

TABLE 1. Summary of all the Reported Cell Fusions in the *C. elegans* Hermaphrodite According to the System, Organ, Tissue, Cell and Number of Nuclei per Syncytial Cell*

System	Organ	Tissue	Cell	Nuclei
Gastro-intestinal	Pharynx[a,b,c]	Pharyngeal muscles	m1	6
			m2 (3X2)	6
			m3 (3X2)	6
			m4 (3X2)	6
			m5 (3X2)	6
		Epithelial marginal cell	mc3	3
		Gland cell in pharynx	g1	3
Excretory	Excretory glands[a,c]	Epithelial	exc. gl.	2
Skin	Hypodermis[a,c,d]	Epithelial	hyp1	3
			hyp2	2
			hyp3	2
			hyp4	3
			hyp5	2
			hyp6+hyp7[e]	139 (144*)
			hyp10	2
			seam (l)	15 (12*)
			seam (r)	15 (12*)
			set (2X5)	— (10*[h])
			hyp8-11	— (5*[h])
Reproductive	Vulva[d,f]	Epithelial	vulA	4
			vulC	4
			vulD	2
			vulE	4
			vulF	4
	Uterus[g]	Epithelial	utse+AC	9
			ut1 (2X4)	8
			ut2 (2X4)	8
			ut3 (2X4)	8
			ut4 (2X6)	12
			sujn (2X4)	8
			plug (2X2)	4
			du	4
4 systems	5 organs	2 tissue types	44 cells	300 nuclei

*Number of nuclei in parenthesis correspond to reported differences for males.
[a]Sulston and Horvitz, 1977; [b]Albertson and Thomson, 1976; [c]White, 1988; [d]Podbilewicz and White, 1994; [e]Yochem et al., 1998; [f]Sharma-Kishore et al., 1999; [g]Newman et al., 1996; [h]Nguyen et al., 1999.

pharynx. Some pharyngeal non-striated muscle cells also fuse. However in *C. elegans* all the striated body-wall muscles are not syncytial (Waterston, 1988). Table 1 summarizes an invariant pattern of syncytial cells in the adult generated by cell fusion during embryonic and postembryonic development.

TRANSIENT CELL FUSIONS

A Tale of Cell Fusion Followed by Programmed Cell Death at the Tip of the Tail

In *C. elegans* embryos a pair of tail spike cells fuse together, form a bundle of filaments in the tip of the tail, and then undergo programmed cell death several hours after birth (Sulston et al., 1983). This invariant chain of events is an example of a transient cell fusion that does not result in a functional binucleate cell because this small caudal syncytium dies and is phagocytosed by another hypodermal cell. Additional transient somatic cell fusions occur in the hypodermis, which is made of four groups of cells: (1) the main body syncytium (hyp-7), (2) lateral seam cells, (3) the hypodermal cells of the head (hyp-1 through hyp-6) and tail (hyp-8 through hyp-11), and (4) interfacial hypodermal cells (White, 1988). For example, hyp6 is formed during embryonic development and remains as a separate ring-shaped syncytium linked anteriorly to hyp5 and posteriorly to hyp7 by cylindrical adherens junctions (Sulston et al., 1983; Podbilewicz and White, 1994). However, this hexanucleated cell remains distinct during the L1 and L2 stages but during the L3 stage hyp6 fuses to hyp7 (Yochem et al., 1998). In a similar way, the most posterior binucleate cell, hyp10, is generated during embryogenesis (Sulston et al., 1983) and in the L4 stage fuses to hyp8, hyp9, and hyp11 in males but not in hermaphrodites (Nguyen et al., 1999). Two ventral hypodermal cells (AB.p[l/r]appppa; see Sulston et al., 1983 for nomenclature) in males but not in hermaphrodites fuse to form a distinct binucleated cell called "hyp13" that is linked by cylindrical adherens junctions to hyp7 and hyp8. At the end of morphogenesis of the male tail and just before adulthood hyp13 fuses to hyp7 (Nguyen et al., 1999). In hermaphrodites the same cells that form hyp13 join hyp7 during elongation of the embryo. Thus, hyp6, hyp10, and hyp13

are transient syncytial cells that remain distinct during embryonic and part of postembryonic development and then invariantly fuse to join the larger syncytium (hyp7). Moreover, cell fusion events are sex specific for hyp10 and hyp13.

Sperm-Egg Fusion

Sperm-egg fusion in *C. elegans* is very efficient. An average wild-type virgin hermaphrodite well fed with *E. coli* under laboratory conditions will produce 300 sperm during her reproductive life (Brenner, 1974). All of these 300 sperm fuse with oocytes as they enter the spermathecae and when there is cross-fertilization one hermaphrodite can produce 1,400 oocytes that fuse with sperm originated in males (Kimble and Ward, 1988). Cross-fertilization between a single male mated with several hermaphrodites is capable of producing more than 2,500 progeny (Hodgkin, 1983). Several mutations affecting sperm-egg fusion have been identified in *C. elegans* (Ward and Carrel, 1979; Ward et al., 1981; L'Hernault et al., 1988; L'Hernault, 1997) and a sperm membrane protein required for fertilization has recently been characterized (Singson et al., 1998). In mammalian sperm, a membrane multidomain protein from the MDC/ADAM family containing A Disintegrin and A Metalloprotease domains (fertilin-α) has been implicated in sperm-egg fusion and/or sperm maturation (Blobel et al., 1992; Myles, 1993; Myles et al., 1994; Almeida et al., 1995; Cho et al., 1998). In *C. elegans*, ADM-1, a member of the ADAM family is expressed in the plasma membrane of mature sperm as well as in hypodermis, pharynx, and vulva where fusion events occur (Podbilewicz, 1996). dsRNA interference experiments (Fire et al., 1998) show phenotypes that include reduced fertility when *adm-1* dsRNA is injected but not when *adm-2,* a related *C. elegans* RNA, is injected (Vardit Adir, G.S. and B.P., unpublished data). A combination of biochemical and genetic approaches will be needed to show whether any member of the ADAM family has a role as a fusogenic molecule in vertebrates and invertebrates.

PERMANENT CELL FUSIONS

Most somatic cell fusion events in *C. elegans* result in the formation of a defined structural differentiated state. The structural differentiated state of a syncytial cell very often has a ring shape (e.g. vulva, hypodermis, and uterus). This section reviews the dynamic cellular events that involve cell migration, cell rearrangements, and cell fusion as an integral process during embryogenesis and organ formation. Similar cellular events involving three to four orders of magnitude more cells may participate in the formation of organs like muscles or placenta in other organisms.

Pharynx: Stereotyped Fusion of Muscles and Epithelial Cells

The pharynx is the organ that serves *C. elegans* in filtering and feeding. It is comprised of non-striated muscle cells arranged as eight muscle layers which engulf the pharyngeal lumen along its four distal regions: the anterior procorpus, the metacorpus, the isthmus, and the posterior terminal bulb that lies proximal to the pharyngeal-intestinal valve and the intestine (Albertson and Thomson, 1976). Three sets of epithelial cells as well as three specialized cells (marginal cells) run along the pharynx, which also contains 20 neurons and two gland cells.

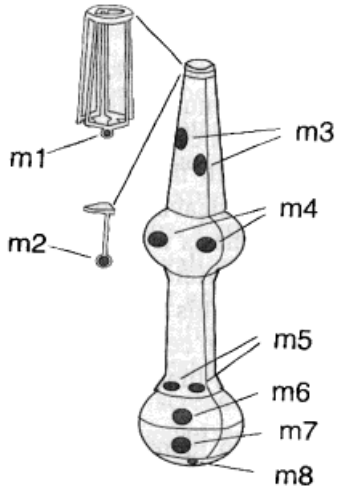

Fig. 1. Organization of the pharyngeal muscles into eight groups of cells (m1–m8). The **m1** cell has six nuclei in a common cytoplasm formed by cell fusion. Muscle cell groups **m2–m7,** each consists of three cells in threefold radial symmetry. The cells **m6** and **m7** are mononucleate while the **m2–m5** cells are binucleate. **m8** is a mononucleate ring-shape cell. Shaded circles illustrate the positions of the nuclei. Anterior is at top; posterior is at bottom. Redrawn from Moerman and Fire, 1997 (originally from Albertson and Thomson, 1976 with corrections in the shape of m2 as described by L. Avery) with permission from The Royal Society.

Stereotypic cell fusion is responsible for the formation of several syncytia in the pharynx, most prominent is fusion in the musculature. The pharyngeal muscular tissue has a three-fold symmetry (Fig. 1). Although 36 of the 77 pharyngeal nuclei are part of syncytia, very little is known about the actual pattern and timing of the fusion events during formation of the pharynx. It has been found that there is heterogeneity in the expression of at least one transcription factor among syncytial nuclei of the **m5** and **g1** cells of the pharynx (Krause et al., 1997). CeE/DA, a helix-loop-helix (HLH) transcription factor related to the E proteins of mammals and the Daughterless (DA) protein of Drosophila is expressed in only two of the six nuclei of the **m5** muscle nuclei. This results in one **m5** binucleated cell with neither of the nuclei positive for CeE/DA and two cells in which only one nucleus is positive for CeE/DA (Krause et al., 1997). Heterogeneity among nuclei sharing the same cytoplasm can be achieved autonomously in a lineage-dependent way before cell fusion and then maintained after cell fusion or only after the syncytium is generated. Transcriptional heterogeneity in response to intercellular signaling has been observed

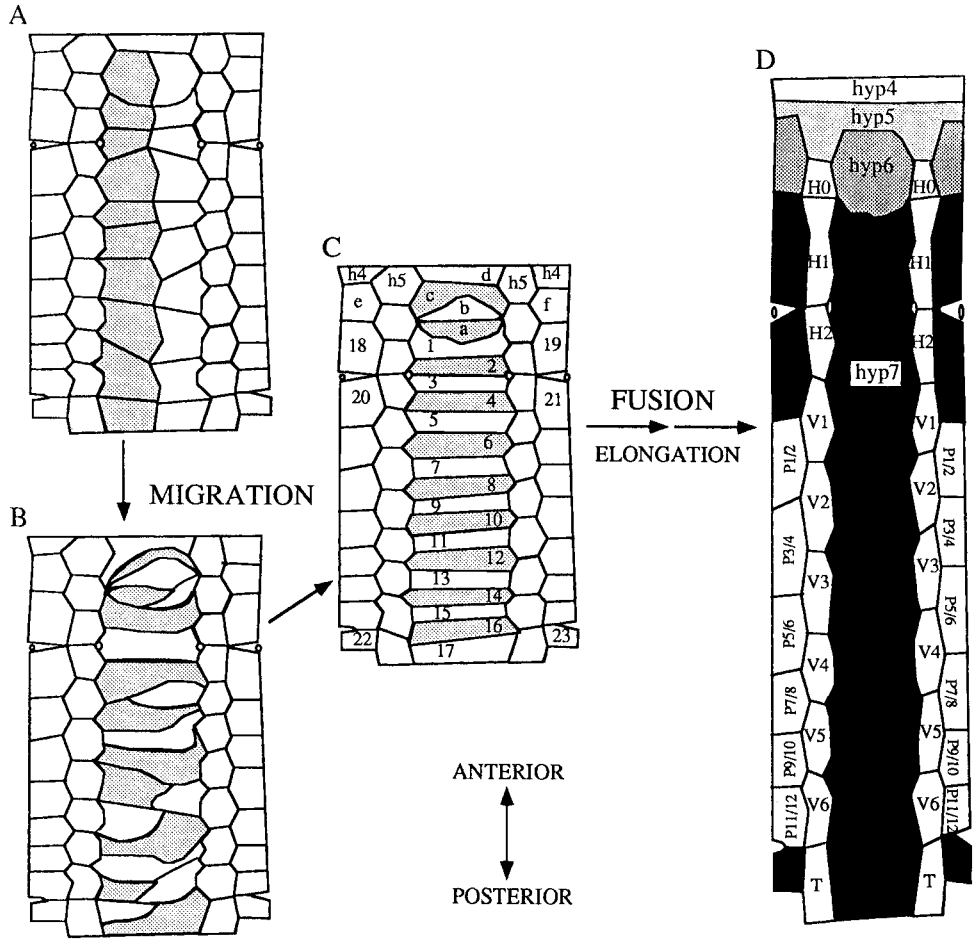

Fig. 2. Cell rearrangements and cell fusion of epidermal cells in the embryo. Cylindrical projection of the epithelial cells making up the central body region of the hypodermis (epidermis) of an embryo cut open along the ventral midline and viewed from outside. **(A)** Epithelial cells are arranged in six parallel rows. **(B)** The central two rows of cells migrate and interdigitate to form a single row of hypodermal dorsal cells. **(C)** After short-range migrations of hypodermal cells, dorsal cells (1–17) fuse with the ventral cells 18–21 (around the excretory pore) and with cells 22–23 (around the anus) to form hyp7. Hyp6 syncytium is formed by the fusion of **a–f** cells. The lateral **h5** cells migrate and fuse to form hyp5. Ventral **h4** cells fuse to form hyp4. The cell fusion events occur before and during elongation (morphogenesis). **(D)** Left and right cells are labeled according to Sulston et al., 1983. [Reprinted from Podbilewicz and White (1994) with permission of Academic Press, Inc.]

among nuclei within the transient syncytial blastoderm of the Drosophila embryo (reviewed in St Johnston and Nusslein-Volhard, 1992), in the germ line of *C. elegans* (Austin and Kimble, 1987) or in the permanent syncytial muscle cells in vertebrates (Burden, 1993). Additional examples of somatic syncytia with heterogeneity among nuclei sharing a common cytoplasm (e.g., in hypodermis, vulva, and uterus) have not been described in *C. elegans*.

Cell Fusion in the Dorsal and Ventral Hypodermis During Elongation of the Embryo

During morphogenesis in *C. elegans* the embryo elongates from a ball of cells to a larva that is fourfold longer (Sulston et al., 1983). *C. elegans* embryo maintains the same volume throughout embryonic development and body elongation is achieved by shortening of circumferentially-oriented microfilaments associated with the adherens junctions on the apical domains of hypodermal (epidermal) cells (Priess and Hirsh, 1986). During elongation, the membranes of the hypodermal cells fuse in a reproducible sequence to form cylindrical syncytial cells linked to each other by adherens junctions (Podbilewicz and White, 1994); Fig. 2).

The external epithelium of *C. elegans* (hypodermis) is responsible for secretion of the cuticle, phagocytosis of dead cells, storage of nutrients and other functions (Singh and Sulston, 1978; White, 1988). The formation of the hypodermis is a paradigm for the contribution of cell fusion to the generation of an organ. In the adult hermaphrodite 183 of the 186 hypodermal nuclei are part of 9 syncytia and in the adult male all the 195 hypodermal nuclei are distributed among 11 syncytia (Table 1). The formation of these syncytia involves

transverse fusion of ventral hypodermal cells, longitudinal fusion of dorsal hypodermal cells and dorso-ventral fusion between these dorsal and ventral intermediate syncytia (see Fig. 10D).

Staining early embryos with the MH27 antibody that recognizes the adherens junctions (ZA) in the apical domains of epithelial cells has revealed the sequential events leading to the formation of the hypodermis (Podbilewicz and White, 1994). Figure 2 illustrates an embryo cut open along the ventral midline and viewed from the outside. Between 200 and 250 min after first cleavage six rows of epithelial cells are born. Two central rows that will become the dorsal hypodermis; two lateral rows that will give rise to the specialized lateral hypodermal cells (seam cells) and two outer rows that will form the ventral hypodermis (Fig. 2A). In the next 140 min two simultaneous events occur: (1) the two most external rows migrate toward the ventral side until meeting with one another and (2) the two central rows of cells migrate and interdigitate (Fig. 2B) (Sulston et al., 1983; Podbilewicz and White, 1994; Williams-Masson et al., 1998). These migrations involve the remodeling of adherens junctions and result in the formation of a cylindrical embryo with two ventral rows, two lateral rows, and one dorsal row of epithelial cells (Fig. 2C) (Podbilewicz and White, 1994; Williams-Masson et al., 1997).

The first fusion events take place just before the onset of embryonic elongation. In the anterior ventral side a pair of cells (left and right) that have met upon enclosure fuse between themselves in a transverse manner ($\mathbf{F_T}$) forming a binucleate syncytium (cells 18 and 19 in Fig. 2C) (Podbilewicz and White, 1994). In the dorsal side, cell fusion occurs between two neighboring cells located anterior to the sensory sensilla (the deirids). This fusion takes place in a longitudinal matter ($\mathbf{F_L}$) as these cells (cells 1 and 2 in Fig. 2C) are positioned next to each other in the anterior-posterior axis as a result of the migration and interdigitation described above (Podbilewicz and White, 1994). The fusion events in the dorsal and ventral side are independent of one another. This pattern is kept as cell fusion in both the dorsal and ventral side progresses during the elongation of the embryo. Although this progression is in general toward the posterior part of the body, the order of fusion events is not completely invariant (Podbilewicz and White, 1994). Beginning ~3 hr after the first hypodermal cells are born and completed between 1.5 and 2-fold elongation, pairs of left-right ventral cells as well as anterior-posterior dorsal cells fuse to form the precursors of 8 of the 11 hypodermal cells. These syncytia are hyp1-6 in the head of the embryo, hyp7 in the midbody and hyp10 in the evolving tail (Sulston et al., 1983; Podbilewicz and White, 1994).

The next stage of hypodermal syncytia formation involves fusion between the dorsal and ventral syncytia. These dorso-ventral fusions ($\mathbf{F_{DV}}$) take place under the lateral seam cells without the involvement of adherens junctions and thus, can be observed only through EM serial sections (Podbilewicz and White, 1994). The dorsal fused cells 1–17 fuse with the ventral 18+19, 20+21, and 22+23 yielding the hyp7 syncytium, which contains 23 nuclei at the end of embryogenesis (Fig. 2D). The dorsal a–d intermediate syncytium fuses with the binucleate ventral e–f accounting for the six nuclei of hyp6. Five additional syncytia (hyp1–5) are formed in the head, each the result of dorso-ventral fusion events (White, 1988). Hyp1–7 are cylindrical syncytia engulfing the anterior and midbody of the embryo. Additional four hypodermal cells, hyp8–11 of which only hyp10 is binucleate (Sulston et al., 1983; White, 1988), are organized in telescopic fashion forming the embryo's tail (Nguyen et al., 1999).

In summary, symmetric and simultaneous migrations generate a spheroidal embryo. During early elongation of the embryo longitudinal fusions in the dorsal side and transverse fusions in the ventral side of the embryo followed by dorso-ventral fusions between the cells in each side give rise to the formation of 11 hypodermal cells, 8 of them syncytial.

Hypodermal Cell Fusion During Postembryonic Development

After hatching, the hyp7 syncytium is not completely cylindrical since two rows of 10 seam cells run along each side of the larva and two rows of 6 P cells are located on each side of the ventral midline (Fig. 2D and 3A).

The seam cells, which are responsible for secreting a specialized cuticle (alae) during specific stages of development and the P cells that are the ancestors of the vulval and the ventral cord cells, contribute nuclei to hyp7 throughout the postembryonic development (Sulston and Horvitz, 1977; Chalfie and White, 1988; White, 1988). Mosaic analysis has revealed that hyp6 also fuses to hyp7 during the mid-L3 stage (Yochem et al., 1998). All this results in the formation of the largest syncytium in *C. elegans* containing eventually 139 nuclei in the adult hermaphrodites (Table 1). In summary, cell fusion during embryogenesis and larval development give rise to the formation of the hypodermis, which is almost totally syncytial (Figs. 2 and 3). These fusion events are important, since mutants that are defective in hypodermal fusions are either embryonic lethal (Tamar Gattegno, and B.P, unpublished results) or have a diverse set of defects during development (Bill Mohler and John White, personal communication).

Ring Cell Formation in the Uterus: Homotypic and Heterotypic Fusions

The uterus is formed between the mid-L2 larval stage and the late-L4 stage with correlation to the formation of nearby organs: the vulva, the spermatheca, and the spermathecal valve (Kimble and Hirsh, 1979).

Two dorsal uterine precursor (DU) and three ventral uterine precursor (VU) cells give rise to 60 cells that

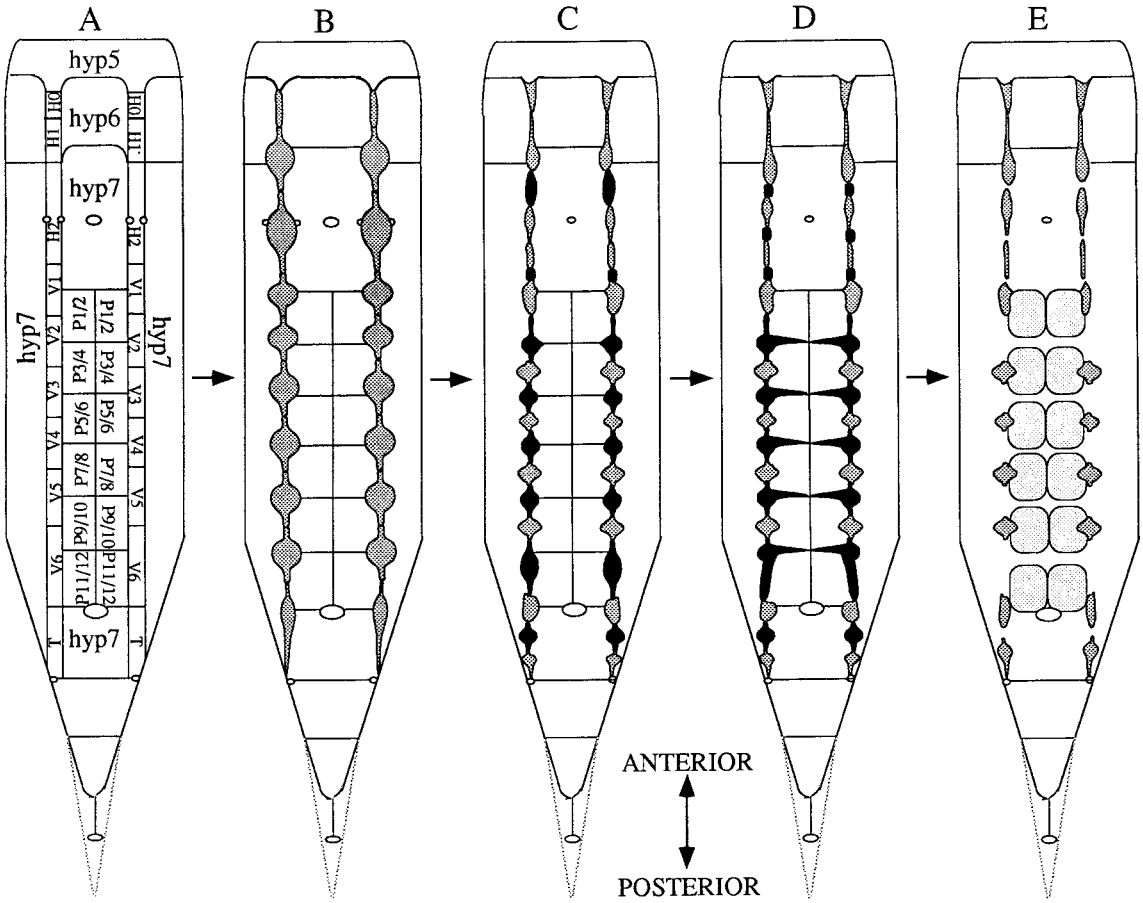

Fig. 3. Generation, migration, and fusion of postembryonic epithelial cells. Cylindrical projections of the body hypodermis of first larval stage (L1) cut open along the dorsal midline and viewed from inside. **(A)** Newly hatched L1 cells are labeled according to Sulston and Horvitz (1977) and Sulston et al. (1983). There are two longitudinal rows of cells, called seam cells (H0-H2, V1-V6, T), that run on the left and right sides of the L1, and two rows of ventral P cells (P1/2-P11/12) all embedded in cylindrical syncytia generated during embryogenesis (hyp5, hyp6, hyp7). **(B)** The seam cells round up and **(C)** divide ~5 hr after hatching except H0 left and right that do not divide. **(D)** The anterior daughters of V2–V6 (left and right) send cytoplasmic processes toward the ventral midline along the junctions between P cells until meeting with their symmetrical pairs. **(E)** About 3 hr after they are generated, black daughter cells fuse to the surrounding hyp7 syncytium isolating six pairs of P cells. Remodeling of adherens junctions by these processes followed by rotation of the isolated six pairs of Pn cells result in reorganization of the 12 P cells in one anterior-posterior row instead of two parallel rows (Podbilewicz and White, 1994). Later, following division of the P cells, their posterior daughters will also fuse to hyp7 except for six cells that will generate the vulva (Sulston and Horvitz, 1977). Except for the most anterior cell, all the seam cells undergo stem cell division at the beginning of each larval stage. Around 3 hr after being born, the non-stem tetraploid daughter cells become constricted in their apical surfaces and fuse to hyp7 (Hedgecock and White, 1985; Podbilewicz and White, 1994). Simultaneously, each stem cell elongates until reaching its neighboring stem cell to form a continuous row of seam cells on each side of the worm. This pattern of division, fusion, and coupled elongation repeats itself in each of the larval stages until mid-L4, when 15 elongated seam cells in each row fuse longitudinally forming two continuous lateral syncytia embedded in the cylindrical hyp7 [Reprinted from Podbilewicz and White (1994) with permission of Academic Press, Inc.]

form the adult uterus (61 uterine cells including the AC) in addition to their other progeny that participate in the formation of the spermatheca and the uterine-spermathecal junction (Table 1). Cell fusions, heterotypic and homotypic, are involved in the formation of three major components of the uterus: the **du** cell, the toroidal **ut** cells that engulf the uterine lumen and the **utse** syncytium that connects the uterus to the lateral seam cells and forms a thin laminar layer between the vulva and the uterus (Fig. 4; Newman and Sternberg, 1996; Newman et al., 1996).

Formation of the utse Syncytium and Heterotypic Fusion With the AC

One of the key players in the formation of the uterus is the anchor cell (**AC**). The **AC** is specified during the late-L2 larval stage and then induces its surrounding **VU** granddaughters to adopt a π fate giving rise to progeny that either become **uv1** cells or part of the **utse** syncytium (Newman et al., 1994; Newman et al., 1999). The **uv1** cells (uterine-vulva cells) are specialized cells that physically connect **vulF**, the most dorsal ring of the vulva to the uterus (Chang et al., 1999;

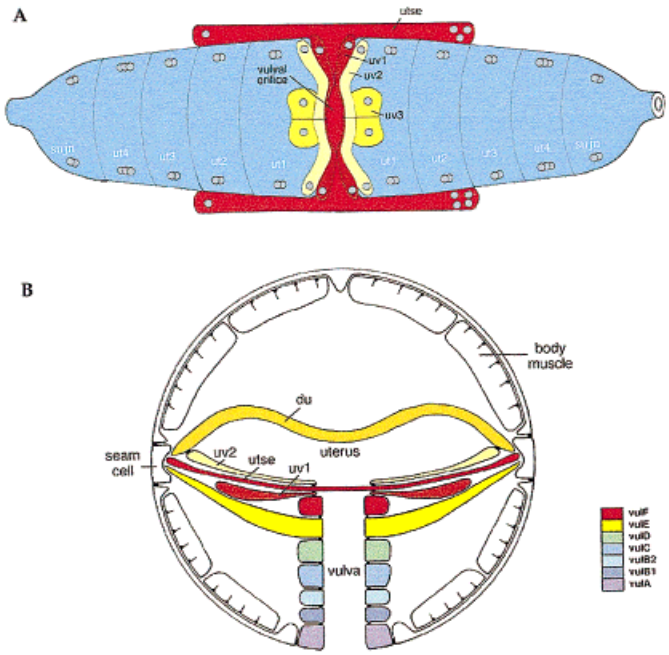

Fig. 4. Schematic representation of mature uterine syncytial structures. **(A)** Ventral view showing **ut** toroids, **utse**, **uv** cells, and spermathecal-uterine valve (i.e., junction: **sujn**). Following the nuclear divisions of the DU and the VU descendants, the primordial uterus is comprised of specialized cells that lie proximal to its midline (the **du** cell and the π cell descendants) and two distal epithelial rows, dorsal and ventral. These rows comprise the **ut** cells and can be divided into eight groups of cells: four anterior and four posteriors. Each group contains four cells (two pairs of dorsal and ventral cells in each side) except for the most distal groups that contain six cells each. The groups are not the result of cell migration but rather are formed as a consequence of the orientation of the cleavage planes. EM and confocal reconstructions have revealed that the cells in each group fuse during mid-L4 to form the **ut1-ut4** toroid syncytia, four anterior and four posterior (Newman et al., 1996). This includes fusions between the dorsal and ventral cells ($\mathbf{F_{DV}}$) and transverse fusions between the left and the right side of each toroid ($\mathbf{F_T}$). The cytoplasm of the toroids becomes thinner when the lumen forms and enlarges resulting in a bi-lobe tube-shaped uterus in the A-P axis with eight toroidal syncytia engulfing the uterine lumen. At the distal ends of the uterus (anterior and posterior) lie the toroidal spermathecal valves (**sujn**), which like the **ut** toroids, are formed from fusion between DU and VU descendants. **(B)** Transverse view of an hermaphrodite showing the uterus where it sits over the vulva. The **uv1** cells are directly dorsal to the vulva, and the **utse** and **uv2** are dorsal to it. Dorsal to the vulva lies the **du** syncytium, which serves as the most dorsal cell in the uterus midline. This syncytium is the result of homotypic longitudinal and transverse fusions ($\mathbf{F_{LT}}$) between four DU descendants. The lateral attachments of the H shaped **utse** and the **vulE** toroid to the seam are also shown. Vulval toroids are color coded as in Figure 5. Modified from Newman et al., 1996; used with permission of the Company of Biologists Ltd.

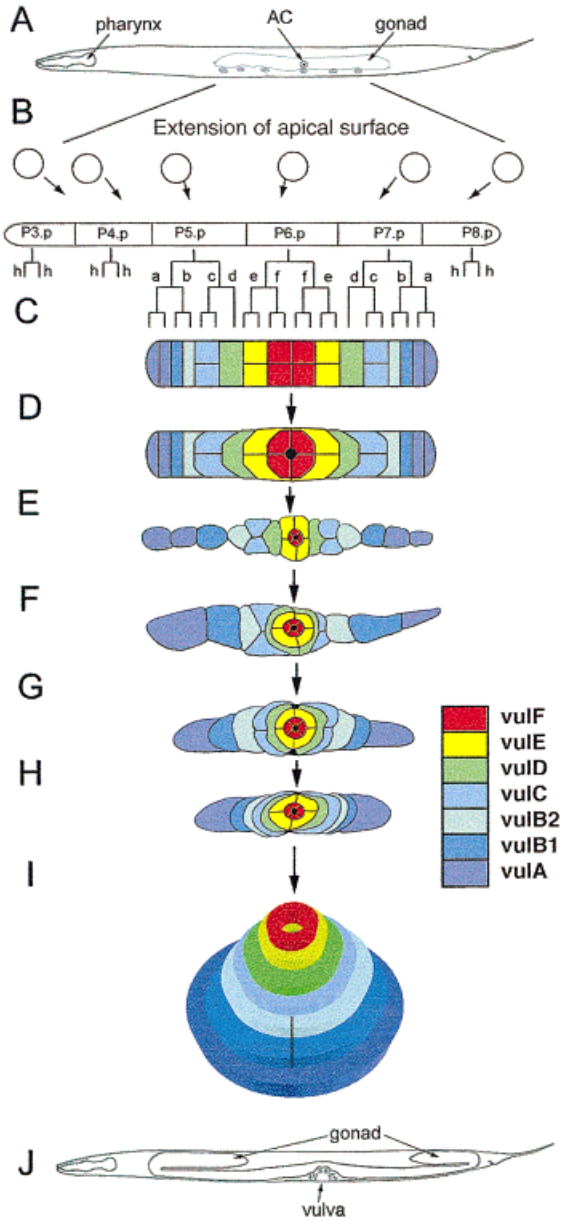

Figure 5.

Hanna-Rose and Han, 1999; Newman et al., 1999). The **utse** (uterine-seam cell) is an H-shaped syncytium (Fig. 4A). The long arms of the "H" are cytoplasmic extensions sent from the uterine midline to the lateral seam cells (Fig. 4B). These extensions serve as an anchor preventing the uterus from everting. The central arm of the "H" is a transverse process that forms a thin laminar layer that defines the uterus from the vulva. This layer is later broken down when the first embryo is laid (reviewed in Newman and Sternberg, 1996; Delattre and Félix, 1999).

Eight VU descendants, **VT5**, **VT6**, **VT7**, and **VT9** (left and right) fuse during mid-L4 as the uterine lumen begins to form and the **AC** starts to migrate laterally. These fusions are homotypic and are followed by a heterotypic cell fusion between this intermediate syncytium and the **AC**. Only after the generation of the nine nuclei syncytium, the cytoplasmic extensions are

sent latterly toward the seam cells and the nuclei migrate to the distal regions of the syncytium (Newman et al., 1996).

In summary, morphogenesis of the primordial cells during the L4 stage give rise to ring-shaped and H-shaped syncytia when part of the uterine specialized cells fuse in a homotypic and heterotypic manner.

Cell Fusion and Ring Cell Formation During Invagination of the Vulva

The vulva of the *C. elegans* hermaphrodite is a tube-shaped sheet of epithelial cells invaginated from the ventral side of the worm, thus serving as a reproductive organ responsible for egg-laying and mating. Because of its simplicity, the vulva has been extensively studied in the last 20 years and much is known about the molecular mechanisms involved in the determination and execution of its cellular fates (Sternberg and Horvitz, 1986; Greenwald, 1997; Kornfeld, 1997). Recently, the complete organogenesis of the vulva has also been described step-by-step (Sharma-Kishore et al., 1999). This developmental process involves heterotypic and homotypic cell fusion that give rise to the formation of seven toroidal cells, which serve as the fundamental structure of the vulva.

Figure 5 illustrates vulva formation in *C. elegans* during the post-embryonic stages of worm development. It starts during the first larval stage (L1) with the generation of six ventral hypodermal cells, each of which carries the potential to participate in the formation of this epithelial organ (Sulston and Horvitz, 1977; Sulston and White, 1980; Kimble, 1981). All of these vulva precursor cells (VPCs), named P(3-8).p, are embedded in the hypodermal syncytium hyp7 (Fig. 5A and B). During the mid-L3 stage, three of the VPCs, P(5-7).p, are induced by the nearby somatic gonad followed by inter-VPC signaling that undergo three rounds of divisions yielding a symmetrical and palindromic row of 22 cells (Fig. 5C). P3.p, P4.p and P8.p divide only once and fuse to the surrounding hyp7, the first fusion event during vulva formation (reviewed in Greenwald, 1997; Kornfeld, 1997).

At the onset of the L4 stage, the cells from each half of the vulva primordium send unidirectional apical extensions toward the midline until meeting with their symmetrical pairs (Fig. 5D–I; Sharma-Kishore et al., 1999). These short-range migrations involve remodeling of adherens junctions with hyp7 and two fusion events. First, the **a** cells in each half fuse longitudinally ($\mathbf{F_L}$) before they start their migration (Fig. 5F) and second, the **c** cells in each half undergo transverse fusion ($\mathbf{F_T}$) after they complete their migration (Fig. 5H) yielding four binucleated syncytia. The result of the short-range migrations is the formation of a stack of seven toroidal cells (**vulA-vulF**), each of them comprised of two or four cells. The anchor cell (**AC**), which lies dorsally, sends a ventral filopodium that penetrates the most dorsal ring (**vulF**) and then fuses with the dorsal **utse** uterine cell, thus forming a hole that connects the uterus with the external ventral hypodermis through the vulval rings (Newman et al., 1996; Sharma-Kishore et al., 1999). After all the migrations have been completed, a process of intratoroidal fusion takes place within the vulval rings. These homotypic intratoroidal fusions are of two kinds: longitudinal ($\mathbf{F_{IL}}$) and transverse ($\mathbf{F_{IT}}$) and are carried out in a temporal and spatial order with the exception of **vulB1** and **vulB2** that do not fuse (Fig. 5I). The result of this process is the formation of seven rings, five of them (**vulA**, **vulC**, **vulD**, **vulE**, and **vulF**) are multinucleated (Sharma-Kishore et al., 1999).

In the final step of vulva organogenesis, vulval muscles attach to specific points on the rings, the **vulE** ring attaches to the lateral hypodermal seam cells (Fig. 4B), the vulval cuticle is secreted and plastic deformations of the vulval structure take place (eversion).

Laser ablation experiments, in which half the vulva was removed and analysis of mutants, which lack some of the cells normally participating in vulva formation, have revealed irregular patterns of cell fusion during vulva organogenesis (Sharma-Kishore et al., 1999; Shemer et al., 2000). In half primordia, the remaining cells showed an autonomous behavior as they migrated, turned toward themselves after passing the midline and self-fused forming vulval rings. Cell fusion takes place in a heterotypic manner as the **a** cells fused to hyp7 in the background of a *ras/let-60* gain-of-function mutation (Shemer et al., 2000). Mutant analyses have also revealed that in the absence of certain rings (e.g., **vulA**) the cells

Fig. 5. Formation of the vulva. **(A)** Lateral view of an L3 larva. **(B)** A somatic gonadal cell, the anchor cell (AC), through inductive signaling induces three (P5.p, P6.p, and P7.p) of the six vulval precursor cells (VPCs), P3.p-P8.p, to adopt vulval fates. The non-vulval cells- P3.p, P4.p and P8.p undergo a single proliferation step and fuse with hyp7 (h cell fate). **(C)** The other cell fates (a–f) are related to the terminal structural differentiated states of the vulval rings and not necessarily to the pattern of cell division of the VPCs (1°, 2°, and 3° sublineages). **(D–H)** Short-range migrations at the onset of the L4 stage, starting from **vulE** precursors and ending with **vulA** precursors, in each half the cells migrate toward the center until meeting with their symmetrical pairs. **(F)** The precursors of **vulA** fuse in each half in a longitudinal orientation prior to their migration. **(H)** The precursors of **vulC** fuse in each half in a transverse orientation at the end of their migration. As a result of these migrations and the successive remodeling of adherens junctions, the inner cells are pushed dorsally forming a stack of seven rings (**vulA–vulF**). **(I)** Intratoroidal fusions. With the exception of **vulB1** and **vulB2**, the cells within the rings fuse to from syncytial rings (toroids). The **AC** is a black circle in panels D–H that penetrates the apex of the vulva and fuses with the dorsal **utse** uterine cell, creating a hole that connects the uterus and the vulva throughout its length (see Fig. 4B). **(J)** Lateral view of an L4 larva showing the invagination of the vulva and the gonad arms. In the last molt, a mature vulva is obtained after eversion of the dorsal rings and connection of specific rings to other tissues (see Fig. 4B). **(C–H)** are dorsal views of the vulva primordium. **(I)** This is a three-dimensional view of the late-L4 vulva. Vulval toroids are color coded as in Figure 4. Modified from Sharma-Kishore et al., 1999; used with permission of the Company of Biologists Ltd.

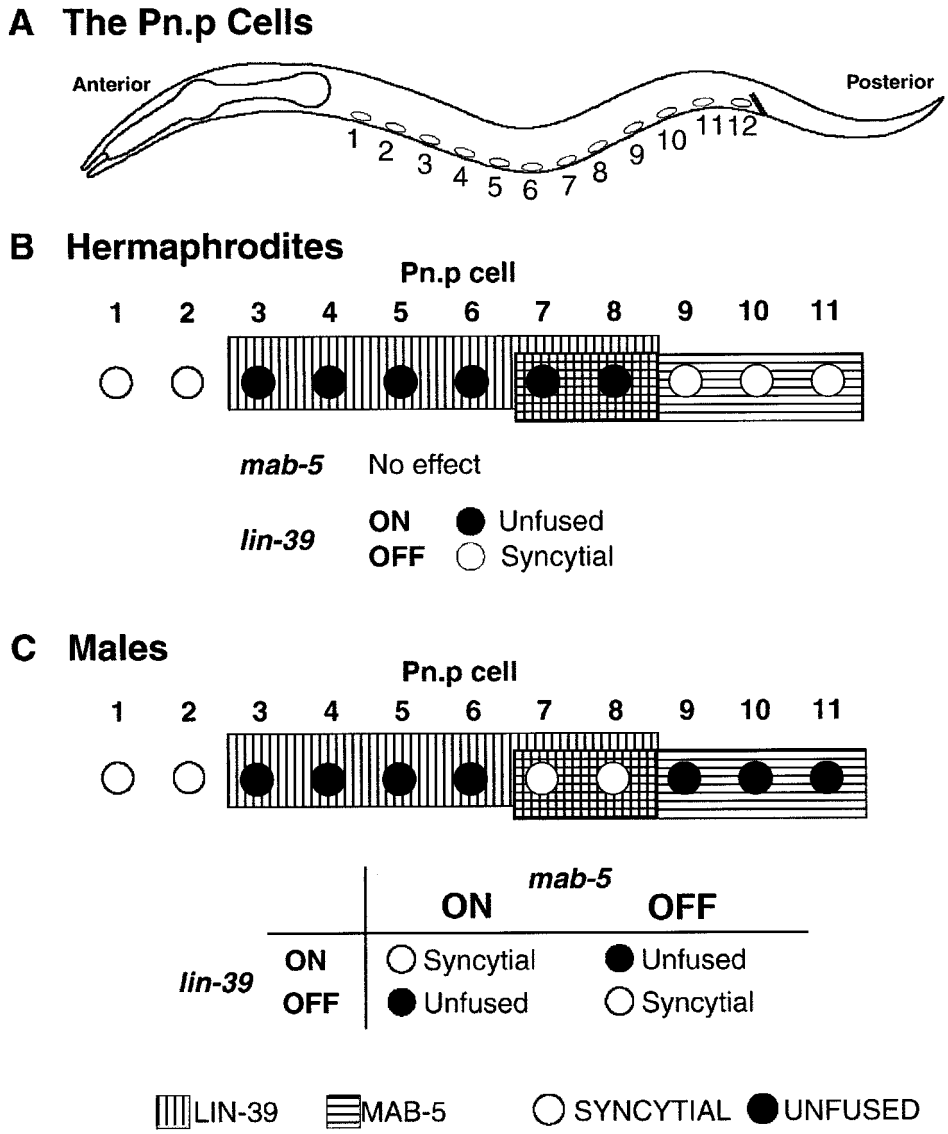

Fig. 6. *Hox* genes pattern Pn.p cell fates in *C. elegans*. **(A)** A drawing of a *C. elegans* first stage larva showing the 12 ectodermal Pn.p cells (numbered 1–12) located in the ventral cord. **(B)** The pattern of Pn.p cell fusion and *Hox* gene expression in hermaphrodites. **(C)** Pattern of Pn.p cell fusion and *Hox* gene expression in males. See text for details. Hatched backgrounds indicate *lin-39* and *mab-5* expression domains; note that overlaps are crosshatched. White circles represent the syncytial fate and black circles represent the unfused fate. Modified from Ch'ng and Kenyon, 1999; used with permission of the Company of Biologists Ltd.)

comprising the **vulB2** ring are also able to fuse. Moreover, the whole pathway of intratoroidal fusion is independent of the components of the primordium but is dependent on the completion of the migration process by the participating cells (Shemer et al., 2000).

In summary, in the early stages of vulva development specific VPCs fuse with hyp7, additional cells fuse during migration of vulval rings precursors at the onset of organogenesis and finally intratoroidal fusions occur after the formation of a stack of vulval rings.

CONTROL OF CELL FUSION BY TRANSCRIPTION FACTORS OF THE HOM-C/HOX GENE CLUSTER

Postembryonic Cell Fusions in the Ventral Hypodermis

The homeotic cluster genes (HOM-C/Hox) have a central role in establishing the anterior-posterior pattern in vertebrates and invertebrates. As a result of the spatial and temporal variations in the expression and activity of the HOM-C genes in different cells, regional identity is established throughout evolution (reviewed in Wolpert et al., 1998).

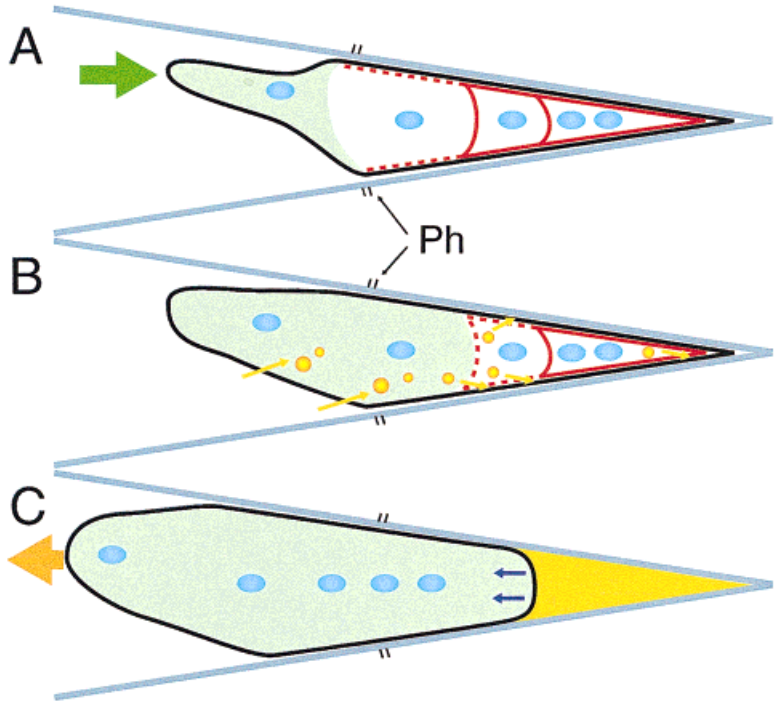

Fig. 7. A working model for the polarized membrane fusion events that occur during morphogenesis of the male tail tip in *C. elegans* (ventral view). **(A)** A hypothetical anterior signal triggers morphogenesis (green arrow). Cell fusions initiate at the anterior adherens junctions and progress to the posterior part of the tail. **(B)** Fluid is transferred through tail tip cells by intracellular vesicular transport (yellow spheres). These vesicles fuse in the posterior region of the plasma membrane. Through vesicle docking and fusion events, fluid is secreted and accumulates in the extracellular space of the tail spike **(C)**. The syncytium (light green cytoplasm) retracts towards the anterior (small arrows), the syncytium moves towards the anterior of the worm (large orange arrow) and the nuclei (blue ovals) are transported towards the anterior part past the phasmid openings (Ph). [Reprinted from Nguyen et al. (1999) with permission of Academic Press, Inc.]

In *C. elegans*, one cluster containing four HOM-C genes that specify the regional identity along the A/P axis has been identified using genetic and molecular approaches (reviewed in Salser and Kenyon, 1994). Three of these genes appear to be key participants in the regulation of cell fusion during embryonic (*ceh-13*) and postembryonic (*lin-39* and *mab-5*) development. The latter two genes act in a sex-specific manner.

The most anterior gene in the HOM-C cluster in *C. elegans* is *ceh-13*, the homolog of the *Drosophila labial* gene, which acts in the anterior part of the embryo. In *ceh-13* mutants 97% of the homozygous animals arrest as embryos or at early larval stages and an extra cell boundary is observed in the dorsal hypodermis in the 2-fold stage indicating incomplete cell fusion during the formation of hyp7 (Brunschwig et al., 1999). This phenomenon could be an indirect result of the mutation since *ceh-13* is multifunctional and the phenotype of the mutant includes cell adhesion defects and misorganized pattern of the anterior lateral hypodermis and the body wall muscle cells.

In hermaphrodites, 12 ventral hypodermal Pn.p cells are generated near the end of the first larval stage (P(1-12).p). Soon after their generation (P(1,2).p) and (P(9-11).p) cells fuse with the surrounding hyp7 syncytium (Fig. 6). The remaining cells in the midbody (P3-8).p) do not fuse and are involved later in the formation of the vulva (see above; (Sulston and Horvitz, 1977)). This switch between fusion and vulval fates is primarily regulated by the Hox gene *lin-39*, the *C. elegans* homolog of the *Drosophila* homeotic genes *Deformed* and *Sex combs reduced* and the vertebrate Hox-4 genes (Clark et al., 1993; Wang et al., 1993). P(3-8).p cells expressing *lin-39* remain unfused while P(1,2,9-11).p cells that do not express this gene undergo fusion to hyp7 (Fig. 6B). When *lin-39* is expressed in the posterior P(9-11).p cells, as a result of a mutation, these cells acquire the ability to remain unfused (Ch'ng and Kenyon, 1999). Conversely, in *lin-39(−)* mutants the P(1-11).p cells fuse with the surrounding syncytium implying that *lin-39* expression is necessary and sufficient to prevent cell fusion of Pn.p cells (Fig. 9C). *lin-39* also takes part in later developmental events when it functions in parallel and downstream to RAS/LET-60 to switch between vulval and non-vulval fates of P(3-8).p and to contribute specificity to the RAS signaling pathway (Maloof and Kenyon, 1998). It is not known whether regulation of cell fusion is directly involved in the later functions of *lin-39*.

In males, some of the Pn.p cells are kept unfused in a *lin-39*-dependent way (Fig. 6C; Clark et al., 1993;

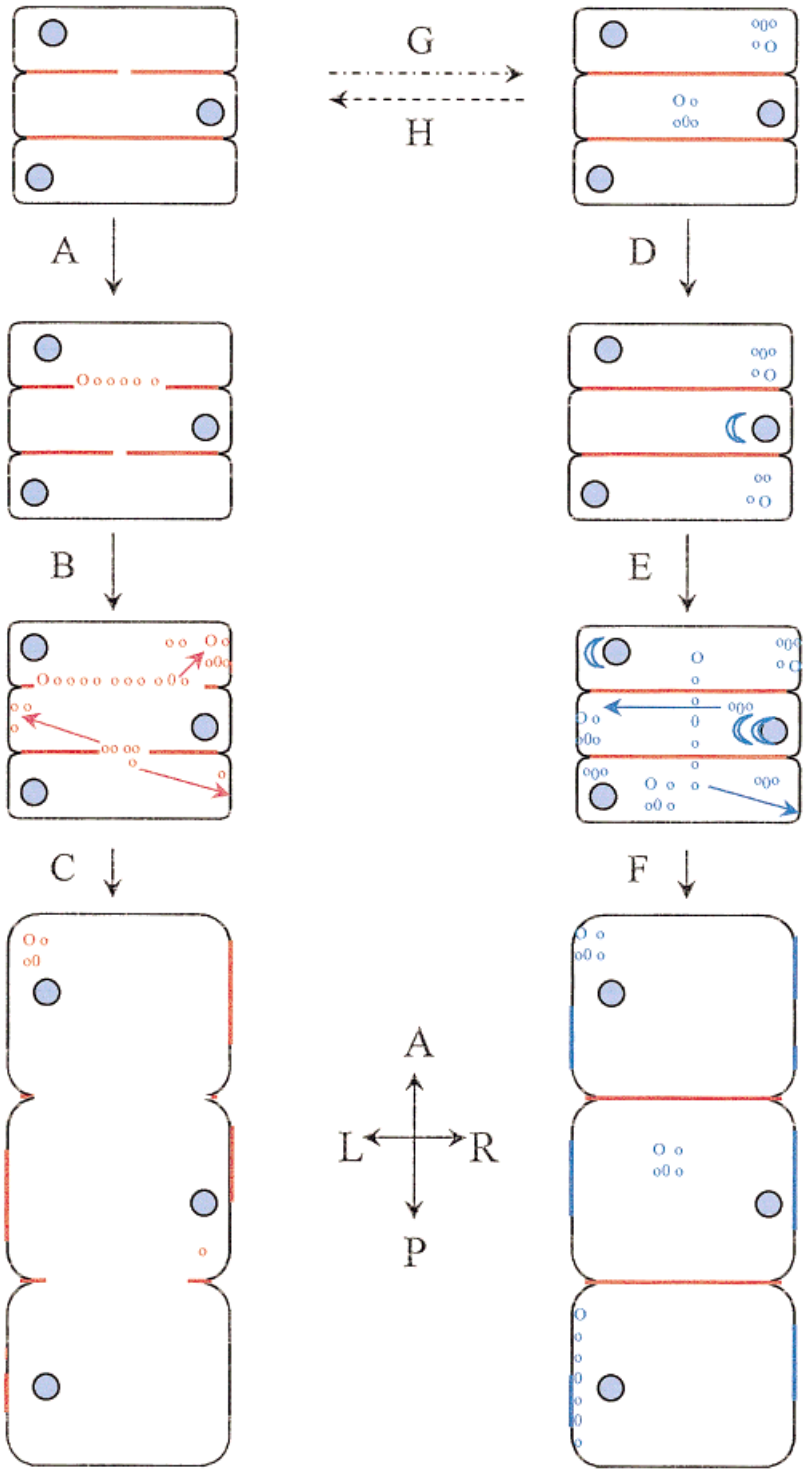

Fig. 8. The fusomorphogenetic model. **(A)** Three cells fuse in an anterior to posterior sequence. A pore is formed between the membranes of the anterior pair of cells, the pore(s) dilate and then the membranes vesiculate. **(B)** The posterior cells fuse and follow the same ultrastructural steps shown for the anterior pair. Vesicles derived from the transverse membranes that fused together are specifically transported to the lateral membranes (arrows). **(C)** The vesicles dock and fuse with the lateral plasma membranes. These polarized fusion events are coupled to a change in cell shape that results in longitudinal elongation. **(D)** Cells may undergo changes in shape without cell fusion by de novo synthesis of membranes through the secretory pathway or by the use of existing reserves of vesicular organelles that will fuse when required. **(E)** Vesicles derived from the Golgi apparatus (blue crescent shaped organelles) are targeted directly to the lateral membranes (arrows) where they fuse **(F)**. The specific fusion of Golgi-derived vesicles to the lateral domains of the plasma membrane (D–F) can bypass the necessity for cell fusion and membrane recycling (A–C). A combination of both strategies (A–C and D–F) and a balance between the two (G and H) may drive morphogenesis. *Caenorhabditis elegans* and other nematodes with syncytial hypodermis may use cell fusion followed by polarized membrane recycling (A–C). Marine nematodes like *Enoplus brevis* may use *de novo* synthesis of membranes coupled to polarized secretion. Blue circles, nuclei; red lines, fusing plasma membranes or recycled membranes through red vesicular carriers. Small blue circles, newly synthesized vesicles in D–F. Adapted from Podbilewicz (2000).

Wang et al., 1993). Although *mab-5* (the *C. elegans* homolog of the *Drosophila Antp* Hox gene) is also expressed in Pn.p cells, its activity as a cell fusion regulator is more complex (Figs. 6 and 9E). MAB-5 protein distribution is identical in hermaphrodites and males at the time of the cell fusion fate determination, however while in hermaphrodites MAB-5 is unable to keep Pn.p cells unfused, in males it is able to do so ((Salser et al., 1993); compare the hatched patterns between Figs. 6B and 6C). It has been proposed that MAB-5 protein activity is inhibited post-translationally in hermaphrodites, and MAB-5 together with LIN-39 proteins can specify a new cell fate in males (Clark et al., 1993; Salser et al., 1993; Wang et al., 1993; Ch'ng and Kenyon, 1999). In contrast to their individual function, when *lin-39* and *mab-5* are coexpressed and active (in males; Fig. 6C), these Hox genes have a combinatorial effect that results in the fusion of these cells to hyp7 (Clark et al., 1993; Salser et al., 1993; Wang et al., 1993). How this phenomenon occurs is still not completely understood. An interesting hypothesis that may explain this combinatorial effect suggests that these proteins act together on another limiting target, preventing them from inhibiting cell fusion (Ch'ng and Kenyon, 1999).

The expression and activity of *lin-39* and *mab-5* during the fusion events are regulated by *egl-27* (Ch'ng and Kenyon, 1999). This gene encodes a protein homologous to the nuclear MTA1, one of the components of the NURD (NUcleosome Remodeling and histone Deacetylation) complex (Herman et al., 1999; Solari et al., 1999). Thus, changes in chromatin structure apparently allow the function of HOM-C/Hox genes of *C. elegans* resulting in position-specific gene expression and in modulation of sex-specific cell fusion events (Ch'ng and Kenyon, 1999).

In summary, *lin-39* and *mab-5* regulate cell fusion in a sex- and a cell-specific fashion. Each of these genes, when expressed and active, prevents cell fusion of the posterior descendants of P cells with the hypodermal syncytium. When both genes are active in males, they show a combinatorial effect resulting in the promotion of cell fusion.

Cell Fusion During Morphogenesis of the Male Tail

Morphogenesis of the male tail occurs during the last stage of larval development (reviewed in Emmons and Sternberg, 1997). The dynamic changes in the shape of the male tail include the transformation from a simple tapered cone to a complex copulatory bursa. These changes include the patterning of male-specific sensilla or rays, the correct specification of anterior-posterior identity of cells in the region (which is controlled by the HOM-C/hox genes), intercellular signaling events and hypodermal cell fusions (Baird et al., 1991; Chow and Emmons, 1994; Chow et al., 1995; Fitch and Emmons, 1995; Zhang and Emmons, 1995).

In *C. elegans* the most posterior cells have identical cell lineages in males and hermaphrodites (Sulston and Horvitz, 1977; Sulston et al., 1983). Four cells in the tip of the tail (hyp8-hyp11) are generated during embryogenesis (Sulston et al., 1983) and have similar morphologies until the late L4 larval stage when sexual dimorphism arises as the four cells fuse and retract in the male but not in the hermaphrodite (Nguyen et al., 1999). This male epithelial tail syncytium secretes fluid by exocytosis to the extracellular space between the syncytial cell and the cuticle and the shape of the tail changes from pointed to round while fluid accumulates on the posterior end (Fig. 7). This is a classic example where identical cell sublineages in males and hermaphrodites generate four cells that adopt different structural differentiated states.

In summary, the stereotyped cell fusions during morphogenesis of the male tail tip change intercellular junctions, cell shapes and relative positions of membranes and nuclei. Mutations in at least six loci interfere with morphogenesis of the male tail tip and result in pointy spikes that protrude ventrally and posteriorly from the adult male tail (leptoderan tail tips). One of this mutations (*lep-1(bx37)*) results in a delay of shape changes and cell fusions suggesting that these two events are interrelated (Nguyen et al., 1999). However, shape changes sometimes occur before cell fusion suggesting that cell fusion is not absolutely necessary for the morphological changes that occur during the same developmental stage.

A FUSOMORPHOGENETIC HYPOTHESIS

To explain the function of cell fusion in the context of intracellular membrane traffic during morphogenesis we have recently proposed a new working hypothesis. Fusomorphogenesis proposes that the driving force responsible for changing cell shapes that result in organ formation is the redistribution of plasma membrane domains through the combination of polarized fusion and targeting of membrane components (Podbilewicz, 2000). The fusomorphogenetic model is based on ultrastructural and dynamic analyses of cell fusion in living and fixed specimens of *C. elegans* (Podbilewicz and White, 1994; Mohler et al., 1998; Nguyen et al., 1999; Sharma-Kishore et al., 1999).

Figure 8 shows the basic principles proposed by the fusomorphogenetic model. Cell fusion is initiated as one or more pores are formed between the plasma membranes of a pair of cells, the pore(s) located at or close to the apical domains of the plasma membranes dilate and then the membranes vesiculate. Thus, the initial ultrastructural steps of cell fusion are: (1) pore formation, (2) dilation of the pore, and (3) vesiculation of the fusing membranes (Mohler et al., 1998; Nguyen et al., 1999). The newly formed vesicles derived from specific domains marked by the adherens junctions that link the pair of cells are transported to a different domain of the membranes of the newly formed syncytium (e.g., to the lateral or the basal plasma mem-

brane). Fusion of the vesicles to the lateral, basal, or basolateral plasma membranes is coupled to a change in cell shape.

Alternatively, changes in shape without cell fusion may be obtained by biosynthesis of new membranes through the secretory pathway or by the use of existing stocks of vesicular organelles that can fuse to generate polarized changes in cell shapes when needed. Thus, there are two alternative pathways to obtain morphogenetic events mediated by membrane fusion and intracellular vesicular traffic. First, vesicles derived from fusing membranes can be targeted directly to a different domain of the plasma membrane where they fuse (Fig. 8A–C). Second, specific insertion of newly synthesized membranes to the basolateral domains of the plasma membrane can bypass the necessity for cell fusion and membrane recycling (Fig. 8D–F). Since most cells have the potential of synthesizing new membranes the later may be the default pathway. A combination of both strategies and a balance between the two reactions involve membrane fusion as a proposed driving force for morphogenesis (Fig. 8G and H). It is hypothesized that nematodes with syncytial hypodermis like *Caenorhabditis elegans* use cell fusion followed by polarized membrane recycling and marine nematodes with cellular hypodermis like *Enoplus brevis* use *de novo* synthesis of membranes coupled to polarized exocytosis. The fusomorphogenetic model predicts that specific mutations that disrupt the cell fusion machinery may be bypassed by the use of a more active biosynthetic pathway and polarized secretion. Changes in the cellular and molecular mechanisms that control the fusomorphogenetic reactions may be involved in the evolution of developmental mechanisms.

Fusomorphogenesis as described here followed by cytoskeletal reorganization that certainly will be coupled to the membrane fusion and traffic events may also be the force that drives organ formation in bones, muscles, and placenta (Fig. 10). Alternatively, cell fusion may act in a permissive manner. The actin and tubulin dependent motors are likely to provide the motive force for morphogenetic movements and cell fusion may allow the rapid deployment of membranes to specific domains of the plasma membrane increasing the surface area thereby facilitating the shape changes.

GENETIC AND BIOCHEMICAL DISSECTION OF FUSOMORPHOGENESIS

To analyze the molecular basis for epithelial cell fusion and to test the fusomorphogenetic hypothesis we and others have sought mutations in *C. elegans* that specifically affect cell fusion. To identify mutations causing abnormal fusions in the hypodermis we looked for mutants with abnormal patterns of adherens junctions (zonula adherens; ZA). The presence of ZA is a good marker for the integrity of the membrane between two adjoining cells. In *C. elegans* ZAs can be efficiently detected using the monoclonal antibody MH27, which recognizes a cloned ZA protein (Waterston, 1988; Francis and Waterston, 1991; Podbilewicz and White, 1994). Using MH27 immonufluorescence staining we performed a clonal screen of 5,252 haploid genomes, and identified many mutants with abnormal cell boundaries and fusions. The disappearance of an adherens junction between two cells defines a particular fusion event. In summary, four classes of mutations that affect epithelial development and fusions were obtained (B.P. unpublished results):

(1) Jigsaw puzzle cell pattern in the hypodermis of embryos (abnormal fusions; n=34)
(2) More cell fusions than in wild-type embryos (hyperfusion; n=44)
(3) Disorganized epithelia with defects in morphogenesis (n=57)
(4) Postembryonic defects in hypodermal cell fusions (n=34).

The pilot screens did not yield mutants with fewer fusions or no fusions. Other screens of nearly 100 chromosomal deficiencies have identified regions in the genome that when deleted result in phenotypes similar to the four classes described above (Ahnn and Fire, 1994; Chanal and Labouesse, 1997; Labouesse, 1997; Terns et al., 1997).

To identify mutations that result in no fusions or fewer fusions (hypofusion) than in wild-type, we performed alternative screens with a transgenic strain expressing a Green Fluorescent Protein (GFP) reporter gene fused to MH27 that localizes to the adherens junctions (Bill Mohler, John White, G.S. and B.P., unpublished data). This reporter protein (MH27-GFP) localizes to the specific hypodermal cells before, during and after the cell fusion events (Mohler et al., 1998). Expression of MH27-GFP is very similar to the staining obtained with MH27 antibody in all stages (Mohler et al., 1998).

The strain containing the MH27-GFP reporter protein was mutagenized with EMS (Brenner, 1974). A screen was performed in which the MH27-GFP expression patterns in living worms were observed with fluorescence microscopes. A candidate mutation (*hy2*) causing L1 arrest and fewer fusions in the dorsal hypodermis was isolated among other mutations from the four classes described above. More recently a viable mutant called *elf-1(oj55)* was identified in a similar screen which used MH27 fused to GFP to monitor adherens junctions (Bill Mohler, personal communication). The mutant *elf-1(oj55)* shows defects in vulva formation, elongation, and male tail formation strongly suggesting that cell fusion is required for various aspects of *C. elegans* morphogenesis.

The zygotic lethal mutation *zu316* was isolated in a screen for elongation defective embryos (Mike Costa and Jim Priess, personal communication). *zu316* arrested embryos have fewer dorsal fusions than in wild-

type. At 15°C the *duf-1(zu316)* embryos arrest between 1.5 to 2-fold stage with fewer fusions in the dorsal hypodermal cells (Dorsal Un-Fused phenotype). At the permissive temperature (25°C) most embryos arrest at 3-fold stage, some L1 larvae hatch with normally fused hypodermal cells and variable posterior defects, and some embryos arrest with the 15°C elongation arrest phenotype (Tamar Gattegno and B. P., unpublished results). Further genetic, cellular, and molecular characterization of *duf-1(zu316cs)* and *elf-1(oj55)* will help to test the fusomorphogenetic hypothesis and the function of cell fusion during embryonic and postembryonic development.

Additional mutations have been isolated that affect the regulation of some, but not all, postembryonic cell fusions. Cell fusions in the lateral hypodermal seams are affected by mutations in the heterochronic genes (reviewed in Ambros and Moss, 1994; Slack and Ruvkun, 1997). *lin-29* encodes two predicted zinc finger proteins that have a role in executing the terminal differentiation of the seam cells during the L4-to-adult molt (Ambros and Horvitz, 1984; Ambros, 1989; Rougvie and Ambros, 1995; Bettinger et al., 1996; Bettinger et al., 1997; Euling et al., 1999). During the last molt the seam cells exit from the ectoblastic cell cycle and fuse longitudinally to form the two adult seam syncytia on each side of the animal that secrete specialized cuticular structures called alae (Sulston and Horvitz, 1977; Singh and Sulston, 1978) in a *lin-29*-dependent fashion (Ambros and Horvitz, 1984; Ambros, 1989; Rougvie and Ambros, 1995). Mutations in *lin-29* also affect the morphogenesis of the male tail and the vulva (Bettinger et al., 1997; Euling et al., 1999) but cell fusion defects have not been reported in these organs.

There are other mutations that affect specific cell fusion events in some but not all cells. For example, *lin-25* is a gene involved in late stages in vulva induction that affects seam cell fusion (Nilsson et al., 1998) and *ref-1*, a newly identified regulator of cell fusion that when mutated causes that specific Pn.p cells fail to fuse to hyp7 (Scott Alper and Cynthia Kenyon, personal communication).

Thus, genes such as *lin-25*, *lin-29*, *lep-1*, and *ref-1* may regulate the spatial and temporal specificity of some cell fusion events. Genes such as *ceh-13*, *mab-5*, *lin-39*, and *egl-27* appear to be involved in the generation of anteroposterior patterns of cell fusion that can be modified due to homeotic cell type transformations. However, mutations in homeotic genes (e.g. *ceh-13* and *lin-39*) may transform a cell that normally fuses into a different cell type that would not normally fuse. *duf-1* and *elf-1* are candidate genes that may be directly needed for the mechanism of cell fusion in *C. elegans*. A combination of genetic and biochemical approaches will certainly be needed before a good understanding of the regulation and mechanisms of cell fusion in *C. elegans* is obtained.

FUTURE PERSPECTIVES

Is Cell Fusion Autonomous or Do You Need Two to Tango?

Based on the following evidence cell fusion in *C. elegans* is proposed to be cell autonomous and not an induced process by cells that do not fuse. First, specific cells derived from the founder cells AB, C, MS, and P4 but not the descendants of E and D are fusion-competent (Sulston and Horvitz, 1977; Sulston et al., 1983; Podbilewicz and White, 1994). Thus, as with other cell fates in *C. elegans* cell fusion is not restricted to the descendents of a few founder cells. Second, the combinatorial expression of genes of the Hox cluster (homeotic selector genes) regulate patterning of the anteroposterior body axis in the ventral ectodermal Pn.p and control cell fusion autonomously (Salser et al., 1993; Wang et al., 1993; Cowing and Kenyon, 1996;

Fig. 9. Schematic representation (left panel) and Nomarski photomicrographs (right panel) of ventral epidermal cell fate specification in *Caenorhabditis elegans* and *Pristionchus pacificus*. **(A)** *Caenorhabditis* wild-type: the vulval equivalence group is formed by P(3-8).p; P(3,4,8).p have the 3° sublineage (yellow); P(5-7).p form the vulva with P(5,7).p having the 2° sublineage (red) and P6.p having the 1° sublineage (dark blue). P12.pa forms part of the rectum (light blue). **(B)** *Pristionchus* wild-type: P(1-4, 9-11).p undergo programmed cell death; P(5,7).p form the vulva; P7.p (pink) is limited in its developmental competence, P8.p (green) is incompetent to adopt vulval fates. **(C)** *Caenorhabditis lin-39(n1872)* mutant: all vulval precursor cells (VPCs), P(3-8).p, adopt the fate of their anterior-posterior lineage neighbors and fuse with the hypodermis. **(D)** *Pristionchus lin-39(sy319)* mutant: VPCs undergo programmed cell death. **(E)** *Caenorhabditis mab-5(e1239)* mutant: most animals have a normal vulva. However, P(7,8).p differ in their developmental competence from wild-type animals. **(F)** *Pristionchus mab-5(tu74)* mutant; P8.p remains competent to form vulval tissue and eventually differentiates (D; arrow). V, vulva; E, epidermal; F, fusion. The Nomarski photomicrographs show only the central body region. Reprinted from *Trends in Genetics,* volume 15, Eizinger et al., Evolutionary change in the functional specificity of genes, pp. 197–202. ©1999, with permission from Elsevier Science.

Fig. 10. The fusomorphogenetic hypothesis applied to organ formation in different organisms. Cell fusion coupled to polarized membrane recycling may be a morphogenetic force in vertebrates and invertebrates. **(A)** Anterior-posterior elongation of the *C. elegans* embryo may be similar to the formation of myotubes in muscles (dorsal view). **(B)** Several mononucleated osteoclast precursors may fuse to form giant multinucleated osteoclasts that recycle the membranes to the specialized domain where bone resorption occur. Syncytial giant cells are also generated in certain tumors. **(C)** Intratoroidal fusions in the vulva result in a change in ring shape. The resulting ring has a larger lumen because the lateral membranes may be inserted into the central apical domain by intracellular vesicular traffic followed by docking and fusion (dorsal view). **(D)** Transverse section of the elongating *C. elegans* embryo showing two dorso-ventral cell fusions and one ventral left-right cell fusion that result in a hypodermal ring cell. The lateral membranes are transferred to the basal (internal ring) domain and the cytoplasm becomes thinner as the embryo elongates (dorsal is toward the top of the page). **(E)** Fusomorphogenesis applied to the formation of the syncytial trophoblast of the placenta to accomplish dynamic changes in cell and organ shapes (transverse section). Blue circles, nuclei; red lines, fusing plasma membranes or recycled membranes through intracellular red vesicular carriers.

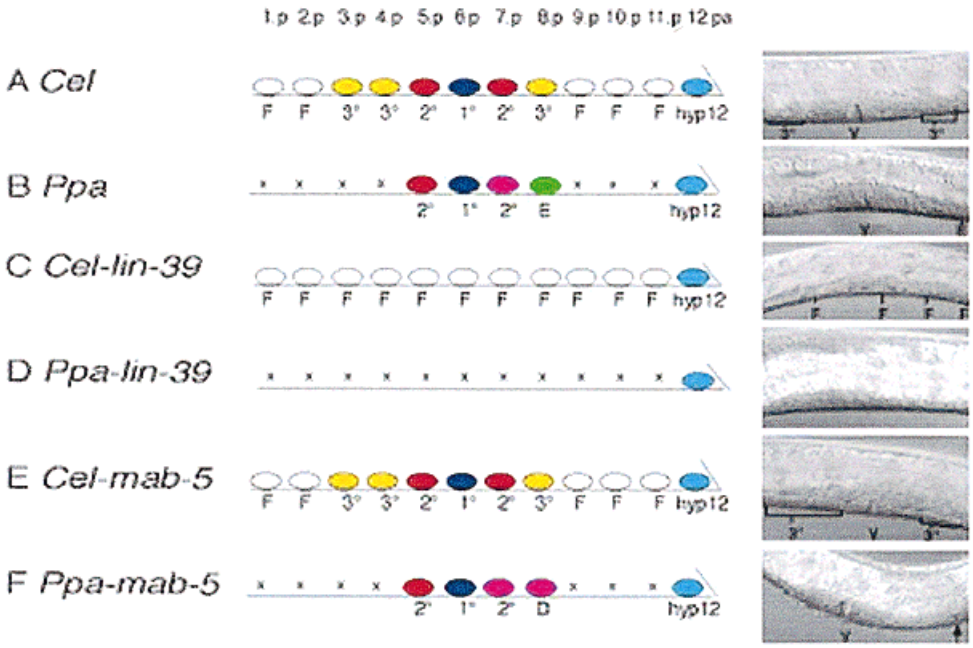

Figure 9.

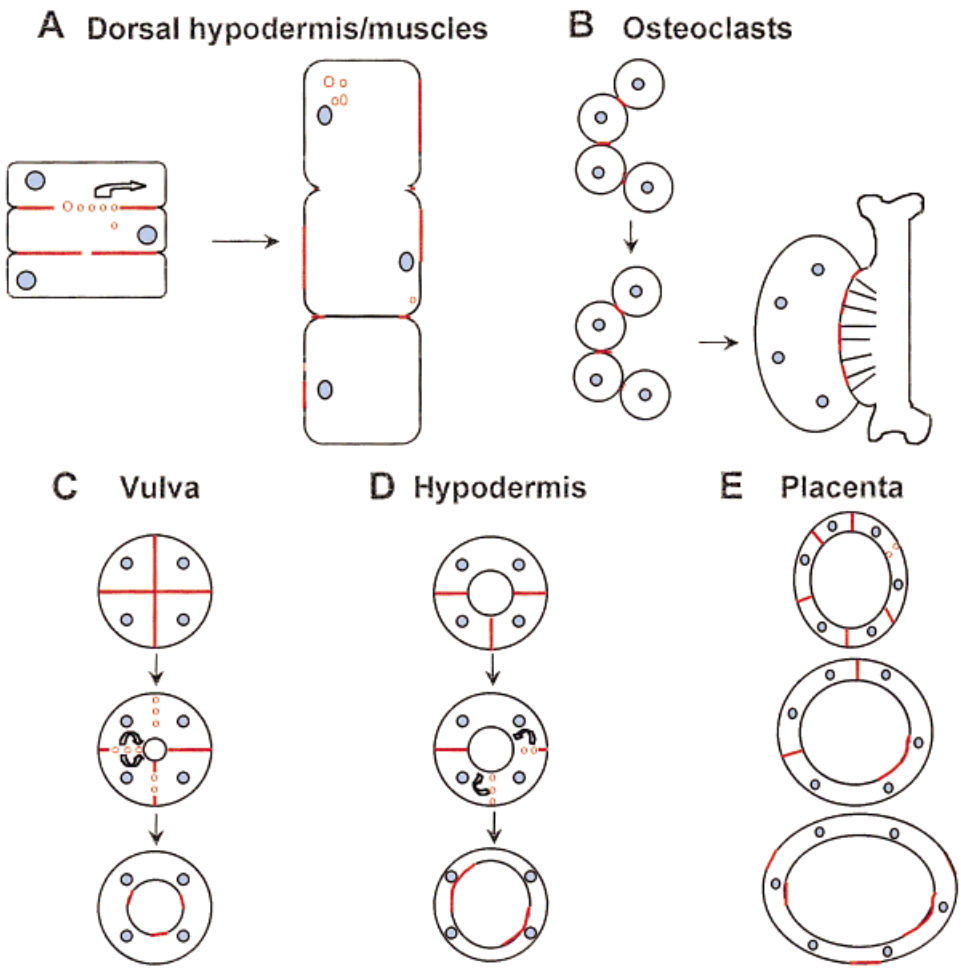

Figure 10.

Ch'ng and Kenyon, 1999). Third, *lin-15* expression in a pre-existing hyp7 syncytium, that is one of the fusion partners, is required for the fusion of certain uninduced vulva precursor cells (VPCs) to the hyp7 syncytium itself (Herman and Hedgecock, 1990; Thomas and Horvitz, 1999). Fourth, laser ablation of one of the two cells that fuse in the vulva results in self-fusion of the surviving cell (Sharma-Kishore et al., 1999). In other words separate domains of a single fusion-competent cell can fuse to one another in the absence of its natural fusion partner. Fifth, incomplete "real" and pseudovulvae in a *let-60*/ras gain-of-function mutant form complete rings that undergo self-fusion autonomously (Shemer et al., 2000). In summary, specific spatial and temporal patterns of many cell fusion events appear to be regulated autonomously within the fusion-competent cells. However, the evidence cited above does not rule out certain forms of cell-cell interactions. Inductive regulation of cell fusion by cells that do not themselves play a role in the fusion process has not been found in *C. elegans*.

Contrary to all the data described above that suggest that the process of cell fusion is cell autonomous, it has been proposed that *lin-29* function is cell non-autonomous for cell fusion in the seam cells (Bettinger et al., 1997). This conclusion is based on elegant mosaic analysis that showed that two adjacent cells must both be *lin-29(+)* in order to fuse (Bettinger et al., 1997). However, the fact that it was found that *lin-29* function is cell autonomous with respect to cell division and alae synthesis in the same mosaic analyses may indicate that *lin-29(−)* cells escape fusion to the adjacent *lin-29(+)* cells because the mutant cells are dividing instead of undergoing endoreduplication and three hours later becoming fusion-competent (Hedgecock and White, 1985; Podbilewicz and White, 1994). In summary, we propose that the *lin-29(−)* cells do not fuse with adjacent *lin-29(+)* because they are in a different stage in the cell cycle that is fusion-incompetent and not because *lin-29* function is cell nonautonomous for cell fusion. The decision of whether to fuse or not to fuse in *C. elegans* appears to be cell autonomous but at the end of the day either you need two to tango or you need a self-fusion.

The apparent autonomous behavior of cell fusion has not been tested directly in all the organs involved and future experiments may prove that some but not all cell fusions are induced as occurs in leeches (Isaksen et al., 1999).

Evolution of Cell Fusion in Nematodes

C. elegans and many related nematodes both free living and parasitic have syncytial epidermal organs. However, nematoda is such a vast and diverse group of organisms at the morphological, developmental, ecological, physiological and molecular levels (Chitwood and Chitwood, 1974; Fitch et al., 1995; Blaxter, 1998; Blaxter et al., 1998; Goldstein et al., 1998; Félix, 1999) that it is not surprising to find that many marine nematodes have cellular (non-syncytial) hypodermis (Smith and Stephenson, 1970; Chitwood and Chitwood, 1974; Voronov and Panchin, 1998).

An evolutionary comparison of cell fusion between closely related but distinct nematodes and between species from far-related groups may help to resolve some conflicts assigning phylogenetic relationships and will provide the opportunity to understand the evolution of cellular and molecular developmental mechanisms of cell fusion (Fig. 8). The evolutionary history of cell fusion has been analyzed looking at the different Rn.p cells that contribute to male tail seam syncytia (set) in different species (Fitch and Emmons, 1995; Fitch, 1997). Thus, changes in tail morphology apparently result from small changes in cell position, cell shape, cell adhesive properties and cell fusion. Fusogen localization (presumably to the adherens junctions) may determine whether a cell will fuse or not fuse at a specific time and location. This simple choice may be sufficient to change the morphology of an organ according to the fusomorphogenetic model (Figs. 8 and 10).

Male tail tip morphogenesis also provides a good model system to study the role of cell fusion in evolution. For example, in the tail of *Oscheius myriophila* and *Rhabditella axei* the tail tip cells do not fuse and there is no retraction during male tail morphogenesis resulting in the generation of fine pointed ("leptoderan") tail tips. In a *Rhabditis* species, the anterior tail tip cells do fuse, but hyp10 fails to fuse and retraction fails. The situation in *Rhabditis* nematodes is very similar to the phenotypes observed in mutants of *Caenorhabditis elegans* that result in the transformation from a peloderan ("retracted") morphology in the wild-type to a leptoderan phenotype in the *lep-1* mutants (Fitch, 1997; Nguyen et al., 1999). Changes of cell fusion resulting in distinct structural differentiated states may help to understand how genetic and developmental programs are modified through evolution.

The first molecular analyses of the developmental regulation of cell fusion in nematodes involve the function of genes of the homeotic cluster (reviewed in Eizinger et al., 1999; Félix, 1999). In *C. elegans lin-39* and *mab-5* have a combinatorial function in the determination of cell fusion fates in the ventral Pn.p cells (Fig. 6). Whereas in *C. elegans* and other rhabditid nematode hermaphrodites, the P(1-2,9-11).p cells fuse with hyp7, these cells undergo programmed cell death in *Pristionchus pacificus* and other diplogastrids (Fig. 9; Sommer et al., 1996; Sommer and Sternberg, 1996; Jungblut and Sommer, 1998; Félix et al., 1999). In *P. pacificus* P(1-4,9-11)p die in the L1 stage and LIN-39 permissively inhibits programmed cell death in P(5-8).p, or promotes their survival (Eizinger and Sommer, 1997; Sommer et al., 1998) (Fig. 9). In *Halicephalobus sp*., P(1-4,9-11).p die and the gonad sends a survival signal that prevents programmed cell death of P(5-8).p that forms the vulva (Félix and Sternberg, 1998). It is not known whether the survival signal involves activation of *lin-39* in *Halicephalobus sp*. (Félix, 1999).

Both *Ce-lin-39* and *Ppa-lin-39* mutants are vulvaless, but in the *P. pacificus* mutants the VPCs undergo programmed cell death instead of cell fusion (Eizinger and Sommer, 1997). In the double mutant *Ppa-lin-39*; *Ppa-ced-3* characterized by being cell death defective, a functional vulva is formed in the absence of *lin-39* activity, indicating that *lin-39* has a permissive function by repressing apoptosis (Sommer et al., 1998). In *C. elegans, lin-39* may promote vulval fates in the VPCs, repress cell fusion, or both. We hypothesize that if in the double mutant *Ce-lin-39*; *Ce-elf-1* characterized by being cell fusion defective, a functional vulva was formed in the absence of *lin-39* activity, it would indicate that *lin-39* has a permissive function by repressing cell fusion in *C. elegans*. Developmental and molecular comparisons between *P. pacificus* and *C. elegans* with respect to *lin-39* activity will define how *lin-39* in *P. pacificus* represses programmed cell death and in *C. elegans* autonomously represses programmed cell fusion. Cell fusion and cell death may be somehow linked to one another in development, and the different mechanisms of vulval formation in different species may be uncovering this connection.

Evolutionary comparisons between cell fusion in different organs and between different species will help clarify the role of cell fusion in organ formation

Are There Universal Conserved Molecular Mechanisms for Cell Fusion?

Although many fusion events have been described in biological systems, to date, the best characterized fusogenic mechanisms are those involving glycoproteins of enveloped viruses (Weis et al., 1988; Stegmann et al., 1989; Carr and Kim, 1993; Carr and Kim, 1994; Hernandez et al., 1996; Chernomordik et al., 1997; Chernomordik et al., 1998). Cells infected with enveloped viruses (e.g., HIV and Influenza) are often induced to form multinucleated cells through the process of cell fusion. With the exception of viral fusions, little is known about the molecular and biochemical mechanisms involved in cell fusion. To understand the function of cell fusion during development and to study its molecular mechanisms it is necessary to identify the proteins directly involved in this process. Only within closely related families of enveloped viruses are there some sequence similarities between fusogenic molecules (Hernandez et al., 1996). Based on the large number of known viral fusogens and their enormous molecular diversity it is unlikely to assume that there will be universal fusogenic molecules even within the same species. Intracellular protein complexes responsible for membrane docking and fusion have been studied using genetic, molecular, and biochemical approaches (Pryer et al., 1992; White, 1992; Prevsner and Scheller, 1994; Rothman, 1994; Denesvre and Malhotra, 1996; Weber et al., 1998). Since cell fusion originates at the extracellular part of the plasma membrane ("exoplasmic"), the protein complexes implicated in vesicle docking and fusion ("endoplasmic") are most probably not directly involved in cell fusion.

Many genes from different organisms have been implicated in cell fusion in vertebrates (Entwistle et al., 1988; Blobel et al., 1992; Myles et al., 1994; Yagami-Hiromasa et al., 1995; Barnoy et al., 1996), sea urchin (Foltz et al., 1993; Ohlendieck and Lennarz, 1995), yeast (McCaffrey et al., 1987; Trueheart et al., 1987; Trueheart and Fink, 1989; Elion et al., 1995; Elia and Marsh, 1996; Elia and Marsh, 1998; Gammie et al., 1998; Philips and Herskowitz, 1998), and Drosophila (Paululat et al., 1995; Rushton et al., 1995; Doberstein et al., 1997; Erickson et al., 1997; Paululat et al., 1997; Nolan et al., 1998; Magie et al., 1999). For example, two related ADAM proteins, fertilin and meltrin-α have been implicated in sperm-egg fusion and myoblast fusion respectively (Primakoff et al., 1987; Blobel et al., 1992; Myles et al., 1994; Yagami-Hiromasa et al., 1995). Metalloprotease activities may participate in membrane fusion events (Roe et al., 1988) but the direct involvement of many putative fusogens specific to cell fusion including members of the metalloprotease-disintegrin ADAMs family in *C. elegans* remains to be proved in a rigorous way using a combination of genetic and biochemical analysis. We propose that the fusomorphogenetic model may be applied to the formation of different organs not only in *C. elegans* but also in the formation of myotubes in muscles, multinucleated osteoclasts in bones, and syncytial trophoblast in the placenta (Fig. 10). Future studies will have to test the following questions:

(1) Is there cell fusion-mediated redistribution of membranes during organ formation?
(2) If the answer to question (1) is positive then what will happen if different steps in the fusomorphogenetic pathway are blocked by biochemical or by genetic means?
(3) What are the molecular mechanisms of fusomorphogenesis?

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