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Maternal Control of Pattern Formation in Early *Caenorhabditis elegans* Embryos

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Genetic screens for recessive, maternal-effect, embryonic-lethal mutations have identified about 25 genes that control early steps of pattern formation in the nematode *Caenorhabditis elegans*. These maternal genes are discussed as belonging to one of three groups. The par group genes establish and maintain polarity in the one-cell zygote in response to sperm entry, defining an anterior/posterior body axis at least in part through interactions with the cytoskeleton mediated by cortically localized proteins. Blastomere identity group genes act downstream of the par group to specify the identities of individual embryonic cells, or blastomeres, using both cell autonomous and non-cell autonomous mechanisms. Requirements for the blastomere identity genes are consistent with previous studies suggesting that early asymmetric cleavages in the *C. elegans* embryo generate six "founder" cells that account for much of the *C. elegans* body plan. Intermediate group genes, most recently identified, may link the establishment of polarity in the zygote by par group genes to the localization of blastomere

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identity group gene functions. This review summarizes the known requirements for the members of each group, although it seems clear that additional regulatory genes controlling pattern formation in the early embryo have yet to be identified. An emerging challenge is to link the function of the genes in these three groups into interacting pathways that can account for the specification of the six founder cell identities in the early embryo, five of which produce somatic cell types and one of which produces the germline.

I. The Early *Caenorhabditis elegans* Embryo

One fascinating question in developmental biology, put simply, is how does it all get started? How does a single cell, such as an oocyte or a zygote, initiate the processes that generate a specific and complex multicellular body? For many years, the fruit fly *Drosophila melanogaster* was the only metazoan used for large-scale genetic screens to study the early steps in pattern formation (St. Johnston and Nusslein-Volhard, 1992). However, genetic screens in the nematode *Caenorhabditis elegans* and in *Arabidopsis thaliana* have begun to broaden our view of early development by identifying additional regulatory loci in another animal and in a plant embryo (Jurgens, 1995; Kemphues and Strome, 1997; Schnabel and Priess, 1997). Mechanistic comparisons of pattern formation in these and other early embryos may reveal not only how different life forms develop but perhaps also how they evolve.

For a comparison of early development, the insect and nematode embryos are impressively different. In *Drosophila*, the embryo is a 500-μm-long syncytium in which a peripheral monolayer of nuclei share a common cytoplasm until completion of the 13th round of mitosis (St. Johnston and Nusslein-Volhard, 1992). Diffusion of transcriptional and translational regulators from localized sources forms morphogenetic gradients of positional information that pattern large fields of nuclei. In dramatic contrast, the 50-μm-long *C. elegans* embryo is completely cellularized, and the early events that control patterning must negotiate the plasma membranes that partition all nuclei (Sulston *et al.*, 1983). Not surprisingly, mechanisms very different from those in *Drosophila* appear to pattern the early *C. elegans* embryo (see the following).

Another notable difference between flies and worms is the relative importance of pattern formation during oogenesis versus after fertilization. In an early insect embryo, many of the events that establish anterior–posterior and dorsal–ventral asymmetry begin during oogenesis, with fertilization activating previously localized regulators (St. Johnston and Nusslein-Volhard, 1992). In *C. elegans*, the body axes form not during oogenesis but sequentially during embryogenesis, with sperm entry establishing an anterior–posterior axis (see below). Such seemingly dramatic differences in early development are not unusual. For example, in *Xenopus* embryos the localization of maternal mRNAs in the oocyte, the point of sperm entry, and rotation of the zygote's cytoplasmic cortex all appear necessary

for specifying the body axes. One largely unmet challenge in developmental biology is to understand how and perhaps even why early embryos begin development in such remarkably different ways. As one route to a broader understanding, the genetics and sequenced genome of *C. elegans* provide rapid access to the molecules and mechanisms that initiate pattern formation in a cellularized animal embryo.

A. Maternal Control of Early Embryogenesis

Although the fly and worm embryos seem very different, they are alike in developing rapidly and in depending extensively on maternally supplied regulatory factors to control early steps in pattern formation. As with *Drosophila*, extensive genetic screens for recessive, maternal-effect, embryonic-lethal mutations in *C. elegans* have identified many regulatory factors that govern the early steps of patterning. Thus, for the genes discussed here, homozygous mutant mothers become fertile adults but then produce broods in which all the embryos die. The mutant embryos differentiate well, but exhibit highly penetrant and specific defects in pattern formation. In almost all cases, the mutations are strictly maternal: mating wild-type males into homozygous mutant mothers to produce $-/+$ embryos does not rescue the defect, as strictly maternal genes must be expressed by the mother during oogenesis. In this chapter, I refer to the embryos produced by self-fertilization in homozygous mutant mothers as mutant embryos or mutants.

In *C. elegans*, maternal genes are transcribed in the germline nuclei that line the syncytial distal arm of the tubular nematode ovary, sharing a common cytoplasmic "core" (Fig. 1). *In situ* hybridization studies have shown that maternal mRNAs are transcribed in germline nuclei and accumulate in the cytoplasmic core of the distal arm (Seydoux and Fire, 1994). As the ovary bends back on itself to form the proximal arm, single nuclei arrested in meiosis become cellularized to form a short, single row of oocytes (Schedl, 1997). Each oocyte appears to acquire maternal gene products from the core cytoplasm that is pinched off with a nucleus during cellularization. Nutritional yolklike factors appear to be secreted by intestinal cells, which are in close proximity to the ovary, and taken up in oocytes by endocytosis. Oocytes enlarge as they near the proximal end of the ovary and eventually enter the spermatheca, where fertilization occurs. Sperm entry initiates the completion of meiosis by the maternal pronucleus, the rapid production of a tough chitinous eggshell secreted by the zygote, and an extensive cytoskeleton-dependent reorganization of the maternally derived cytoplasm, setting the stage for maternal gene products to initiate different cell fate programs in different regions of the embryo (Kemphues and Strome, 1997; see the following).

Recessive, nonconditional, embryonic-lethal mutations that specifically affect pattern formation have been identified in about 25 maternally expressed genes in

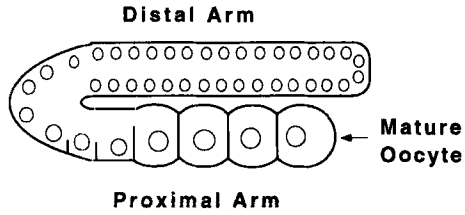

Fig. 1 One arm of the bilaterally symmetrical *C. elegans* ovary. The distal arm of the ovary is a syncytium of nuclei maintained in a mitotic state near the tip of the ovary but that enter into meiosis as they move proximally (Schedl, 1997). At the bend of the ovary, individual nuclei become cellularized, acquiring maternal gene products from the common cytoplasmic core of the ovary. As oocytes approach the spermatheca (not shown) at the proximal end of the ovary, the oocyte pronucleus moves to the side of the oocyte opposite the spermatheca. In this and all subsequent figures, see the text for additional details and references.

C. elegans (Table I). Mutations in these genes result in patterning defects without affecting more general cellular processes such as mitosis, metabolism, or even differentiation. Because homozygous mutant mothers must survive to adulthood to reveal maternal-effect phenotypes, and because many maternal genes also have essential zygotic functions, it is likely that important regulatory loci have been missed in spite of extensive screening for nonconditional maternal-effect mutations.

The ability to efficiently eliminate maternal gene functions from *C. elegans* by using antisense RNA has provided a new and powerful tool for the identification of regulatory loci (Guo and Kemphues, 1996a,b). By microinjecting antisense RNA into the syncytial ovary of wild-type hermaphrodites, one can test maternal requirements for genes that mutate to give zygotic-lethal phenotypes or for genes in which no mutations have been identified in *C. elegans* but that are related by sequence to known regulatory genes in other organisms. The use of antisense RNA microinjection has led to the identification of additional genes required for pattern formation in the early embryo and is certain to play an increasingly important role with the nearly complete sequence of the worm genome now available. Finally, genetic screens for temperature-sensitive mutations and molecular and genetic screens for loci that interact with previously identified genes should continue to identify new regulatory factors and clarify how developmental regulators interface with more general cellular machineries to pattern the early embryo.

B. Three Groups of Maternal Genes in *C. elegans*

For heuristic purposes, it is useful to group the maternal-effect mutants identified in *C. elegans* on the basis of their shared phenotypic traits. In fact, very few of the mutants identified thus far have identical or even nearly identical phenotypes,

Table I Maternal Loci in *C elegans*: Gene Names and Molecular Identities (See Text for References)

Gene	Name	Molecular identity
	Par Group Genes	
let-99	*Let*hal	?
par-1	*Par*titioning-defective	Ser–Thr kinase; binds a nonmuscle myosin
par-2	Same	Novel; ATP-binding site
par-3	Same	Novel; two PDZ domains
par-4	Same	Ser–Thr kinase
par-5	Same	?
par-6	Same	?
mes-1	*M*aternal-e*ff*fect *s*terile	?
	Blastomere Identify Group Genes	
P_1 subgroup		
pal-1	*P*osterior *al*ae defective	Homeodomain protein; putative transcription factor
pie-1	*P*harynx and *i*ntestine *e*xcess	TIS-11-like Zn^{2+} finger ptn
skn-1	*Skn* excess	bZIP-like putative transcription factor; lacks a leucine zipper
pop-1	*P*osterior *p*harynx defective	HMG domain protein; putative transcription factor
mom-1	*M*ore *m*esoderm	Porcupine homologue; ER protein required for Wnt secretion
mom-2	Same	Wingless/Wnt homologue; putative secreted glycoprotein ligand
mom-3	Same	?
mom-4	Same	?
mom-5	Same	Frizzled homologue; putative receptor for Wnt ligands
AB subgroup		
aph-2	*A*nterior *ph*arynx defective	Novel membrane-associated extracellular protein
apx-1	*A*nterior *p*harynx *ex*cess	Delta-like transmembrane protein; putative GLP-1 ligand
glp-1	*G*erm*l*ine *p*roliferation defective	Notchlike transmembrane protein; putative receptor
	Intermediate Group Genes	
mex-1	*M*uscle *ex*cess	TIS-11-like Zn^{2+} finger ptn
mex-3	Same	Two KH domains; putative RNA-binding protein
pos-1	*P*osterior localized mRNA	TIS-11-like Zn^{2+} finger ptn

but for this review I have grouped all maternal genes into one of three groups. The first, the par group, consists of eight genes, *par-1* through *par-6*, *let-99*, and *mes-1*. Mutational inactivation of any one of these genes results in losses of

asymmetry in early embryos and widespread defects in cell fate patterning. As summarized here, the *par* genes are essential for establishing or maintaining anterior–posterior polarity in the one-cell zygote. Inactivation of maternal genes in the second blastomere identity group causes much more specific defects in the fates of individual blastomeres, without causing the more general defects in asymmetry and cell fate patterning characteristic of par group mutants. These genes include *aph-2*, *apx-1*, *glp-1*, *pal-1*, *pie-1*, five *mom* genes, and *skn-1*. The mutant phenotypes caused by inactivation of these genes fall into several distinct subgroups, as reflected by the different gene names. The genes in this group thus appear to regulate development more specifically and probably act later than the *par* genes. The third and smallest category consists of only three genes: *mex-1*, *mex-3*, and *pos-1*. Mutations in these intermediate group genes result in phenotypes with a pleiotropy in between those observed for the par group and blastomere identity group mutants. The intermediate group genes may link the establishment and maintenance of polarity in the one-cell zygote to the specification of individual blastomere identities in the early embryo.

C. Polarization of a Dynamic Cytoskeleton and the Establishment of Anterior–Posterior Asymmetry

Genes in the par group provide at least a glimpse of the machinery that polarizes a *C. elegans* zygote after fertilization. This usage of the term polarization specifically refers to the generation of asymmetry within individual cells and therefore is distinct from another common usage of polarity, which is in reference to morphogenetic patterning across a field of cells. Because the polarization of single cells in different organisms appears to require a functional cytoskeleton (Drubin and Nelson, 1996), an overview of our understanding of cytoskeletal dynamics and polarity in the early *C. elegans* embryo is prerequisite to a discussion of the par group genes.

As in *Drosophila*, studies of pattern formation in *C. elegans* indicate that the polarization of a dynamic cytoskeleton is an early and fundamental step for generating polar asymmetries (Grunert and St. Johnston, 1996; Guo and Kemphues, 1996b). In wild-type *C. elegans* embryos, three early asymmetries are evident at the one-cell stage, and all three require functional microfilaments. One obvious early asymmetry is the posterior displacement of the first mitotic spindle, which results in the production of two daughters with very different fates: a smaller posterior blastomere called P_1 and a larger anterior blastomere called AB (Fig. 2). This polar asymmetry is lost upon treatment of wild-type embryos with cytochalasin D for only brief intervals to transiently disrupt the actin cytoskeleton during part of the first cell cycle (Hill and Strome, 1988, 1990). Cytochalsin D treatment results in some embryos having apparently reversed polarity, with a smaller P_1-like cell located anteriorly and a larger AB-like cell located

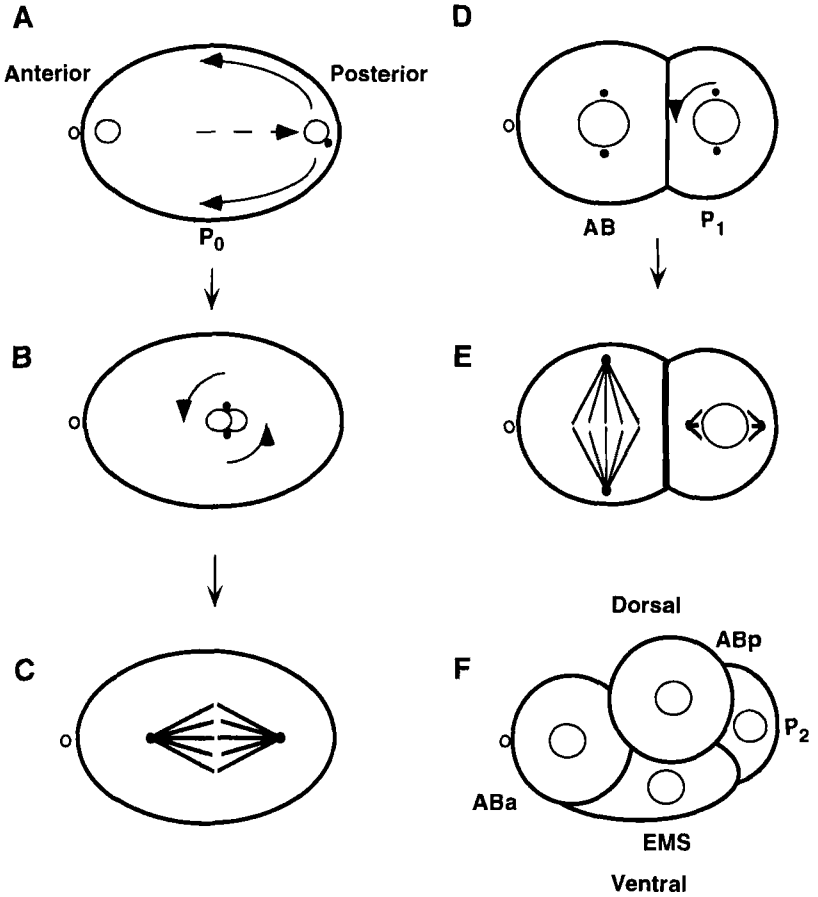

Fig. 2 Early asymmetries in the *C. elegans* zygote at the one-cell (A–C), two-cell (D, E), and four-cell stages (F) of embryogenesis. (A) The position of the sperm pronucleus and its accompanying centriole define the posterior pole of the zygote and initiate cytoplasmic flux in the posterior half of the zygote (curved and dashed arrows). (B) After congression of the pronuclei, the initially transverse spindle, indicated by solid dots to represent centrosomes, rotates to lie along the long axis (curved arrows). (C) The first mitotic spindle becomes displaced slightly to the posterior, resulting in the production of a smaller posterior daughter called P_1 and a large anterior daughter called AB. (D) Both mitotic spindles in the two-cell stage embryo initially set up transversely, but the P_1 spindle rotates before the completion of mitosis to lie along the long axis (curved arrow). (E) P_1 divides slightly after AB, again with a posteriorly displaced spindle, while AB divides transversely and equally. (F) In a four-cell stage embryo, the two daughters of AB, called ABa and ABp, are of equal size and initially have equivalent developmental potential. The two P_1 daughters, called P_2 and EMS, are of different size and are born with different fates.

posteriorly. In other cases, two equally sized P_1-like cells or two equally sized AB-like cells resulted, with the similarities to P_1 and AB based on the orientation of their mitotic spindles during subsequent mitosis (Fig. 2). Although the cell fate patterns produced by P_1 and AB were not examined further in these experiments, the cytochalsin D treatments appeared to severely disrupt AP axis formation.

The discovery of cytoplasmic structures called P-granules provided the first example of molecular asymmetry in the early *C. elegans* embryo (Strome and Wood, 1983; Wolf *et al.*, 1983). Although their function remains largely unknown, P-granules are ribonucleoprotein complexes present specifically in germline precursors (Strome and Wood, 1983; Seydoux and Fire, 1994; Draper *et al.*, 1996; Guedes and Priess, 1996). Ultrastructurally, P-granules resemble polar granules, which are present in the *Drosophila* germline and are known to be important for germline specification (Wolf *et al.*, 1983; St. Johnston, 1993). Mutations in a *C. elegans* gene called *pgl-1* were found to result in the loss of detectable P-granules and sterility, providing the best genetic evidence that P-granules are important for germline development (Susan Strome, personal communication). P-granules initially are present throughout the cytoplasm of the oocyte and the one-cell zygote, but after fertilization they are actively segregated to the cortical cytoplasm at the posterior pole before the first embryonic mitosis, localizing most P-granules to P_1. P-granules continue to be segregated to germline precursors at each subsequent division until the birth of the final germline progenitor, P_4, at the 24-cell stage. Treatment of one-cell-stage wild-type embryos with brief pulses of cytochalasin D prevents P-granule segregation, indicating that this asymmetry also requires functional microfilaments (Hill and Strome, 1990).

A third asymmetry in early embryos is a cytoplasmic flux that occurs posteriorly shortly after fertilization and the ensuing completion of meiosis (Fig. 2). By using time-lapse videomicroscopy to observe the movements of individual cytoplasmic "yolk" droplets—visible throughout the cytoplasm of all early blastomeres—one can readily detect cortical cytoplasm flowing anteriorly while more internal cytoplasm flows posteriorly (Hird and White, 1993; Hird, 1996). This cytoplasmic flux occurs in the posterior half of the one-cell zygote, and similar cytoplasmic fluxes may occur in P_1 and AB. Cytochalasin D treatment prevents the cytoplasmic flux from occurring, although disruption of microtubules with nocodazole treatment does not (Hird and White, 1993).

Intriguingly, the asymmetric positioning of the first mitotic spindle, the localization of P-granules, and the posterior flux of cytoplasm all require functional microfilaments. Moreover, the time period in which cytochalasin D treatment can disrupt spindle positioning and P-granule localization precisely corresponds to the time of cytyoplasmic flux; treatment with pulses of cytochalsin D before or after the flux does not affect positioning of the first mitotic spindle or the localization of P-granules (Hill and Strome, 1990; Kemphues and Strome, 1997). These results suggest that, after fertilization, actin-dependent processes generate

a cytoplasmic flux required for localizing P-granules and possibly for posteriorly displacing the first mitotic spindle.

D. Sperm Entry Provides the Initial Cue for Anterior–Posterior Polarity

The importance of the actin cytoskeleton in establishing anterior–posterior asymmetry begs the obvious question: What factor(s) initiate establishment of these asymmetries and distinguish the anterior and posterior poles of the oblong zygote? Observations of developing oocytes and fertilization provide some clues. In the ovary of a wild-type worm (Fig. 1), the occytes line up in a single row that terminates at the spermatheca, a socklike structure that houses hermaphrodite and male sperm. As an oocyte approaches the spermatheca, its pronucleus becomes displaced toward the prospective anterior pole, and a sperm usually enters at the prospective posterior pole, the end that first engages the spermatheca (Figs. 1 and 2). This sequence of events typically results in the anterior end of an embryo being marked by two polar bodies extruded during the completion of meiosis by the maternal pronucleus. However, polar bodies are present at the anterior end in only about 90% of embryos with the remainder positioned laterally or even posteriorly, suggesting that the position of the oocyte pronucleus is not an accurate predictor of polarity in the zygote (Albertson, 1984; Bowerman *et al.*, 1993).

Rather than having prepatterned oocytes, sperm entry appears to provide the initial asymmetric cue that polarizes the *C. elegans* zygote and thereby determines the anterior–posterior body axis (Goldstein and Hird, 1996). This conclusion follows from experiments using genetically feminized nematodes that lack sperm. In these female nematodes, oocytes enter the spermatheca before fertilization. Consequently, if such females are then mated, male sperm contact the oocyte in a more random manner, sometimes entering at the side or even at the same end occupied by the maternal pronucleus. Wherever the sperm enters, its pronucleus and accompanying centriole appear to generate a cytoplasmic flux that appears to push the male pronucleus and centriole to the nearest pole of the oblong embryo, with that end becoming the posterior pole. Thus, if the oocyte does possess any polarity, it can be overridden by altering the position of sperm entry. This finding suggests that, upon entering an oocyte and taking up residence at one end, the sperm-donated pronucleus or centriole, or some other sperm-donated product(s), defines the posterior pole and initiates the cytoplasmic flux that redistributes maternally provided factors such as P-granules along the AP axis. Genetic studies show that sperm in fact do donate a factor(s) critical for early embryogenesis, beyond their genetic material and mitotic spindle apparatus. The paternally expressed *spe-11* gene encodes a novel protein associated with the sperm pronucleus that is required for development to proceed beyond the one-cell stage (Browning and Strome, 1996). The function of *spe-11* remains

unknown but can also be provided by using a transgene to express *spe-11* in oocytes.

One important but unresolved issue is how the circulating cytoplasm in an early *C. elegans* zygote results in localization of P-granules to the posterior cortex. Presumably, anchoring molecules active only at the posterior pole trap P-granules as they pass by. If so, the localization of such anchoring complexes presumably would precede that of P-granules and might require a different mechanism. It is conceivable that the hydrodynamic properties of the cytoplasm imposed by the narrowing of the eggshell at the pole could result in localized sheer forces mechanically activating ubiquitously distributed anchors. Alternatively, the sperm might donate or activate anchoring molecules that accompany the sperm pronucleus and centriole in moving to the future posterior pole. In these latter two models, the localization of P-granules and the localized function of anchoring molecules would represent two different responses to one event, the sperm-induced flux of cytoplasm.

II. The Par Group of Maternal Genes and Polarization of the Embryonic Cytoskeleton

Tantalizing insights into how regulation of the cytoskeleton might polarize the *C. elegans* zygote have come from the identification of six maternally expressed *par* genes, *par-1* through *par-6* (Kemphues *et al.*, 1988; Guo and Kemphues, 1996b; Watts *et al.*, 1996). For reviews of the *par* genes, see Guo and Kemphues (1996b) and Kemphues and Strome (1997). Mutational inactivation of *par* genes causes defects in cytoplasmic reorganization after fertilization, a failure to partition P-granules properly, and abnormal positioning of mitotic spindles (Kemphues *et al.*, 1988; Kirby *et al.*, 1990). The first mitotic spindle fails to move posteriorly in all but *par-4* mutant embryos, producing equal-sized posterior and anterior two-cell-stage daughters (Fig. 3). In all six *par* mutants, the two-cell-stage blastomeres divide synchronously and equally, in contrast to the asynchronous and unequal divisions of P_1 in wild-type embryos (Fig. 3). Cytoplasmic flux appears defective to some degree in *par-1* through *par-4* mutants, with *par-1* and *par-4* perhaps exhibiting less severe defects (Kirby *et al.*, 1990). Because the first mitotic cleavage is equal in *par-1* but unequal in *par-4* mutants, the relationship between cytoplasmic flux and positioning of the first mitotic spindle is not clear. Finally, the characteristic orientations of two-cell-stage mitotic spindle axes in *par* mutant embryos define three *par* subgroups: (i) In *par-1* mutant embryos, P_1 and AB have longitudinally and transversely oriented spindles, respectively, as in wild-type embryos. But unlike wild-type embryos, both two-cell-stage blastomeres in *par-1* embryos divide synchronously. (ii) In *par-2* and *par-5* mutant embryos, both spindles divide transversely and synchronously, and

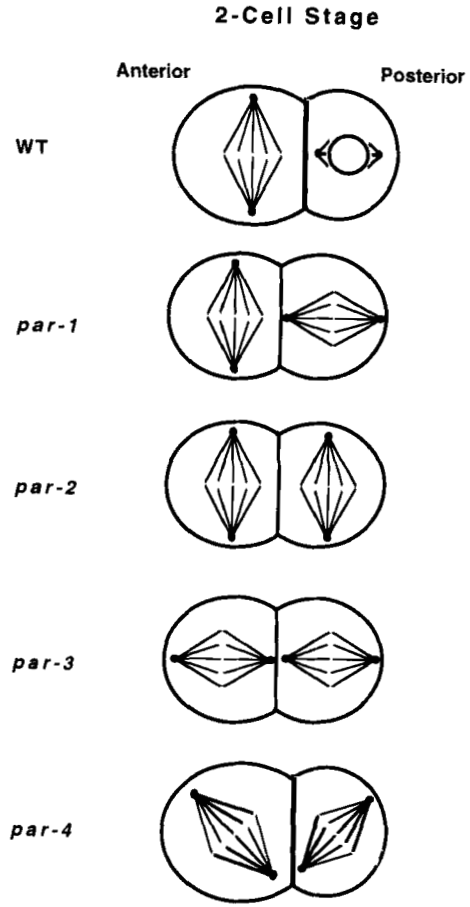

Fig. 3 Two-cell-stage mitotic spindle orientations in *par* mutant embryos. In *par-1* mutant embryos, the posterior blastomere (for convenience called P_1) and the anterior blastomere (for convenience called AB) divide with normal spindle orientations, but synchronously instead of, as in wild-type embryos, asynchronously. In *par-2* and *par-5* mutant embryos, P_1 and AB both divide transversely and synchrononously. In *par-3* and *par-6* mutant embryos, both divide longitudinally and synchronously. In *par-4* mutant embryos, P_1 and AB divide with random orientations; one arbitrary example is shown. In all but *par-4* mutant embryos, the first cleavage produces two blastomeres roughly equal in size. In *par-4* mutants, the first cleavage produces a smaller posterior and larger anterior blastomere, as in wild type, but both blastomeres subsequently divide synchronously.

(iii) in *par-3* and *par-6* mutants, both divide longitudinally and synchronously (Kemphues and Strome, 1997).

Because *par* mutants are defective in cytoplasmic flux, P-granule localization, and spindle positioning—the same processes disrupted by cytochalasin D treatment of wild-type embryos—the *par* genes may regulate pattern formation at

least in part by interacting with and perhaps polarizing the actin cytoskeleton (Guo and Kemphues, 1996b). Consistent with this hypothesis, studies have shown that the C-terminus of PAR-1 binds a conventional nonmuscle myosin required for proper polarization of the early embryo (Guo and Kemphues, 1996a). Inactivation of this myosin by RNA microinjection results in the production of embryos with transversely oriented two-cell-stage spindles, in addition to defects in cytokinesis and oogenesis. Affinity column chromatography has been used to identify 17 oocyte proteins that bind filamentous actin (Aroian *et al.*, 1997). Antibodies to three such proteins show distinct localization patterns in the zygote and early embryo. CABP1 localizes to the actin-rich cortex throughout the zygote and in all early blastomeres. CAPB14 is dynamic, cycling from the nucleus during prophase, to the cortex during metaphase, and to the cleavage furrow during cytokinesis. Perhaps most intriguing, CABP11 is localized to the cortex but only in the anterior part of the embryo, indicating that asymmetries involving proteins that physically interact with actin microfilaments exist as early as the time of pronuclear congression in the one-cell zygote. The powerful molecular, genetic, and biochemical methods now available for identifying and inactivating gene products in *C. elegans* make it seem likely that the discovery of important mechanistic links between the PAR proteins, the cytoskeleton, and embryonic polarity in *C. elegans* are close at hand.

A. Polarized Distribution of the PAR Proteins in the One-Cell Zygote and in Germline Precursors

At a molecular level, the DNA sequences of *par-1*, *par-2*, *par-3*, and *par-4* are known, and all four of the proteins they encode are present in the cytoplasm and enriched in the cytoplasmic cortex. PAR-1 contains a predicted N-terminal Ser–Thr kinase domain and a C-terminal domain that interacts with the nonmuscle conventional myosin (Guo and Kemphues, 1995, 1996a). Before fertilization, PAR-1 is not polarized in its cortical distribution, but after fertilization and cytoplasmic flux, by the time the maternal and paternal pronuclei meet, PAR-1 is present at the cortex only posteriorly in the one-cell zygote, called P_0 (Fig. 4). Homologues of *par-1* have been identified in yeast and in mammals (Levin *et al.*, 1987; Levin and Bishop, 1990; Drewes *et al.*, 1997). The involvement of these related kinases in regulating polarity in yeast and microtubule stability in mammalian cells suggests that the functions of the *par* genes are likely to be of general importance in studies of cell polarity and the cytoskeleton. PAR-2 is a protein of unknown function with a putative ATP-binding site and a zinc-binding domain of the "RING finger" class (Levitan *et al.*, 1994). Like PAR-1, PAR-2 is enriched in the cortex but only in the posterior part of the zygote shortly after fertilization (Boyd *et al.*, 1996). PAR-3 is a novel protein with three PDZ repeats that presumably mediate protein–protein interactions. PAR-3 is present cortically

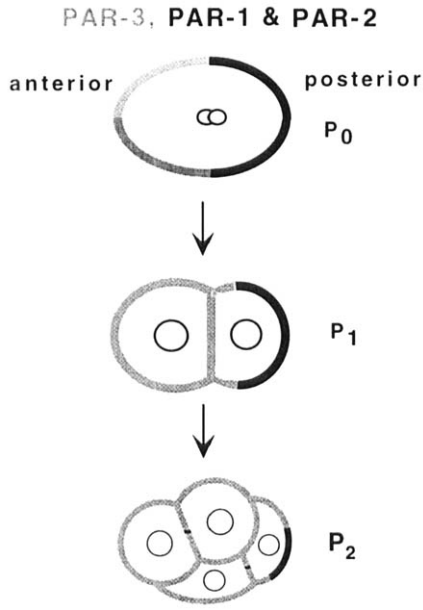

Fig. 4 Polarized distributions of PAR-1, PAR-2, and PAR-3 cortically localized proteins. PAR-1 and PAR-2 are both enriched in the cytoplasmic cortex and only posteriorly shortly after fertilization in P_0 (dark lines). PAR-3 is also enriched cortically but only in the anterior portion of P_0 (gray lines). PAR-1, PAR-2, and PAR-3 continue to show a polarized distribution in the germline precursors, P_1 and P_2, at the two-cell and four-cell stages of embryogenesis, respectively. This polarized distribution is also maintained in the P_3 daughter of P_4 (not shown, but see Fig. 15). PAR-1 and PAR-2 are absent from somatic blastomeres, while PAR-3 is enriched cortically throughout the entire cortex of all somatic blastomeres.

only in the anterior cortex of the one-cell zygote; its posterior boundary precisely coincides with the common anterior boundary of the cortical domains of PAR-1 and PAR-2 (Etemad-Moghadam and Kemphues, 1995). PAR-4 also contains a Ser–Thr kinase domain different from that in PAR-1. PAR-4 is unique in having a nonpolarized distribution, localized to the cortex throughout the zygote and early blastomeres (J. Watts and K. Kemphues, personal communication). The polarized distributions of PAR-1, PAR-2, and PAR-3 are inherited by the germline precursors P_1, P_2, and P_3, but not by P_4, the final germline progenitor (Guo and Kemphues, 1996b; Kemphues and Strome, 1997; see Fig. 4). It is not known whether the PAR proteins are required for maintaining polarity in germline precursors. However, the temperature-sensitive periods for mutant alleles of *par-2* and *par-4* are both over by the end of the one-cell stage, consistent with *par* function being required only during the first zygotic cell cycle (Kemphues and Strome, 1997).

Studies of PAR protein distributions in *par* mutant embryos indicate that interactions among the *par* genes are important for regulating embryonic polarity. Four interactions have been noted. First, the cortical localization of PAR-1 requires *par-2* function: In *par-2* mutant embryos, PAR-1 is present throughout the cytoplasm with no enrichment at the cortex (Boyd *et al.*, 1996). Furthermore, PAR-2 and PAR-3 each depends on the other for their polarized distributions (Etemad-Moghadam and Kemphues, 1995; Boyd *et al.*, 1996; Kemphues and Strome, 1997). In *par-2* mutants, cortical PAR-3 extends posteriorly, and in *par-3* mutants cortical PAR-2 extends anteriorly. Finally, the cortical localization of PAR-3 requires *par-6* function, and in turn *par-3* function is required for proper cortical localization of CABP11, an actin-binding protein present in oocytes that shows a distribution similar to that of PAR-3 (Watts *et al.*, 1996; Aroian *et al.*, 1997). Finally, none of the other PAR proteins require *par-4* function for their polarized distribution, and PAR-4 appears normal in all other *par* mutants (Kemphues and Strome, 1997).

Genetic studies, together with the known distributions of the PAR proteins just described, suggest that the only function of *par-2* may be to limit the posterior extension of PAR-3. This conclusion is based on the observation that eliminating one copy of *par-6* rescues *par-2* mutant embryos: *par-6(−)/par-6(+); par-2(−)/par-2(−)* mothers produce viable embryos (Watts *et al.*, 1996). As cortical PAR-3 in *par-2* embryos forms a gradient that fades posteriorly, one possible explanation for the suppression of *par-2* is that reducing the *par-6* function by one-half causes the loss of enough cortical PAR-3 to reduce its posterior extension sufficiently for normal development to occur. If so, then the only function of *par-2* might be to restrict cortical PAR-3 to the anterior portion of the zygote (Watts *et al.*, 1996). Another observation consistent with *par-2* functioning only to define the posterior boundary for cortical PAR-3 is that *par-1*, but not *par-2*, mutants fail to partition P-granules to P_1; *par-2* embryos do fail to partition P-granules during subsequent divisions (Kemphues *et al.*, 1988). Thus *par-2* embryos partition P-granules to P_1 even though cortical PAR-1 is absent from *par-2* embryos (discussed earlier). Therefore, the cortical localization of PAR-1 is not necessary to partition P-granules to P_1, and the loss of cortical PAR-1 in *par-2* mutant embryos may not be relevant to the *par-2* mutant phenotype. This result raises the important caveat that cortical localization of a PAR protein may not be important for its function. Instead, it is possible that cortical localization could be a secondary consequence of cytoplasmic PAR functions that more directly regulate polarity in the zygote. The relative importance of cytoplasmic and cortical pools of the PAR proteins remains unknown.

B. *par* Gene Functions: A Network, Not a Pathway?

The observations that proper cortical localization of some PAR proteins requires some *par* gene functions suggest that the *par* genes interact to regulate polarity.

Additional evidence for *par* genes functioning in linear pathways has come from studies of *par-2* and *par-3* and their requirements for proper orientation of the mitotic spindles in P_1 and AB (Kemphues *et al.*, 1988; Cheng *et al.*, 1995). In wild-type two-cell-stage embryos, both the P_1 and AB initially set up transversely oriented mitotic spindles (Fig. 2). AB continues to divide transversely, slightly ahead of P_1 in timing. Just before P_1 divides, however, its mitotic spindle rotates to lie along the longitudinal axis. Rotation of the P_1 spindle appears to involve the capture of astral microtubules emanating from either end of the P_1 spindle by a largely uncharacterized complex present in the P_1 cortex near the center of P_1's border with AB (Hyman and White, 1987; Hyman, 1989). Other studies have shown that microtubules, actin, and actin-capping protein colocalize to this cortical site during the time that the P_1 spindle rotates (Waddle *et al.*, 1994). Whatever its nature, something associated with the anterior cortex of P_1 can trap and pull one end of the P_1 spindle toward the center of P_1's border with AB. This attachment can be severed by delivering pulses from a laser microbeam in between the cortex and the attached centrosome (Hyman, 1989). Severing of the connection causes the rotation to stop, but it then resumes in one or the other direction upon recapture of a centrosome. On the basis of analogies to bud site selection and orientation of the mitotic spindle in *Saccharomyces cerevisiae* (Drubin and Nelson, 1996), it is tempting to speculate that a spindle-rotating complex in *C. elegans* might assemble in association with proteins left upon the termination of cytokinesis at the birth of P_1 and AB.

As illustrated in Fig. 3, both spindles orient transversely in two-cell-stage *par-2* mutants while they both orient longitudinally in *par-3* mutants, suggesting that *par-2* and *par-3* interact to regulate the longitudinal and transverse orientations of wild-type P_1 and AB mitotic spindles (Kemphues *et al.*, 1988). Because both spindles orient longitudinally in *par-2;par-3* double mutants, *par-3* is epistatic to *par-2* with respect to spindle rotation in two-cell-stage blastomeres, and neither *par-2* nor *par-3* is required for spindle rotation (Cheng *et al.*, 1995). Rather, *par-3* is required to prevent spindle rotation in AB while *par-2* appears to prevent *par-3* from functioning in P_1, thereby permitting some other process to rotate the P_1 spindle.

Models in which PAR-3 directly prevents spindle rotation in AB received support from immunohistochemical studies showing that *par-2* restricts cortical PAR-3 mostly to the AB blastomere (Etemad-Moghadam and Kemphues, 1995; Boyd *et al.*, 1996). On the basis of these genetic and molecular studies of *par-2* and *par-3*, it has been proposed that cortical PAR-3 may stabilize spindle axes by interacting with astral microtubules in AB (Guo and Kemphues, 1996b; Kemphues and Strome, 1997). The presence of cortical PAR-3 only at the anterior of P_1 presumably would be insufficient to prevent spindle rotation in P_1. Mutations in *par-2* result in PAR-3 being present throughout the cortex in both P_1 and AB, consistent with stabilization of the transverse spindles in both two-cell-stage blastomeres in *par-2* mutant embryos. However, mutations were identified in another maternal gene, named *let-99*. In about 50% of *let-99* embryos, the AB

spindle orients longitudinally and the P_1 spindle orients transversely, the opposite of the pattern observed in wild type (Rose and Kemphues, 1997). Remarkably, PAR-1, PAR-2, and PAR-3 all show a normal polarized distribution in *let-99* mutant embryos (Rose and Kemphues, 1997). Therefore, spindle rotation in AB is not prevented by PAR-3, and the P_1 spindle can be stabilized without altering the distribution of PAR-3, calling into question the notion that stabilization of the spindle by cortical PAR-3 is either necessary or sufficient to prevent spindle rotation. Alternatively, *par-2* and *par-3* might function to polarize the distribution of other factors, resulting in only P_1 acquiring the machinery necessary for spindle capture and rotation. Consistent with this latter possibility, mutations in *par-3* also are epistatic to mutations in *par-2* with respect to the distribution of SKN-1, a putative transcription factor (see the following). As in wild-type embryos, SKN-1 is present at high levels only in the posterior blastomeres in *par-2* mutant embryos, but in *par-3* and *par-3;par-2* double mutant embryos SKN-1 is present at equal levels in anterior and posterior blastomeres (Bowerman *et al.*, 1997). Thus, it may be simpler to explain the functions of *par-2* and *par-3* in terms of the polarized distributions of other maternal factors, rather than in terms of PAR-3 mediating interactions between the cortex and the mitotic spindle.

While the epistatic relationship between *par-2* and *par-3* indicates that some par group genes function in linear pathways, the *par* genes more often appear to act independently of each other to regulate pattern formation. This conclusion is based in part on the dissimilar phenotypes of most par group mutants and on the distributions of four regulatory proteins in *par-1*, *par-2*, *par-3*, and *par-4* mutant embryos (Crittenden *et al.*, 1996; Bowerman *et al.*, 1997). These four proteins, discussed in more detail later, are distributed asymmetrically in wild-type embryos. Two, GLP-1 and MEX-3, are present at high levels only in anterior blastomeres at the two-cell and four-cell stages. Two others, SKN-1 and PAL-1, are present at high levels only in posterior blastomeres at the four-cell stage. Because the maternal mRNAs for all of these proteins are distributed throughout the early embryo, either translational regulation or regional differences in protein stability must account for their asymmetric distributions. Remarkably, no correlation is seen in how the distributions of these four proteins respond to mutations in the *par* genes (Fig. 5). For example, mutations in *par-1* result in SKN-1 being present in all four-cell-stage blastomeres, but also result in a complete absence of PAL-1. Furthermore, while mutations in *par-2* do not affect MEX-3 distribution, GLP-1 is present in all four-cell-stage blastomeres in many *par-2* mutants. Thus, mutations in the *par* genes extensively uncouple the mechanisms that localize different regulatory molecules. Perhaps the *par* genes represent components of a network of polarizing factors that in turn regulate the spatial distribution of translational or proteolytic regulators, ultimately localizing more specifically acting regulators like GLP-1, MEX-1, PAL-1, and SKN-1 to specific blastomeres. Whereas some of these events may involve linear pathways using

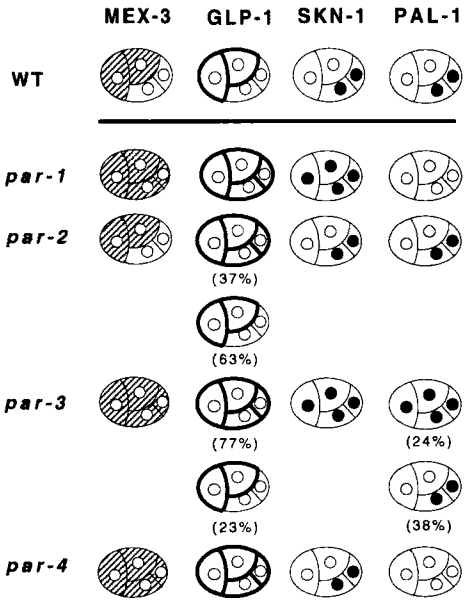

Fig. 5 Summary of the distributions of MEX-3, GLP-1, SKN-1, and PAL-1 in *par* mutant embryos. Wild-type distributions are shown in the upper row, with the distributions in *par-1*, *par-2*, *par-3*, and *par-4* mutant embryos summarized below.

two or more par group genes, the extensive uncoupling of cell fate specification pathways in *par* mutant embryos and the diversity of the *par* mutant phenotypes suggest that the *par* genes also act independently of each other during the regulation of polarity and pattern formation.

C. The *mes-1* Gene and a Reversal of Polarity during Germline Development

The maternal gene *mes-1* also warrants consideration as a par group member. The most obvious defect in *mes-1* mutant embryos is the equal cleavage of the germline precursor P_3 into two daughters that produce body wall muscle (Strome *et al.*, 1995). Thus, *mes-1* mutants make two D-like blastomeres and lack the germline progenitor P_4. However, *mes-1* mutants probably have defects in the development of C, the sister of P_3, and the earliest detectable defect occurs in P_2 when the P_2 mitotic spindle fails to orient properly. Studies by Schierenberg suggest that the germline undergoes a polarity reversal in P_2: If P_1 is extruded from the eggshell and allowed to divide, P_2 is born posteriorly, but then P_2 divides to produce a smaller P_3 anteriorly and P_3 makes a smaller P_4 anteriorly (Schierenberg and William, 1985). When constrained at the narrow end of a rigid

eggshell, the germline daughters P_3 and P_4 are forced to adopt more ventral and anterior positions relative to their somatic sisters, even though their parents divide along a reversed AP axis relative to P_1 and P_0. Thus, it is possible that the defective positioning of the P_2 spindle in *mes-1* mutant embryos reflects a specific defect in a second phase of "reversed" germline polarity. If so, then *mes-1* might be considered a late-acting example of a par group gene, reversing polarity within germline precursors perhaps after the roles of the other par group genes are complete.

III. The Blastomere Identity Group of Maternal Genes in *C. elegans*

Before a discussion of maternal genes that act more specifically than those in the par group to control pattern formation, it is useful to briefly review the names of early embryonic cells, or blastomeres, and the characteristic cell types they each produce. Cleavage of the one-cell zygote generates the smaller P_1 blastomere and the larger anterior blastomere, AB. AB then divides before P_1 to produce two daughters of equal size, ABa and ABp, which subsequently divide synchronously. P_1 and its descendants undergo a series of asymmetric divisions to produce blastomeres differing in size and in the timing of their cell cycles (Sulston *et al.*, 1983). These early unequal cleavages produce a group of six so-called founder cells, born from the 2-cell to the 24-cell stage. Five founder cells, called AB, MS, E, C, and D, produce somatic cells, while P_4 is the germline progenitor. The descendants of each founder cell have characteristic and somewhat synchronous cell cycle times and in sum produce the 558 surviving cells that form a hatched larva. Founder cell descendants are named according to their position at birth relative to their sisters. For example, ABpr is the right-hand daughter of ABp, and ABp is the posterior daughter of AB (Sulston *et al.*, 1983).

Each founder cell produces an essentially invariant pattern of cell fates, with one or two cell types sufficing to distinguish each of the six founder fates (Sulston *et al.*, 1983). Three founder cells produce descendants that all share roughly the same fate: P_4 divides to make Z2 and Z3, the two progenitors of the germline; D produces only body wall muscle cells; and E makes all of the worm's intestinal cells. The remaining founder cells produce more complex patterns of cell fate. For example, MS generates body wall muscle and the somatic gonad in addition to several cell types that form the posterior part of the pharynx, an organ in the head of the animal used for feeding. C produces body wall muscle cells and most of the dorsal epidermis. ABa makes many neurons, some epidermal cells, and the anterior half of the pharynx. ABp also makes many neurons and some epidermal cells, and a number of specialized cell types, including the excretory cell and cells associated with the rectum and anus.

As described earlier, the position of sperm entry determines the anterior–posterior body axis in *C. elegans*. However, a dorsal–ventral axis is not evident until P_1 and AB are nearly done dividing (Priess and Thomson, 1987). As the spindle of AB elongates, it becomes longer than the eggshell is wide and skews to one side or the other, forcing P_1 to become skewed in a complementary fashion (Fig. 5). This sequence of events results in one AB daughter, ABa, being the anterior-most four-cell-stage blastomere, while ABp becomes the dorsal-most. The asymmetric division of P_1 produces the smaller germline progenitor P_2, the posterior-most blastomere in a four-cell-stage embryo, and EMS the ventral-most blastomere. Thus, the dorsal–ventral axis appears to be defined by the positions of two four-cell-stage blastomeres, ABp and EMS, the fates of which are specified using largely independent mechanisms (see the following). Left–right differences become apparent at the eight-cell stage, when for unknown reasons the left-side daughters of ABa and ABp adopt positions more anterior than their right-side sisters (Wood, 1991); the constraints of the eggshell and the left–right skewing of the EMS spindle may contribute to this asymmetry.

A. P_1 and AB Descendants: Mechanisms Controlling the Specification of Blastomere Identities

As noted earlier, the first cleavage of the *C. elegans* embryo produces two daughters, P_1 and AB, with dramatically different fates, indicating that mosaic mechanisms play an important role in patterning the early embryo, beginning with the first cleavage (Schnabel and Priess, 1997). The different developmental potentials of AB and P_1 can be demonstrated by separating them upon birth and examining their abilities to develop in isolation (Priess and Thomson, 1987). If AB is physically removed from a two-cell-stage embryo, P_1 still produces many if not all of the cell types it normally makes. Thus, P_1 appears to inherit a largely intrinsic ability to develop, suggesting that localization of embryonic determinants to P_1 might specify its fate, with cell signals from AB descendants apparently playing at most a minor role. In contrast, if P_1 is removed and AB develops in isolation, AB descendants fail to produce many cell types, including the anterior pharyngeal cells normally made by ABa and the intestinal–rectal valve cells characteristic of ABp fate. While AB development appears to depend extensively on cell signals from P_1 descendants, presumably factors that act cell-autonomously in AB and its descendants are also important for their proper development.

The relative importance of cell-autonomous and non-cell-autonomous mechanisms for patterning the fates of P_1 and AB descendants was demonstrated most clearly by a landmark experiment in 1987 that has shaped much mechanistic thinking about embryogenesis in *C. elegans* for the past 10 years. Priess showed that if one uses a micromanipulator to switch the positions of ABa and ABp just

as they are being born, a normal embryo results with an expected reversal in left–right asymmetry (Priess and Thomson, 1987). This one simple experiment showed that ABa and ABp are born with equivalent developmental potentials. Because ABa and ABp can adopt appropriate fates according to their positions at the beginning of the four-cell stage, cell–cell interactions must distinguish their fates. Furthermore, the ability to switch ABa and ABp without disrupting embryogenesis indicates that the DV axis in *C. elegans* can be reversed at the beginning of the four-cell stage: The blastomere switch puts ABa in the region formerly occupied by EMS and forces EMS to move dorsally to the former position of ABp. A second experiment by Priess in 1987 suggested that P_1 descendants develop using more mosaic mechanisms. After switching the positions of P_2 and EMS, each P_1 daughter appeared to produce its normal complement of cell types but in the wrong place, resulting in morphologically abnormal embryos that fail to form a worm or hatch (Priess and Thomson, 1987). In summary, both cell-autonomous and non-cell-autonomous mechanisms pattern the early embryo, with AB descendants apparently relying more on cell signaling and P_1 descendants relying more on the asymmetric segregation of development potential during early cleavages.

Over the past 10 years, genetic studies have in large part supported the conclusion that P_1 and AB descendants develop using substantially different mechanisms (see the following). These differences in P_1 and AB development make it convenient to discuss members of the blastomere identity group by referring to two subgroups of maternal genes, a P_1 subgroup and an AB subgroup.

B. Maternal Genes That Specify the Identities of P_1 Descendants

1. *skn-1*, *pal-1*, and *pie-1*: Cell-Autonomous Control of Posterior Blastomere Identities

The three maternal genes *skn-1*, *pal-1*, and *pie-1* all encode putative transcriptional regulators that act in P_1 descendants to initiate the different cell fate programs that define the fates of the four P_1-derived somatic founder cells called MS, E, C, and D (Bowerman *et al.*, 1993; Hunter and Kenyon, 1996; Mello *et al.*, 1996). Whereas other genes clearly are essential for the specification of these four fates (see the following), *skn-1*, *pal-1*, and *pie-1* may play the most direct and early roles of perhaps any maternal genes in specifying these blastomere identities. This conclusion is based on the observation that the elimination of both *skn-1* and *pal-1* functions from early embryos results in P_1 failing to produce any differentiated somatic cell types and instead producing many small and apparently undifferentiated descendants (Hunter and Kenyon, 1996). This is the only combination of all the mutants discussed in this review that results in the production of small, undifferentiated cells instead of in trans-fating. As described in the following, *pie1* appears to separate the functions of *skn-1* and *pal-1* in

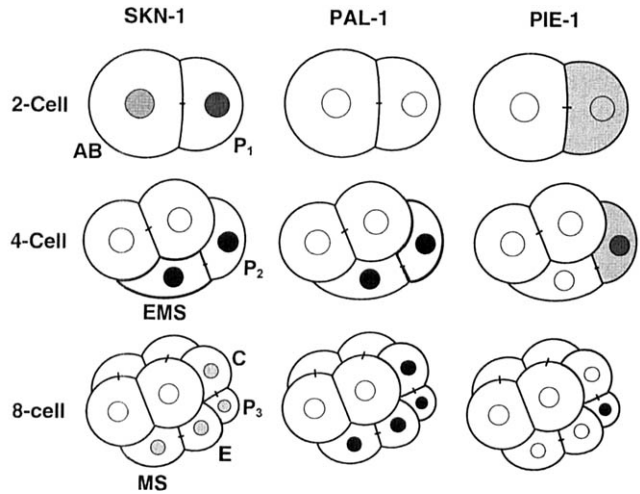

Fig. 6 Distributions of SKN-1, PAL-1, and PIE-1, three putative transcriptional regulators that specify the fates of P_1 descendants in the early embryo. SKN-1 accumulates to higher levels in P_1 than in AB and persists in P_1 descendants until the eight-cell stage. PAL-1 is detectable first at the four-cell stage, only in P_2 and EMS, and persists at high levels in P_1 descendants until the eight-cell stage and beyond. PIE-1 is mostly cytoplasmic at the two-cell stage but becomes localized to the nuclei of germline precursors in subsequent stages.

space and time and may specify germline fate in the remaining founder cell derived from P_1, the germline progenitor P_4.

Before a discussion of the genetic evidence that *pal-1*, *pie-1*, and *skn-1* specify the fates of P_1-derived somatic founder cells, it is helpful first to describe the distributions of the proteins encoded by these three genes (Fig. 6). SKN-1 is a sequence-specific DNA-binding protein with an unusual C-terminal DNA-binding domain related to the basic region of bZIP transcription factors (Bowerman *et al.*, 1992, 1993; Blackwell *et al.*, 1994). However, SKN-1 lacks the leucine zipper motif C-terminal to the basic region in bZIP proteins that is essential for dimerization and bZIP DNA-binding activity. Instead, SKN-1 terminates immediately after its C-terminal basic region and binds DNA as a monomer (Blackwell *et al.*, 1994). NMR studies indicate the SKN-1 DNA-binding domain by itself in solution may be an unstructured "molten globule" and that upon binding DNA it may use a novel fold of short α-helices to position an extended bZIP-like α-helix in the major groove of the DNA-binding site (Keith Blackwell, personal communication). SKN-1 is first detectable at very low levels in the maternal and paternal pronuclei at the end of the one-cell stage (Bowerman *et al.*, 1993). By late in the two-cell stage, SKN-1 accumulates to substantially higher levels in the nucleus of P_1 than in that of AB, and SKN-1 levels peak midway through the four-cell stage in the two P_1 daughters, EMS and P_2 (Fig. 6). SKN-1 is present at lower levels in all four P_1 descendants and undetectable in the four

AB descendants at the 8-cell stage, and SKN-1 is not detectable in any blastomeres by the 12-cell stage. SKN-1 is required to specify the fate of EMS, the parent of the two somatic founder cells, E and MS (Bowerman *et al.*, 1992).

The second putative transcription factor that specifies the fates of P_1-derived somatic founder cells, PAL-1, is a homeodomain protein (Hunter and Kenyon, 1996). The PAL-1 homoedomain is most similar in sequence to that of the *Drosophila* homeodomain protein Caudal, which, like PAL-1 in *C. elegans*, is required for patterning posterior cell fates in *Drosophila* (Hunter and Kenyon, 1996). PAL-1 is first detectable at the four-cell stage, in the nuclei of P_2 and EMS. PAL-1 remains present at high levels in all P_1 descendants until well after the 12-cell stage, when SKN-1 is undetectable. PAL-1 is required to specify the identities of the somatic founder cells C, a daughter of P_2, and D, a daughter of P_3.

Finally, PIE-1 is a Zn^{2+} finger protein that appears to separate the functions of SKN-1 and PAL-1, in part by virtue of its remarkable localization properties in the early embryo (Mello *et al.*, 1992, 1996). At the two-cell stage, PIE-1 is present cytoplasmically in P_1. During mitosis, PIE-1 localizes to the P_1 centrosomes, and as P_1 finishes dividing, PIE-1 leaves the centrosomes. PIE-1 disappears from the somatic daughter EMS but transits to the nucleus in the germline precursor, P_2. At each division of a germline precursor, PIE-1 localizes to the centrosomes during mitosis and then returns to the nucleus of the germline progenitor as mitosis ends. PIE-1 is thought to repress the activation of any somatic cell fate programs in germline precursors, maintaining the germline in a transcriptionally silent, inert, or undifferentiated state (Seydoux *et al.*, 1996).

The temporal and spatial regulation of SKN-1, PAL-1, and PIE-1 expression appears to play an important role in segregating the activities of SKN-1 and PAL-1, both of which are present at high levels in P_2 and EMS, even though each acts in only one of these two P_1 descendants to specify somatic founder cell fates (Mello *et al.*, 1992, 1996; Bowerman *et al.*, 1993; Hunter and Kenyon, 1996). By appearing at high levels before PAL-1, SKN-1 may predominate at the four-cell stage and specify EMS identity. Whereas SKN-1 specifies EMS identity, PIE-1 prevents SKN-1 from functioning in P_2 as part of its more general germline repressor function (Bowerman *et al.*, 1992; Mello *et al.*, 1992, 1996; Seydoux *et al.*, 1996). By the time P_2 divides to produce a C daughter free of PIE-1, SKN-1 is barely detectable and PAL-1 is present at high levels. Perhaps the higher levels of PAL-1 can override the fading levels of SKN-1 to specify C identity and, after P_3 divides, D identity (Hunter and Kenyon, 1996). Of course it is possible that as yet unidentified genes may provide additional means to regulate the time and place of SKN-1 and PAL-1 functions. For example, a factor localized to EMS could serve to block PAL-1 function in EMS much as PIE-1 blocks SKN-1 in P_2. Furthermore, because the maternal mRNAs for *pal-1*, *pie-1*, and *skn-1* are all distributed uniformly throughout early embryos, either translational regulation or differences in protein stability must account for their localized expression. Two

maternal genes, *mex-1* and *mex-3*, are required to prevent high levels of SKN-1 and PAL-1, respectively, from accumulating in anterior blastomeres and ectopically specifying EMS or P_2-like somatic cell fate patterns (see Section IV). Finally, as E, MS, C, and D all have very different fates, other genes must be necessary to distinguish E from MS and C from D (see the following).

Genetic studies of *skn-1*, *pal-1*, and *pie-1* indicate that they each play highly specific roles in specifying the fates of certain P_1 descendants without affecting the identities of other blastomeres in the early embryo. *skn-1* mutant embryos lack the endoderm normally made by E and the mesoderm normally made by MS. Instead, E and MS in *skn-1* mutant embryos each produce epidermal cells and body wall muscle, a fate similar to that of the C daughter of P_2 (Bowerman *et al.*, 1992). In the absence of SKN-1, the PAL-1 present in E and MS appears to respecify them to adopt more posterior C-like fates (Hunter and Kenyon, 1996). As mentioned earlier, in the absence of *skn-1* and *pal-1* function, very few or no differentiated somatic cell fates are produce by either P_2 or EMS. Thus, SKN-1 and PAL-1 appear to act very early to specify blastomere identities: In their absence, pattern formation appears absent even though cell division continues. Other blastomeres in *skn-1* mutant embryos appear to develop completely normally (Bowerman *et al.*, 1992). Both P_2 and ABp in *skn-1* mutant embryos produce normal patterns of cell division and cell fate. Although ABa fails to produce any anterior pharyngeal cells as *skn-1* mutant embryos completely lack a pharynx, the lack of ABa-derived pharyngeal cells is an indirect consequence of the requirement for *skn-1* function in EMS (Shelton and Bowerman, 1996). In the absence of *skn-1* function, MS is incapable of signaling wild-type ABa descendants to produce pharyngeal cells, but a wild-type MS can induce *skn-1* mutant ABa descendants to produce pharyngeal cells (Fig. 7). Thus, *skn-1* appears to specify EMS fate with respect to both the cell fate patterns it produces and the ability of MS to signal ABa.

The *pal-1* gene was first identified by the partial loss of function mutations that affect cell fate patterning postembryonically during larval development but are not lethal (Waring and Kenyon, 1990, 1991). A maternal role for *pal-1* was first suggested by the observation that the PAL-1 protein, in addition to being expressed zygotically in much of the posterior embryo, is also expressed maternally in P_1 descendants, beginning at the four-cell stage in P_2 and EMS (Hunter and Kenyon, 1996) (Fig. 6). The maternal requirements for *pal-1* were tested in two ways. First, for homozygous *pal-1* mutant mothers rescued by an extrachromosomal array containing the wild-type *pal-1* gene produce embryos, about 1 in 200 will lose the array specifically in the germline during mitotic division of early blastomeres. These animals grow up to produce broods of *pal-1* mutant embryos. Phenotypic analysis of these embryos indicates that P_2 usually fails to produce epidermal cells or body wall muscle cells, fates characteristic of C and D. Instead, P_2 in *pal-1* mutant embryos produces two germline-like cells resembling Z2 and Z3, the two germline precursors normally made by P_4, whereas the

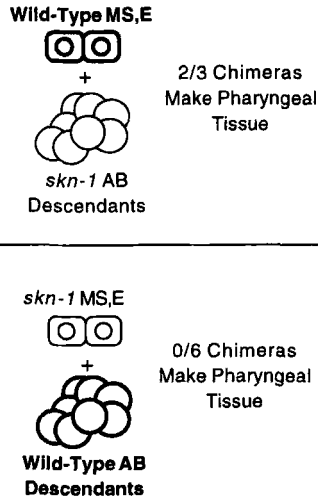

Fig. 7 SKN-1 is required for MS to signal AB descendants to produce pharyngeal cells at the 12-cell stage, but not for AB descendants to respond to MS signaling. By recombining in culture medium blastomeres isolated from wild-type (bold type) and skn-1 mutant embryos (light type), it is possible to reproduce early embryonic inductions and determine which blastomeres require particular gene functions.

remaining P_2 descendants appear small and undifferentiated (Hunter and Kenyon, 1996). Thus, in the absence of PAL-1, the low levels of SKN-1 present in C at the eight-cell stage are not sufficient to specify the production of any of the cell types normally made by E or MS. Similar results are observed when antisense *pal-1* RNA is microinjected into the ovaries of wild-type mothers to inactivate the endogenous maternal *pal-1* mRNA (Hunter and Kenyon, 1996).

Mutational inactivation of *pie-1* results in P_2 adopting a fate nearly identical to that of EMS, producing excess pharynx and intestine (Mello *et al.*, 1992). Thus, in the absence of the P_2-localized nuclear PIE-1 protein, the SKN-1 in P_2 specifies EMS fate ectopically. The smaller P_3 daughter of P_2 develops like E, and the larger daughter, C, usually develops like MS. Consistent with *pie-1* being required to block *skn-1* function in P_2, *pie-1;skn-1* double mutant embryos look like *skn-1* mutant embryos, failing to produce any pharyngeal or intestinal cells and instead making numerous epidermal cells and body wall muscle cells (Mello *et al.*, 1992). However, P_2 still fails to produce germline in *pie-1;skn-1* double mutant embryos, indicating that *pie-1* is required in P_2 for more than just blocking *skn-1* function; it is also required to specify germline. Studies of *pie-1* indicate that it functions as a general repressor of transcription in germline progenitors (Seydoux *et al.*, 1996). Several zygotic genes have been identified that begin transcription as early as the four-cell stage in somatic blastomeres. In all, 12 genes were studied that in wild type are transcribed in the somatic

daughter but not the germline daughter of a germline precursor. In all 12 cases, the gene was transcribed in both the somatic and the germline daughter in *pie-1* mutants. Mutations in the gene *mex-1* (see Section IV) have been shown to result in mislocalization of PIE-1 to somatic blastomeres, which in turn results in the ectopic repression of zygotic gene expression in the somatic blastomeres containing ectopic PIE-1 protein (Guedes and Priess, 1996). Thus, *pie-1* appears to function as a general repressor in germline precursors of somatic transcription programs. The observation that P_2 adopts an EMS-like fate in *pie-1* mutants indicates that SKN-1 may be the first embryonic determinant capable of activating pathways that specify somatic cell fates in P_1 descendants. Perhaps the absence of PIE-1 from the nucleus of P_1 indicates that zygotic gene expression in *C. elegans* does not begin until the four-cell stage. To summarize, during wild-type embryogenesis *pie-1* prevents *skn-1* function in P_2, limiting to EMS the production of *skn-1*-dependent endodermal and mesodermal cell fates and perhaps delaying the specification of P_2-derived somatic founder cells until a time when SKN-1 is nearly absent and PAL-1 can therefore predominate and specify C and D fates.

In addition to *pie-1*, six *mes* genes, *mes-1* through *mes-6*, have been identified that are maternally expressed and required for germline development. However, with the exception of *mes-1*, mutations in the *mes* genes do not affect the fates of embryonic blastomeres but instead result in the apparent degeneration, nonapoptotically, of the germline during larval development (Capowski *et al.*, 1991). Thus, *mes* mutations are maternal-effect sterile, or grandchildless, and *mes-2* through *mes-6* appear to act downstream of *pie-1* to either maintain germline or specify later stages of germline development in late embryos or larvae. Finally, other maternal genes that appear to be required specifically for P_1 and not AB fate have been identified. These include *mes-1*, already discussed, and *pos-1*, which will be discussed in Section IV.

2. The *mom* and *pop* Story: Wnt-Mediated Induction of Endoderm

The simple fates of most P_1 descendants (discussed earlier), the inability of P_2 and EMS to replace each other after having their positions interchanged, and the ability of P_1 to develop roughly normally after the removal of AB all seem to suggest that P_1 develops in a largely autonomous fashion. However, none of the preceding observations rule out important roles for cell interactions among P_1 descendants during development. In another simple and extremely informative set of experiments, in 1985, Schierenberg found that only germline precursors are able to maintain asymmetric divisions when allowed to develop in isolation after being extruded from the early embryos. He concluded that only germline precursors have an intrinsic polarity and predicted that somatic founder cells would require cell interactions with germline precursors in order to be properly polarized (Schierenberg and William, 1985). Seven years later, Goldstein showed

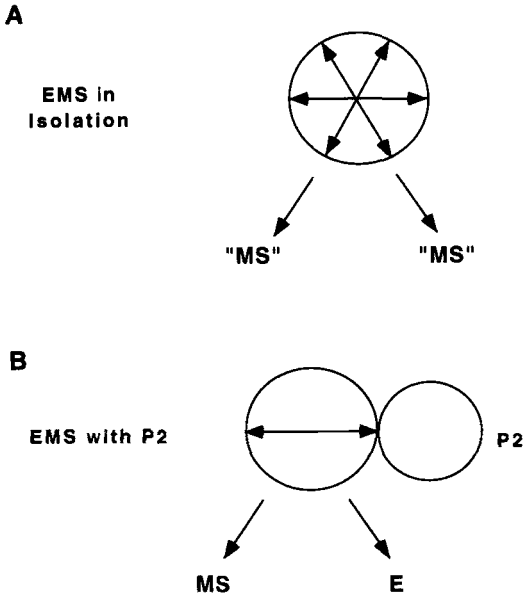

Fig. 8 A polarizing induction from P_2 signals EMS to orient its mitotic spindle axis and to induce the production of endoderm from the daughter of EMS born next to P_2. (A) In the absence of P_2 contact, EMS blastomeres cultured *in vitro* divide with randomly oriented mitotic spindles to produce two mesodermal precursors similar in fate to a wild-type MS blastomere. (B) When placed in contact with P_2, the EMS mitotic spindle rotates to lie along an axis defined by points at the center of P_2 and EMS, and EMS divides to produce one mesodermal precursor, MS, away from the site of contact with P_2 and one endodermal precursor, E, adjacent to P_2.

that the proper development of the somatic P_1 daughter EMS requires a polarizing signal from the neighbor and sister of EMS, the germline precursor P_2 (Goldstein, 1992). Normally, EMS divides to make one daughter, MS, that produces mesoderm and another daughter, E, that produces all of the endoderm in *C. elegans* (Sulston *et al.*, 1983). The signal from P_2 polarizes EMS such that the daughter of EMS born next to P_2 produces endoderm (Fig. 8). In the absence of the P_2 signal, EMS instead divides to make two MS-like daughters (Goldstein, 1992, 1993). Thus, the specification of E fate, the earliest founder cell born that makes a single cell type, requires an inductive signal from P_2 at the four-cell stage. Signals from P_2 not only polarize gut potential to one side of EMS but also orient the EMS mitotic spindle axis: Move P_2 to a different position on EMS, and the EMS spindle will rotate to "point" toward P_2, producing an E daughter close to P_2 and an MS daughter away from P_2 (Fig. 8). In an elegant series of experiments using isolated blastomeres placed in contact with each other at specific times after their birth, Goldstein (1995) found that the timing of the gut polariza-

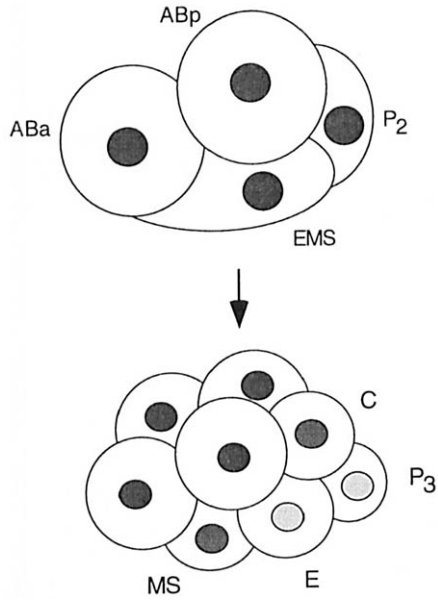

Fig. 9 POP-1 protein distribution in four-cell-stage and eight-cell-stage wild-type embryos. POP-1 contains a single HMG domain and is present in all nuclei at the four-cell stage. At the eight-cell stage, EMS divides to produce one daughter, MS, with high levels of nuclear POP-1 and one daughter, E, with low or undetectable levels of nuclear POP-1.

tion signal and the timing of the mitotic spindle orientation signal appear to be different, suggesting that two different signals from P_2 influence EMS fate.

pop-1 was the first maternal gene identified that is specifically required for distinguishing the fates of E and MS (Lin *et al.*, 1995). In *pop-1* mutant embryos, MS adopts an E-like fate, resulting in a 2-fold excess of intestinal cells at the expense of MS mesoderm. Thus, *pop-1* mutants have a phenotype opposite that caused by the elimination of P_2 signaling, producing two E-like daughters instead of the two MS-like daughters made in the absence of signaling. POP-1 is a putative transcription factor that contains a single HMG domain and is present in all nuclei at the four-cell stage. However, when EMS divides, nuclear POP-1 levels remain high in MS but drop in E (Fig. 9). Thus, down-regulation of nuclear POP-1 correlates with endoderm fate, and elimination of POP-1 from both E and MS in *pop-1* mutant embryos results in both EMS daughters adopting endodermal fates (Lin *et al.*, 1995). These results, together with those of Goldstein, suggest that polarization of EMS by P_2 signaling results in the differential segregation of gut potential to the daughters of EMS, such that nuclear POP-1 is present only in MS. If so, mutations in genes required for P_2 signaling should result in EMS producing two daughters that have high levels of POP-1 and adopt MS fates. Mutations in five such genes have been identified.

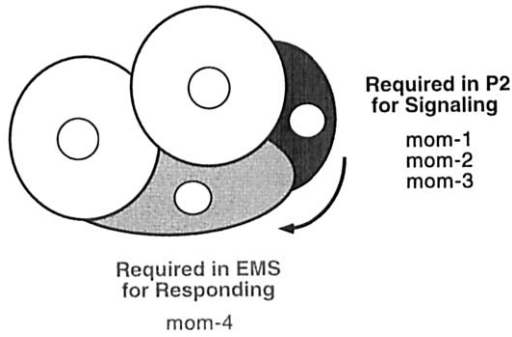

Fig. 10 Summary of which blastomeres require which *mom* gene functions based on an analysis of gut induction using genetically chimeric partial embryos made *in vitro* by recombining blastomeres isolated from mutant and wild-type embryos.

The mutationally identified genes required for the polarizing induction of gut potential in EMS are named *mom-1* through *mom-5*. In *mom* mutant embryos, EMS usually produces two MS-like daughters, resulting in excess mesoderm at the expense of all endoderm (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997). By reassociating in culture medium the early blastomeres isolated from wild-type and *mom* mutant embryos, genetically mosaic partial embryos can be reconstituted to examine the requirements of different blastomeres for the different *mom* gene activities (Thorpe *et al.*, 1997). Three *mom* genes, *mom-1*, *mom-2*, and *mom-3*, are required in P_2 for signaling but not in EMS for responding, while *mom-4* is required in EMS for responding but not in P_2 for signaling (Fig. 10). All mutant alleles of the *mom* genes show incomplete penetrance for the gut defect. For example, even strong loss of function mutations in *mom-2* result in only about 75% of the mutant embryos lacking endoderm (Thorpe *et al.*, 1997). In almost all cases, mutations in the *mom* genes result in a completely penetrant defect in morphogenesis, a largely undefined and likely complex process that converts the round ball of cells made by embryonic cleavages into a long, thin worm. Thus, the *mom* genes probably participate in other processes in addition to the P_2 signaling that polarize gut potential in EMS.

Three of the five *mom* genes have been cloned, and all three are predicted to encode components of the widely conserved Wnt signal transduction pathway (Fig. 11). *mom-1* is a homologue of *Drosophila porcupine*, *mom-2* is a homologue of *Drosophila wingless/Wnt*, and *mom-5* encodes a *frizzled* homologue (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997). Porcupine is a multipass transmembrane protein localized to the endoplasmic reticulum and required in signaling cells for proper processing and secretion of wingless (Perrimon, 1995). Wingless is a secreted glycoprotein that is required for many different cell signaling processes that pattern the bodies of both invertebrates and vertebrates

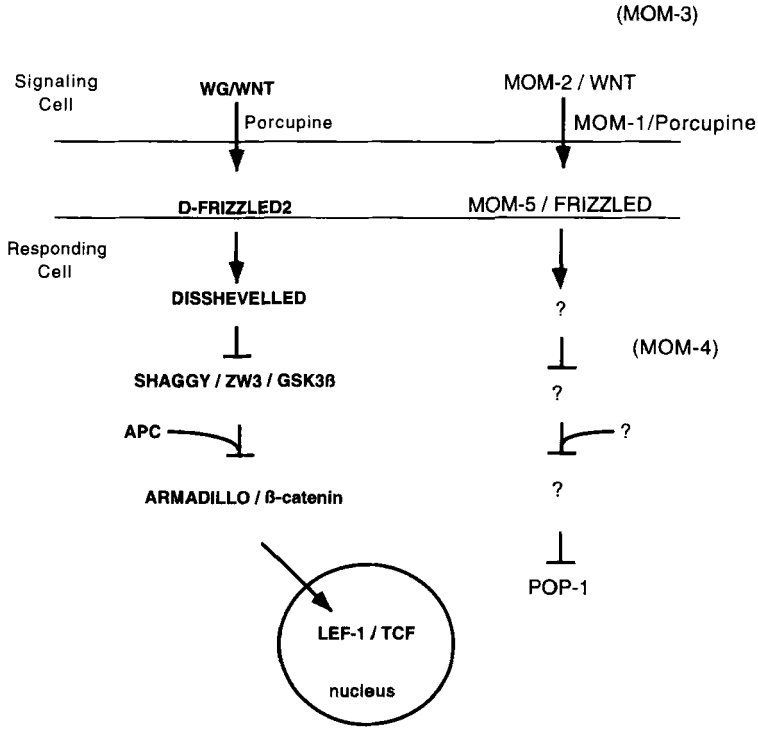

Fig. 11 A summary of the Wnt signal transduction pathway and the corresponding identities of *mom-1*, *mom-2*, and *mom-5*. The *mom-3* gene has not been cloned but is required in P_2 for signaling; *mom-4* has not been cloned but is required in EMS for responding to the polarizing signal from P_2.

(Moon, 1995). Thus, the requirements for *mom-1* and *mom-2* in P_2 are in accord with known functions of their *Drosophila* relatives. However, *mom-3* is also required in P_2 for signaling, and no other genes besides *porcupine* and *wingless* have been identified that are required in signaling cells for Wnt function. *mom-4* remains to be identified and thus could be any one of several components of the Wnt pathway known to act downstream of the signal (Moon, 1995). *mom-5* encodes a frizzled homologue and therefore is likely to be the receptor for MOM-2/WNT based on findings in *Drosophila* (Nusse, 1996).

A detailed discussion of the role of Wnt signaling in gut polarization is beyond the scope of this chapter. Instead, I focus on how the current analysis of Wnt signaling in *C. elegans* sheds new light on Wnt signaling mechanisms. As mentioned earlier, *mom-3* is a third gene required in signaling cells, along with *mom-1/porcupine* and *mom-2/wingless*, and therefore might represent a novel Wnt pathway component. Furthermore, the final step of gut polarization identified thus far is the down-regulation of POP-1 in E to permit endoderm fate. In mom mutant embryos, this down-regulation of POP-1 is lost, and POP-1 levels

remain high in both EMS daugzahters, consistent with POP-1 preventing either from adopting an endodermal fate (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997). We conclude that to polarize gut potential, Wnt signaling down-regulates an HMG domain protein, POP-1, in E. By contrast, in all other studies of the Wnt pathway, signaling results in the activation of an HMG domain protein, such as LEF-1 or TCF, via an interaction with *armadillo*/β-catenin (Moon, 1995). Finally, intriguing defects in mitotic spindle orientation occur in the early blastomeres of *mom-1*, *mom-3*, and *mom-5* single mutant embryos and in *mom-1;mom-2* double mutant embryos (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997). These results suggest that Wnt signaling affects both gut polarization and the P_2-induced orientation of the EMS mitotic spindle (discussed earlier), which were initially proposed to be two different processes (Goldstein, 1995). However, mutations in other Wnt pathway genes do not affect spindle orientation in EMS, and the relationship between these two process remains unclear. Nevertheless, these results provide evidence that Wnt signaling might regulate the polarity of the cytoskeleton in EMS, an intriguing possibility given the association of *armadillo*/β-catenin with the cytoskeleton at adhesion plaques, in addition to its cytoplasmic and nuclear locations during Wnt signaling to the nucleus (Moon, 1995). Finally, mutations in some *mom* genes affect spindle orientation not only in EMS but also in other somatic blastomeres, indicating that Wnt signaling may influence blastomere polarity throughout most of the early embryo (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997). But as Schierenberg first predicted, the embryonic germline precursors may rely more on autonomous polarization mechanisms (Schierenberg and William, 1985), and all germline precursors appear to have normal mitotic spindle orientations and asymmetric divisions in all *mom* mutant embryos examined thus far (Thorpe *et al.*, 1997).

In addition to Wnt signaling, other signals have been identified that influence the fates of MS and D. However, only two other molecules have been identified that, by genetic and molecular criteria, participate in cell interactions that influence blastomere identity. These two genes, *apx-1* and *glp-1*, are the two remaining blastomere identity group genes to be discussed in this review. Rather than influencing P_1 development, these two genes participate in a sequence of early inductions that specify the different identities of AB descendants. Other genes influencing P_1 development are described later in Section IV.

C. Cell Interactions That Specify the Fates of Anterior Blastomeres

Shortly after the finding that ABa and ABp are born with equivalent developmental potential (discussed earlier), the maternal gene *glp-1* was identified and shown to be required for a cell interaction that contributes to making ABa and ABp different (Priess *et al.*, 1987). In wild-type embryos, the MS blastomere signals the two granddaughters of ABa that touch MS to adopt fates that include the production of the cells forming the anterior part of the pharynx (Priess *et al.*,

1987; Hutter and Schnabel, 1994; Mango *et al.*, 1994b). If MS is killed with a laser microbeam at the end of the 8-cell stage, no pharyngeal cells are induced, but if MS is killed later in the 12-cell stage, the induction occurs and pharyngeal cells are made by the two ABa granddaughters. In *glp-1* mutant embryos, the 12-cell-stage induction of pharyngeal cells does not occur, and genetic mosaic studies of *glp-1* during larval development indicated that *glp-1* is required in responding cells (Austin and Kimble, 1987; Priess *et al.*, 1987). *glp-1* encodes a member of the Notch family of transmembrane receptors, which, like the Wnt pathway, participates in many cell interactions that pattern cell fates during vertebrate and invertebrate development (Austin and Kimble, 1989; Yochem and Greenwald, 1989). Antibodies to GLP-1 show that it is present at the cell surfaces of ABa and ABp in the 4-cell stage and persists at high levels in AB descendants until the 28-cell stage (Evans *et al.*, 1994). Thus, GLP-1 is present on the responding cells at the appropriate time to act as the receptor for the 12-cell stage signal from MS that induces the production of pharyngeal cells (Fig. 12). Lower

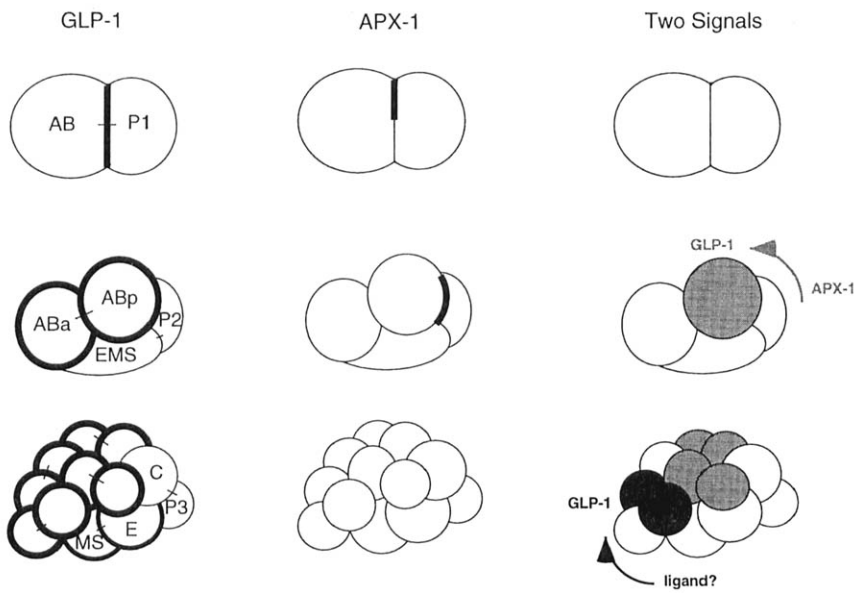

Fig. 12 Summary of GLP-1 and APX-1, Notch and Delta homologues, respectively, in two-, four-, and eight-cell-stage embryos. GLP-1 is first detected at the P_1/AB boundary at the 2-cell stage and on the surfaces of AB descendants at the 4-cell and 12-cell stages. APX-1 is first detected at the interface of P_1 and AB, but only in about one-half of the boundary; mechanisms controlling this early asymmetry and its functional significance remain unknown. APX-1 is present at the interface of P_2 and ABp at the 4-cell stage, but fades to undetectable levels by the 12-cell stage. APX-1 and GLP-1 are required for a cell–cell interaction at the four-cell stage that specifies ABp identity and breaks the initial equivalence of ABa and ABp (light shading). GLP-1 and an unknown ligand produced by MS are required for a second cell–cell interaction that specifies the fates of two granddaughters of ABa at the 12-cell stage (dark shading).

levels of GLP-1 are present in E and MS, and studies indicate that GLP-1 is required to receive a signal required for MS to produce body wall muscle (Schnabel, 1994).

The finding that MS induces ABa to produce pharyngeal cells raises an important question: If ABa and ABp are born equivalent, why does the MS signal influence only ABa descendants and not ABp descendants that touch MS? Because P_2 is the only four-cell-stage blastomere that touches only ABp and not ABa, P_2 was a logical candidate for sending a signal to break the equivalence of ABa and ABp at the 4-cell stage, and an earlier signal from P_2 might render ABp descendants resistant to the inductive signal from MS at the 12-cell stage. A variety of blastomere manipulation experiments provided evidence for such an interaction in wild-type embryos. If P_2 is killed with a laser microbeam just as it is born, ABp often fails to produce intestinal–rectal valve cells, an ABp-specific cell type (Bowerman *et al.*, 1992). Removal of P_2 within 5 min of its birth results in the absence of intestinal–rectal valve cells and the production of excess pharyngeal cells (Mango *et al.*, 1994a). If P_1 is prevented from dividing such that a large undivided P_1 touches both ABa and ABp, extra intestinal–rectal valve cells are made and no pharyngeal cells are induced (Mello *et al.*, 1994). Finally, if P_1 and AB are allowed to divide without an intact eggshell forcing their spindles to skew obliquely, P_2 is born out of contact with both AB daughters, in which case no intestinal valve cells and large numbers of pharyngeal cells are produced (Mello *et al.*, 1994). In sum, these experiments indicate that a signal from P_2 at the 4-cell stage is required for the production of ABp-specific cell types and for prevention of the production of pharyngeal cells by ABp in response to a signal from MS at the 12-cell stage.

Genetic evidence for a P_2 signal specifying ABp fate came both from the identification of the maternal gene *apx-1* and from an elegant analysis of temperature-sensitive alleles of *glp-1* (Hutter and Schnabel, 1994; Mango *et al.*, 1994a; Mello *et al.*, 1994). Mutations in *apx-1* result in ABp adopting a fate much like that of ABa: ABp in *apx-1* mutant embryos fails to produce ABp-specific cell types, and the ABp granddaughters that touch MS produce extra anterior pharyngeal cells in response to MS signaling at the 12-cell stage. Consistent with *apx-1* being required for P_2 signaling, it encodes a transmembrane protein related to the Delta family of Notch ligands, and the APX-1 protein is produced in P_2 at the four-cell stage localized to the interface of P_2 and ABp (Mickey *et al.*, 1996). The localization of APX-1 to the interface of P_2 and ABp could be simply due to clustering of APX-1 by its receptor on ABp. However, APX-1 is also present at the two-cell stage at the interface of P_1 and AB, but on only one side of the embryo (Fig. 12). Thus, APX-1 localization may be polarized in some manner that cannot be explained by contacts with neighboring blastomeres.

The identity of APX-1 as a Delta family member suggests that GLP-1, a Notch family member present on the surface of ABa and ABp at the four-cell stage, might act as the receptor for the APX-1 ligand made by P_2. Temperature shift

experiments using a conditional allele of *glp-1* showed that GLP-1 functions as the receptor both for the 4-cell-stage signal involving *apx-1*, which specifies ABp identity, and for the 12-cell-stage signal from MS, which induces pharyngeal cell production by ABa (Hutter and Schnabel, 1994; Mello *et al.*, 1994). If *glp-1* function is blocked by raising embryos at the restrictive temperature but then restored shortly after the four-cell stage by shifting to permissive temperature, the P_2 signal is blocked and ABp develops just as it does in *apx-1* mutant embryos, failing to produce ABp-specific cell types and insteading making excess anterior pharyngeal cells in response to the MS inductive signal. If *glp-1* function is blocked at both the 4-cell and 12-cell stages, neither ABp-specific cell types nor anterior pharyngeal cells are produced; instead all AB descendants adopt one of two "uninduced" ABa fates, that of a wild-type ABala or ABarp blastomere. Therefore, GLP-1 appears to function as the receptor for both the 4-cell-stage signal from P_2 and the 12-cell-stage signal from MS, which induces pharyngeal cell production by the two granddaughters of ABa that touch MS. Whereas the ligand for the MS signal remains unknown, MS requires SKN-1 function to express signaling activity, and presumably the MS ligand is also a Delta family member that activates the GLP-1/Notch receptor (Shelton and Bowerman, 1996).

The specific responses by AB descendants to the P_2 and MS signals—ABp fate vs induced ABa granddaughter fates—appear to depend not on the specific nature of the ligand or signaling blastomere but rather on time-dependent differences in the response of AB descendants to activation of the GLP-1 receptor. By using isolated blastomeres, it is possible to induce 12-cell-stage AB descendants to produce pharyngeal cells, either by placing them in contact with MS, the normal signaling blastomere from a 12-cell-stage embryo, or by placing them in contact with P_2 to form a "heterochronically" chimeric partial embryo *in vitro* (Shelton and Bowerman, 1996). Pharyngeal induction by P_2, but not by MS, requires *apx-1* function, indicating that APX-1 in P_2 can substitute for the MS signal to induce 12-cell-stage AB descendants to produce pharyngeal cells. Factors that presumably change with time in AB descendants to mediate the different responses to GLP-1 signaling at the 4-cell and 12-cell stages have yet to be identified, as do any autonomously acting factors responsible for specifying the uninduced fates of 12-cell-stage AB descendants.

A third maternal gene required for proper regulation of GLP-1 signaling in the early embryo, called *aph-2*, has been identified (C. Goutte and J. Priess, personal communication). Mutations in *aph-2* frequently result in mutant embryos having a phenotype similar to that of *glp-1* mutant embryos in which only the MS signal is defective: *aph-2* mutant embryos usually lack anterior pharyngeal cells and instead produce extra neurons and epidermis. However, about 10% of *aph-2* mutant embryos resemble *apx-1* mutants, with both ABa and ABp producing anterior pharyngeal cells. Thus, mutations in *aph-2* appear in some cases to result in a loss of *glp-1* function only at the 12-cell stage and in other cases to result in a loss of *glp-1* function only at the 4-cell stage. The APH-2 protein is novel but has a signal sequence and is predicted to be secreted and bound to the outer surface of

the plasma membrane via a GPI linkage. By making chimeric partial embryos in culture medium using isolated blastomeres from wild-type and *aph-2* mutant embryos, it has been shown that either signaling or responding blastomeres can supply *aph-2* function. Thus, APH-2 appears to be a membrane-bound extracellular protein that can be produced by either responding or signaling cells and somehow influences *glp-1* function during early embryonic inductions. It will be interesting to learn whether similar factors modulate Notch function during embryogenesis in other animals.

Finally, other signals have been identified that serve to specify the eight different fates of the AB descendants present in a 12-cell-stage embryo (Hutter and Schnabel, 1995). However, the gene products that mediate these other interactions remain to be identified, and thus these other interactions are not covered further in this chapter.

IV. The Intermediate Group Genes: *mex-1, mex-3,* and *pos-1*

This third and final group of mutants exhibits specific but relatively widespread defects in blastomere fates compared to the blastomere identity group mutants described earlier. Intermediate group mutant embryos usually have normal cleavages up until the division of P_3, which in wild-type embryos divides asymmetrically to produce a smaller germline progenitor, P_4, and a larger somatic daughter, D (Fig. 2). In *mex-1*, *mex-3*, and *pos-1* mutant embryos, P_3 divides equally and produces two daughters with similar fates (Mello *et al.*, 1992; Draper *et al.*, 1996; R. Hill and J. Priess, personal communication). However, the changes seen in blastomere identity are distinct for each intermediate group mutant, and in each mutant other blastomere identities are also affected (see the following). In addition to the P_3 defect, some *mex-1* mutant embryos show more global, *par*-like defects, including a small fraction that have an equal first cleavage (Schnabel *et al.*, 1996). P-granule localization defects parallel the cleavage defects: Normal or roughly normal segregation occurs until the division of P_3, at which time no P-granule segregation occurs. Again, *mex-1* mutants appear to be more severely defective: P-granules usually are segregated to P_1, P_2, and P_3, but they fail to associate with the posterior cortex in germline precursors, and many are lost to somatic daughters during cell division (Mello *et al.*, 1992; Schnabel *et al.*, 1996). Thus, *mex-1* mutants may be defective not in moving P-granules but in anchoring them to the posterior cortex.

A. Intermediate Group Gene Products

Molecular analysis has shown that two of the genes in this group, *mex-1* and *pos-1*, are related and both encode proteins with a Zn^{2+} finger domain (Guedes

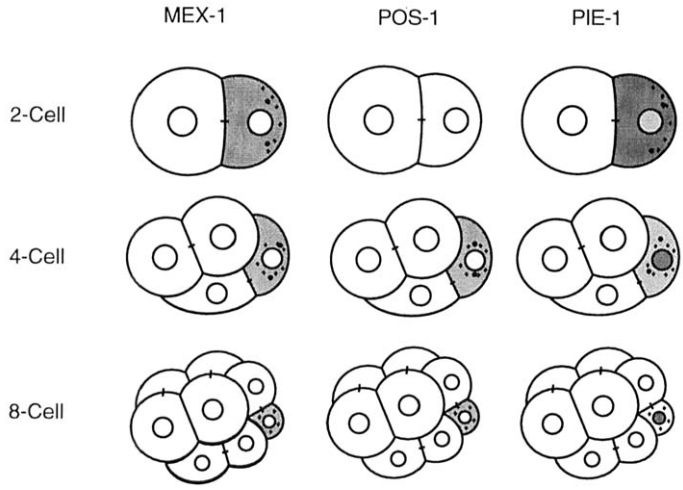

Fig. 13 Summary of the distributions of three TIS-11-like Zn^{2+} finger proteins in the early embryo. MEX-1, POS-1, and PIE-1 are all present in germline precursors and are associated with P-granules. All three proteins are first detectable at the two-cell stage in P_1. MEX-1 and POS-1 are cytoplasmic and associated with P-granules; PIE-1 at first is cytoplasmic and associated with P-granules in P_1 but becomes nuclear, localized, and associated with P-granules in later stages. No P-granule association is detectable for any of these proteins in oocytes.

and Priess, 1996; R. Hill and J. Priess, personal communication). The spacing of cysteine and histidine residues found in both *mex-1* and *pos-1* is also found in vertebrate TIS-11 genes, which are expressed early in response to treatment of fibroblasts with triphorbol esters but are of unknown function (Guedes and Priess, 1996). The MEX-1 and POS-1 proteins also show nearly identical distributions in the early embryo (Fig. 13). They are present in the cytoplasm of germline precursors, and after each division of a germline precursor they rapidly fade from the somatic daughter. Intriguingly, both proteins associate with P-granules in the early embryo. Like *mex-1* and *pos-1*, the maternal gene *pie-1* also encodes a TIS-11-like Zn^{2+} finger protein (Mello *et al.*, 1996). *pie-1* mutant embryos resemble *mex-1* and *pos-1* mutants in that P_3 divides equally, and the PIE-1 protein also is progressively localized to germline precursors and associates with P-granules at the two-cell stage (Mello *et al.*, 1992, 1996). PIE-1 is different from MEX-1 and POS-1 in that it localizes to centrosomes during mitosis and to nuclei during interphase beginning at the four-cell stage (Fig. 13). For all three TIS-11-like proteins, no association with P-granules is detectable in the maternal germline, due either to their absence or perhaps to masking by other proteins. In either case, these results indicate that P-granules are dynamic structures, raising the interesting possibility that P-granules load and perhaps unload different factors at different times during the early cleavages that generate found-

er cells. Finally, *mex-1* is required to restrict PIE-1 to the germline when P_3 divides: In *mex-1* mutant embryos, PIE-1 is mislocalized to and represses the transcription of zygotic genes in both P_3 daughters, indicating that in addition to being related these genes also have some regulatory interactions (Guedes and Priess, 1996). To summarize, three maternal genes, *mex-1*, *pos-1*, and *pie-1*, are all related by sequence and show similar localization to the germline and an association with P-granules. The functions of TIS-11-like Zn^{2+} finger domains are not known, but this group of genes, and how they interact, is likely to become an important focus in future studies of embryonic patterning in *C. elegans*.

B. Intermediate Group Mutant Phenotypes

Aside from the equal division of P_3, the phenotypes that result from inactivation of intermediate group genes are different from one another (see the following). However, in all three mutants, most affected blastomeres are transformed into more posterior fates (Draper *et al.*, 1996; Guedes and Priess, 1996; R. Hill and J. Priess, personal communication). On the basis of these observations, it seems reasonable to consider the possibility that intermediate group genes mediate assignments of blastomere identity based on interpretations of the AP polarity generated more directly by the par group gene products (discussed earlier). Nevertheless, the phenotypes of these three mutants are all quite distinct. As summarized in the following, *mex-1* and *mex-3* mutants have extensive defects in AB development in addition to P_3 defects, whereas *pos-1* mutants have extensive defects in P_1 development.

mex-1 mutant embryos are the most *par*-like of the intermediate group mutants. In some embryos, the first cleavage is equal, and P-granule partitioning is defective throughout the early cleavages of all mutant embryos (Mello *et al.*, 1992; Schnabel *et al.*, 1996). Most obvious in *mex-1* mutant embryos, the four granddaughters of AB often adopt fates similar to that of MS, a descendant of the posterior blastomere, P_1. Consistent with this finding, SKN-1 protein is mislocalized in *mex-1* mutant embryos, accumulating to high levels in ABa and ABp at the four-cell stage and in their daughters at the eight-cell stage (Bowerman *et al.*, 1993). Thus, *mex-1*, like *par-1* and *par-3*, is required for restricting the accumulation of high levels of SKN-1 to P_1 descendants (Bowerman *et al.*, 1997). Although many AB descendants develop like MS, in most cases they appear to adopt fates that are mosaics of AB and MS fates, as determined by cell lineage studies (Schnabel *et al.*, 1996). Thus, transformations in blastomere identities in the early *C. elegans* are by no means absolute and can instead be partial in nature. In some mutant embryos, E fails to produce gut and instead adopts a C-like fate, which is a more posterior fate in the sense that C is a daughter of P_2 and the posterior sister of EMS, the parent of E. The equal cleavage of P_3 in *mex-1* mutants results in a loss of germline and the production

of two D-like daughters that produce body wall muscle cells, as does a wild-type D blastomere. Finally, although some *mex-1* mutant embryos exhibit extensive defects in the development of both P_1 and AB descendants, in many *mex-1* mutant embryos, P_2 and EMS development, aside from the abnormal development of P_3, appears largely normal. Thus, whereas *mex-1* mutant embryos resemble *par* mutant embryos in many respects, *mex-1* mutant embryos may have less extensive losses of AP polarity.

mex-3 mutant embryos are like *mex-1* mutant embryos in that their most obvious defect is the abnormal development of AB granddaughters (Draper *et al.*, 1996). However, instead of adopting MS-like fates, they adopt C-like fates. As described earlier, *pal-1* is required for specifying C fate, and PAL-1 is present at high levels only in the nuclei of P_2 and EMS at the four-cell stage. In *mex-3* mutant embryos, PAL-1 is evenly distributed in all four-cell-stage blastomeres, suggesting that PAL-1 acts ectopically to specify C-like fates in the granddaughters of AB (Hunter and Kenyon, 1996). Consistent with this hypothesis, AB descendants no longer develop like C in *mex-1;pal-1* double mutant embryos, in that they no longer produce body wall muscle cells (Hunter and Kenyon, 1996). The MEX-3 protein contains two KH domains, found in RNA-binding proteins, and is present at higher levels in the cytoplasm of AB than in P_1 at the beginning of the two-cell stage and through the four-cell stage (Draper *et al.*, 1996). Furthermore, *pal-1* mRNA is present throughout the early embryo, and the 3′-UTR of *pal-1* mRNA is necessary and sufficient to localize the translation of a lacZ reporter RNA to P_1 descendants (Hunter and Kenyon, 1996). Thus, it has been proposed that MEX-3 directly regulates PAL-1 expression by acting as a translational repressor in AB, perhaps binding 3′-UTR sequences in *pal-1* mRNA (Draper *et al.*, 1996; Hunter and Kenyon, 1996). In later stage embryos, MEX-3 is present at very low levels only in P_2 descendants and is undetectable in other blastomeres.

How the complex expression pattern of MEX-3 is regulated remains largely unknown, but it appears to occur in part by localization of the *mex-3* maternal mRNA (Fig. 14). *mex-3* is unique among the maternal genes discussed thus far in showing an enrichment of mRNA in AB (Draper *et al.*, 1996). At the beginning of the one-cell stage, *mex-3* mRNA is distributed evenly but it fades to lower levels posteriorly by the time the zygote divides. During the two-cell and four-cell stages, higher levels of *mex-3* mRNA are present in AB and its daughters than in P_1 and its daughters. Subsequently, *mex-3* mRNA levels fade to below detectable levels except in the germline, an observation true of many maternal mRNAs in *C. elegans* (Seydoux and Fire, 1994). The stability of maternal mRNAs in the germline may simply reflect the apparently inert status of the germline, maintained at least in part by *pie-1* function (discussed earlier).

Finally, *mex-3* is unique among mutants with equal P_3 cleavages (*pie-1*, *mex-1*, *mes-1*, *mex-3*, and *pos-1*) in that both P_3 daughters adopt a germline fate (Draper *et al.*, 1996). In all the other mutants, both P_3 daughters adopt a D-like

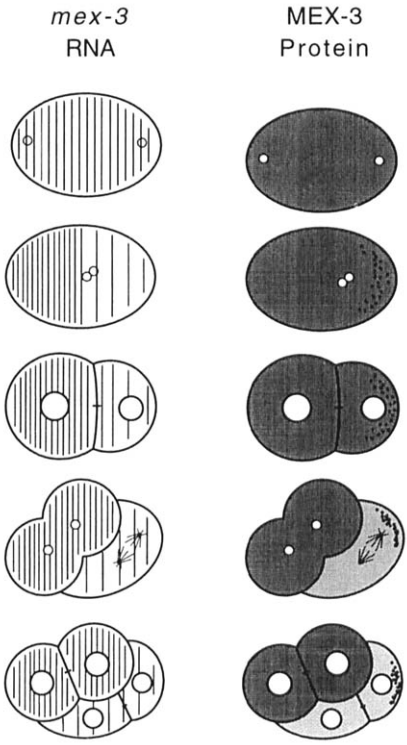

Fig. 14 Distribution of *mex-3* RNA and protein in one-, two-, and four-cell-stage wild-type embryos. The maternally expressed *mex-3* mRNA is evenly distributed in oocytes and early one-cell-stage embryos, but becomes enriched in the anterior part of the zygote shortly before mitosis and remains enriched in AB and its daughters at the two-cell and four-cell stages. Similarly, MEX-3 is first detected throughout oocytes and the one-cell-stage embryo, but begins to fade to lower levels in P_1 as it divides and remains enriched in ABa and ABp at the four-cell stage. MEX-3 is also associated with P-granules beginning late in the one-cell stage. The only other maternal gene that produces a localized mRNA is *pos-1*, which is present in P_1 but not in AB at the two-cell stage (not shown).

fate and produce body wall muscle cells (discussed earlier). Thus, not all blastomere transformations in the intermediate group mutants are to more posterior fates, as the transformation occurs in both directions for P_3 daughters depending on the mutant background. Also, as P-granules are mislocalized in all mutants when P_3 cleaves equally, their mislocalization is not sufficient to specify germline. However, as mentioned earlier, mutations in the maternal gene *pgl-1* result in a loss of detectable P-granules and sterility, indicating that P-granules are necessary but not sufficient for specifying germline fate and that somatic blastomeres may be resistant to any germline specification activities that P-granules may possess (S. Strome, personal communication).

As described earlier, *pos-1* also encodes a TIS-11-like Zn finger protein present in the cytoplasm of germline precursors and in P-granules beginning at the two-cell stage. Mutations in *pos-1* cause widespread defects in the development of P_1 descendants (R. Hill and J. Priess, personal communication). MS fails to produce body wall muscle, while E develops like C as in some *mex-1* mutant embryos. C development appears normal, but P_3 divides evenly as in *mes-1*, *mex-1*, *mex-3*, and *pie-1*. As in *mex-1* and *mes-1* mutant embryos, both P_3 daughters adopt a D-like fate, producing excess body wall muscle at the expense of germline. Finally, in both *pos-1* and *pie-1* mutant embryos, ABa and ABp produce anterior pharyngeal cells due to a lack of APX-1 expression (Mango *et al.*, 1994a; R. Hill and J. Priess, personal communication). Thus, mutations in *pie-1* and *pos-1* alter P_2 identity not only in terms of the cell fate patterns produced by P_2 but also in terms of P_2's ability to signal ABp via APX-1 to break the initial equivalence of ABa and ABp (discussed earlier). In contrast, P_2's ability to induce gut is normal, at least in *pie-1* mutants. Thus, mutations in genes that pattern the identities of P_1 descendants uncouple different temporal aspects of P_2 identity, even when comparing activities that both depend on maternally expressed regulatory factors.

V. Pathways of Blastomere Development

Thus far I have summarized a substantial body of work devoted to identifying maternal genes in *C. elegans* that regulate the early steps in pattern formation. This effort continues, but an even more challenging problem now is to understand how these gene functions are related. What are the genetic pathways that specify the eventual fates of individual blastomeres, and how do these pathways interact to coordinate their activities? Presumably the coordinated function of a limited set of such pathways results in the proper temporal and spatial regulation of transcription factors that regulate undefined sets of zygotic genes to specify blastomere identities. One example of such a genetic pathway involves the genes *par-1* and *mex-3* temporally and spatially regulating the expression of the putative transcription factor PAL-1 (Fig. 15).

A *par-1*, *mex-3*, and *pal-1* pathway can be understood most easily by referring first to the distributions of MEX-3 and PAL-1 in *par-1* mutant embryos (Fig. 15). MEX-3, normally present at higher levels in ABa and ABp than in P_2 and EMS, is present at high levels in all four-cell-stage blastomeres in *par-1* mutant embryos (Draper *et al.*, 1996). Consistent with the proposed role of MEX-3 as a translational repressor of *pal-1* in ABa and ABp in wild-type embryos, high levels of MEX-3 throughout *par-1* embryos correlate with the complete loss of PAL-1 protein (Hunter and Kenyon, 1996). Thus, mislocalization of MEX-3 to P_1 descendants causes ectopic repression of *pal-1* translation and hence a complete absence of PAL-1 from early *par-1* mutant embryos. Moreover, elimination

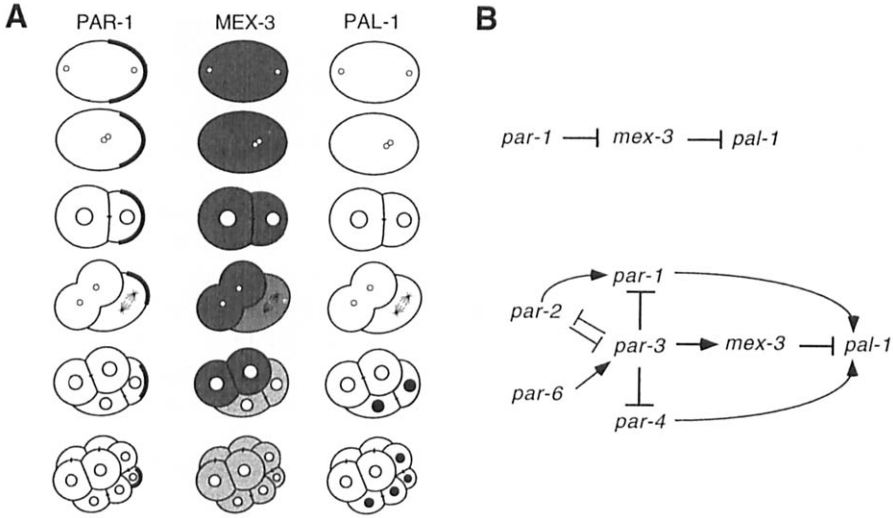

Fig. 15 Summary of PAR-1, MEX-3, and PAL-1 protein localization and models for the genetic interactions. See previous figure legends for a description of the protein localizations, illustrated in (A) for one-, two-, four, and eight-cell-stage wild-type embryos. (B, top) Simple pathway proposed for the regulation of PAL-1 expression by PAR-1 and MEX-3. (B, bottom) More complex network of gene function that attempts to account for all known functional interactions and protein localization data for the genes shown. *par-2* and *par-3* interact based on genetic studies and on analysis of their respective protein localization patterns in wild-type, *par-2*, and *par-3* mutant embryos. *par-2* is required for cortical localization of PAR-1, and *par-6* is required for cortical localization of PAR-6. *par-1* and *par-4* are both required for PAL-1 expression in posterior blastomeres, whereas *par-3* is partially required and *mex-3* is absolutely required to prevent PAL-1 expression in AB descendants. Therefore, *par-3* might indirectly limit PAL-1 expression to posterior blastomeres by restricting the *par-1*- and *par-4*-dependent activation of PAL-1 expression to posterior blastomeres. In this latter model, *par-3* is only partially required to limit PAL-1 expression to posterior blastomeres, and *mex-3* always functions to repress PAL-1 expression unless other factors, including *par-1* and *par-4*, overcome that repression to activate PAL-1 expression. By proposing such genetic pathways that connect the functions of the par group genes, through the intermediate group genes, to the localized function of blastomere identity group genes, it is now possible to begin to account more fully for the linkage between the establishment of AP polarity and the activation of blastomere identity programs in early founder cells. Bruce Draper generously provided materials for Figures 14 and 15.

of *mex-3* function in *par-1* mutant embryos restores PAL-1 expression and function in all four-cell-stage blastomeres (Draper *et al.*, 1996; Hunter and Kenyon, 1996). Finally, the localization of PAR-1, a putative Ser–Thr kinase, to the posterior cortex of P_1 in wild-type embryos correlates with the loss of *mex-3* mRNA and protein in P_1 and its daughters. Thus, PAR-1 may block *mex-3* function in the posterior part of the zygote, perhaps even directly by phosphorylating MEX-3, permitting the translation of *pal-1* in posterior blastomeres and eventually leading to the specification of the somatic founder cell fates derived from P_2 (Fig. 15).

However, studies of *par-3* mutant embryos indicate that the regulation of PAL-1 expression by *par-1* and *mex-3* may be more complex (Bowerman *et al.*, 1997). Like *par-1* mutant embryos, *par-3* mutant embryos mislocalize MEX-3 such that it is expressed at high levels in all four-cell-stage blastomeres. However, unlike *par-1* embryos, PAL-1 is expressed at normal levels and sometimes even mislocalized in *par-3* mutant embryos. One interpretation of this result is that *par-1* might act independently of *mex-3* to derepress the translation of *pal-1* mRNA (Fig. 15). While the linearity of a *par-1*, *mex-3*, and *pal-1* pathway in the early embryo requires additional testing, these studies provide an example of how genetic analyses in *C. elegans* are beginning to identify pathways that link the establishment of polarity in the zygote to the specification of individual blastomere identities in early stage embryos.

VI. Concluding Remarks

One purpose of this review has simply been to summarize progress in identifying maternal genes that regulate early steps in pattern formation. A more ambitious goal is to integrate this information into the construction of genetic pathways that can begin to explain at a molecular level how a cellularized embryo develops. Toward this latter end, I have tried to group the known maternal genes into categories that correspond not to close similarities in phenotype but rather to how early or late a particular gene might fit into a pathway of blastomere development. From what we know thus far, these pathways must link the specification of AP polarity initiated by sperm entry and PAR protein polarization into a reproducible pattern of founder cell identities specified by localized transcription factors such as PAL-1 and SKN-1. Genes within each of the three groups that I have defined in some cases appear to regulate each other, in addition to regulating a gene(s) in the group downstream of them. However, only one such pathway can be constructed that links members of all three groups: the possible *par-1*, *mex-3*, and *pal-1* pathway just described (Fig. 15). Almost certainly, these three groupings will prove inadequate to either describe all maternal genes or even integrate our understanding of those it can describe. Nevertheless, similar and perhaps more elaborate efforts should aid attempts to understand how a presumably complex network of gene regulation functions to specify the *C. elegans* body plan.

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