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Ras Is Required for a Limited Number of Cell Fates and Not for General Proliferation in *Caenorhabditis elegans*

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Experiments with mammalian tissue culture cells have implicated the small GTPase Ras in the control of cellular proliferation. Evidence is presented here that this is not the case for a living animal, the nematode *Caenorhabditis elegans*: proliferation late in embryogenesis and throughout the four larval stages is not noticeably affected in animals lacking Ras in various parts of their cell lineages. Instead, genetic mosaic analysis of the *let-60* gene suggests that Ras is required only, at least later in development (a maternal effect cannot be excluded), for establishment of a few temporally and spatially distinct cell fates. Only one of these, the duct cell fate, appears to be essential for viability.

The small G protein Ras participates at the plasma membrane in several signal transduction mechanisms, the best known being a pathway from receptor tyrosine kinases to transcription factors phosphylated by activated mitogen-activated protein (MAP) kinases. In addition to its involvement in cellular differentiation, Ras has been implicated in the control of cellular proliferation (2). Activated forms, many of which have a decrease in intrinsic GTPase activity, promote growth of certain tumors and certain cells in culture. A requirement for Ras in the stimulation of growth of NIH 3T3 cells has been demonstrated with neutralizing antibodies (22), and Ras is required for NRK cells to proliferate in medium lacking serum (7). Although these observations and others suggest a requirement for Ras in stimulation or maintenance of certain proliferative states, an unanswered question is the generality of this requirement during embryonic and postembryonic development and during adulthood.

In *Caenorhabditis elegans*, Ras appears to be encoded by a single gene, *let-60* (3, 8). Based on extensive genetic studies, the product of *let-60* participates in a signal transduction cascade that includes Raf, MEK, MAP kinase, and other proteins, as in mammals and *Drosophila melanogaster* (for a review, see reference 27). In postembryonic induction of the vulva of hermaphrodites, *let-60* functions genetically downstream of *let-23*, a gene whose product resembles a receptor for epidermal growth factor (1). Genetic evidence also indicates that *let-60* acts to some extent downstream of a fibroblast growth factor receptor (6, 28), the product of the *egl-15* gene (6).

The involvement of *let-60* in transducing signals from at least two receptor tyrosine kinases suggests an extensive role for the gene during development. This is consistent with an absolute requirement for *let-60* for viability: animals homozygous for *let-60*(*sy101sy127*), a likely null allele, die in the first of the four larval stages (L1 to L4) with a fluid-filled morphology (9, 10). Although the cause of death has remained unknown, its occurrence in the first larval stage is consistent with problems in embryogenesis. Because the homozygotes must be derived from mothers containing a functional copy of the *let-60* locus, it is possible that maternally provided *let-60* mRNA and/or protein also contributes to early development.

Analyses of reduction-of-function or dominant negative alleles also indicate multiple requirements for *let-60* throughout the life cycle. Properties observed for these nonnull alleles in hermaphrodites can include a total or partial failure of vulval induction during the L3 stage (3, 9), imprecise migration of sex myoblasts during the L3 stage (28), and a failure of germ nuclei to exit pachytene during oogenesis (5). These alleles can also affect the morphology of the copulatory spicules in males (4). However, because animals homozygous for *let-60*(*sy101sy127*) die in the first larval stage, the best assessment, based on the best candidate for a null allele, of requirements for *let-60* for cellular proliferation and for establishment and maintenance of differentiated states is lacking. In order to investigate whether lethality results from a general or specific loss of *let-60* activity during development, animals genotypically mosaic for the likely null allele were generated, and the consequences of the presumed lack of Ras activity in particular parts of the cell lineage with respect to development and proliferation were examined.

MATERIALS AND METHODS

Construction of MH809. The extrachromosomal array *kuEx72* was formed in vivo by intermolecular recombination following standard microinjection (20) of a solution of cloned DNA into the gonad of a strain with the genotype *ncl-1(e1865) unc-36(e251)III; let-60(sy101sy127)/dpy-20(e1282)IV; him-5(e1490)V*. The solution contained C33C3 (75 μg/ml), R1p16 (50 μg/ml), and pMH90 (10 μg/ml), and transformants were initially screened for correction by the plasmid R1p16 (12) of uncoordinated behavior conferred by the *unc-36* mutation. Complementation of the *ncl-1* mutation by the cosmid C33C3 (21) and of *let-60* by the plasmid pMH90 (28) was then determined. A control strain, MH888, with the genotype *ncl-1(e1865) unc-36(e251); him-5(e1490); kuEx72(ncl-1$^+$ unc-36$^+$ let-60$^+$)* was constructed by genetic crosses in order to be certain that properties in mosaic animals were associated with the presence or absence of wild-type *let-60* DNA.

Mosaic analysis. Images of living L3 or L4 larvae were obtained at a 1,000-fold magnification with Nomarski optics, and the nuclei of the following cells were examined for the phenotype conferred by *ncl-1(e1865)*: RID, ALA, and RMED (from ABala); m3L, m3VL ASKL, and ADLL (from ABalp); mc2DL, mc2DR, m3R, I5, and mc2V (from ABara); AMshL, G2.p, ASIL, TL.pa(a/p), and P(3/4,5/6,7/8).p (from ABpla); excretory duct cell, excretory gland L, excretory cell, DVB, hyp8/9, and F (from ABplp); ASKR, ADLR, ILsoDR, AMshR, ASIR, TR.pa(a/p), and P(3/4,5/6,7/8).p (from ABpra); excretory gland R, hyp8/9, B, DVA, and body muscle (from ABprp); body muscles, various pharyngeal cells, coelomocytes, posterior distal tip cell, and AC (from MSa); various pharyngeal cells, body muscles, coelomocytes, anterior distal tip cell, and AC (from MSp); DVC, body muscles, and hyp 11 (from C); and body muscles (from D). Losses in ABarp were not scored, thereby preventing an assessment of ABar losses. Also,

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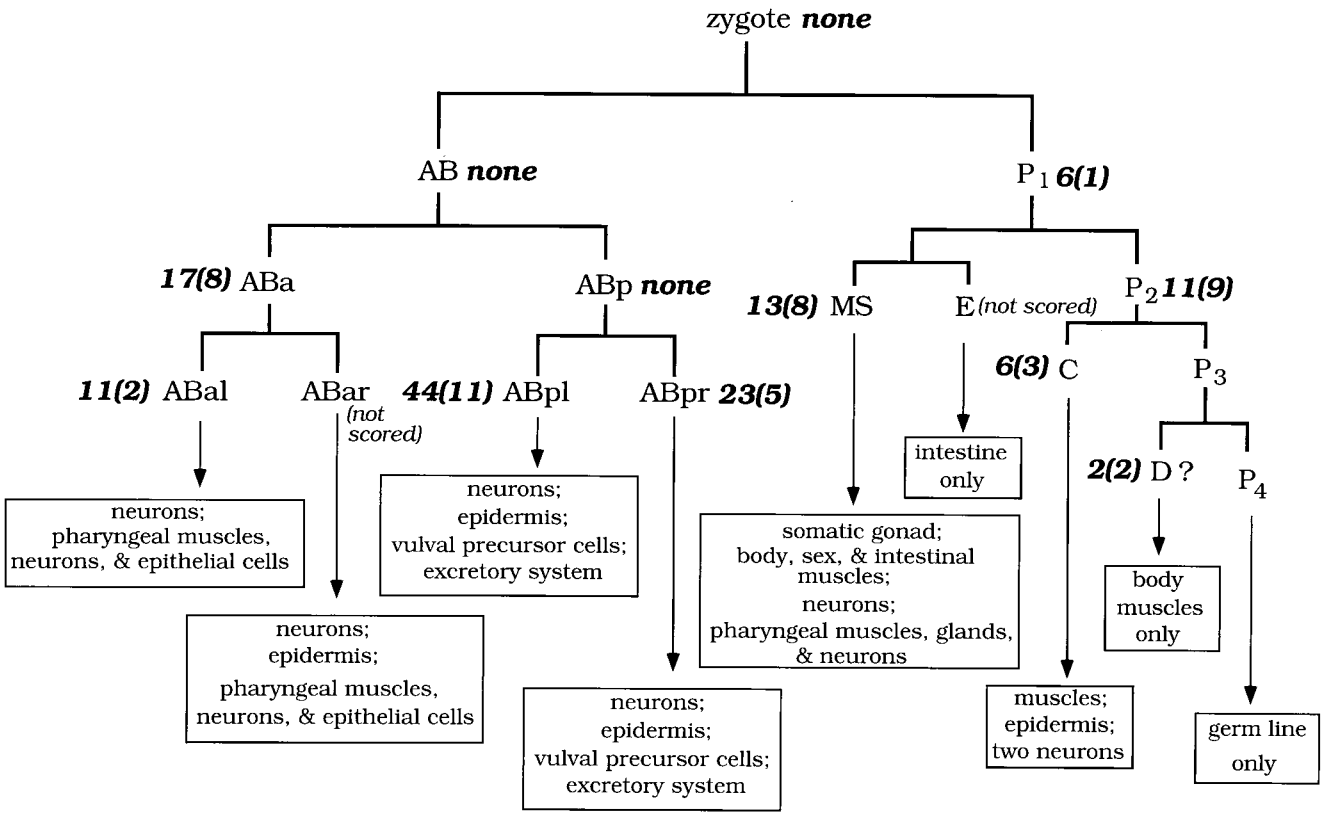

FIG. 1. Large parts of the cell lineage need not inherit wild-type *let-60* DNA. The number of L3 or L4 animals, from a set of 4,077 animals examined individually by Nomarski microscopy, in which a particular cell of the early embryo (26) was deduced not to have inherited *kuEx72* is indicated next to that cell. In order to determine whether these types of losses affected further growth, animals were occasionally rescued from the microscopy and allowed to resume growth. None of the losses prevented growth to adulthood or survival as adults, and the total number assessed in this manner is indicated in parentheses.

losses in D could not be distinguished from losses in P_3; this ambiguity arises from an inability to score the nucleoli of the germ line (11). Conclusions as to the genotype of the germ line were based on losses in P_1 or P_2.

In order to determine whether losses affected further growth and development, animals were occasionally rescued from the microscopy and allowed to resume growth. Pachytene arrest was determined by microscopy of fixed adults whose DNA had been stained with a fluorescent dye as described previously (5). Hermaphrodites with losses of the array in all or in a subset of the vulval precursor cells when examined at the early L3 stage were rescued from the initial microscopy so that induction could be scored later. Because the results are very similar to those observed in mosaic analyses of the *let-23* gene (16, 24), they are not presented in detail but are available upon request. Effects on apoptosis or resistance to UV light or other stresses were not examined.

RESULTS

Absence of wild-type *let-60* DNA from most parts of the cell lineage does not affect viability or cellular proliferation. Animals genotypically mosaic for *let-60* were derived by a standard method for *C. elegans* that relies on occasional nondisjunction of extrachromosomal DNA during mitosis (11). A daughter cell not inheriting this DNA will be genotypically mutant for recessive, chromosomal mutations complemented by the DNA and, if able to divide, will produce genotypically mutant cells or tissue in an otherwise wild-type background.

One source of extrachromosomal DNA for mosaic analysis can be an array that can form by intermolecular recombination following microinjection of DNA clones into the gonadal syncytium (18). This approach was used here. A strain [MH809; the complete genotype is *ncl-1(e1865) unc-36(e251); let-60 (sy101sy127)*; *him-5(e1490)*; *kuEx72(ncl-1$^+$ unc-36$^+$ let-60$^+$)*] was constructed to have mutant alleles for the chromosomal copies of *let-60* and of a second gene, *ncl-1*, and an extrachromosomal array (*kuEx72*) of cloned DNA that complements both. *let-60(sy101sy127)* was chosen because it is a likely null allele. It was isolated as a revertant of a dominant negative allele (*sy101*) and contains a stop codon at position 123, which should produce a truncated product lacking domains conserved among Ras proteins, including the fourth nucleotide-binding domain and the CAAX box for association with the plasma membrane (10). *ncl-1(e1865)* confers in a cell-autonomous manner an enlarged nucleolus that can be identified by Nomarski differential interference contrast microscopy (11). Because the functional copies of *let-60* and *ncl-1* cosegregate with the *kuEx72* array, cells that fail to inherit it are genotypically mutant for both genes. Since the nearly invariant cell lineage from zygote to adult has been completely determined (25, 26), it is possible to infer the genotypes of nearly all parts of the lineage for *let-60* based on the sizes of the nucleoli of a large number of representative cells that have descended from various parts of the cell lineage.

A surprising result was the ability to obtain animals that had suffered losses of the array very early during embryonic development (Fig. 1). For example, from a set of 4,077 L3 or L4 larvae randomly chosen for microscopy, six had losses of the array in P_1, one of the daughters of the zygote, and 17 had losses in ABa, one of the cells constituting a four-cell embryo. Furthermore, one of the animals with a loss in P_1 and eight of those with losses in ABa were rescued from the microscopy and assessed for growth to adulthood. Each of these nine animals attained and survived in adulthood. Thus, the vital

functions of *let-60* must be restricted in the lineage rather than general, because ABa and P_1 each generates a very large portion of an animal and a number of different cell types over many hours of embryonic and postembryonic development.

The only early losses not seen were in AB and one of its daughters, ABp, suggesting that lethality results before the L3 stage when ABp fails to inherit the array. (Descendants of the early embryonic cells are designated according to the axes of the cell divisions that generate them [26]; a and p represent anterior and posterior, respectively, and l and r represent left and right, respectively.) As discussed below, lethality appears to be very restricted even within the descendants of ABp.

The ability to obtain animals with very early losses also indicates that *let-60* activity is largely dispensable for cellular proliferation, at least later in development. One example is the expansion of the somatic gonad of hermaphrodites from two cells at hatching to 143 cells at the L4 stage 2 days later (15). Animals with losses in P_1 or MS, the early embryonic progenitors of the somatic gonad, clearly possessed a gonad containing a uterus and two spermathecae composed of many cells. Although the cell divisions of the somatic gonad were not directly observed, raising the possibility that subtle transformations of cell fates may have been overlooked, seven of eight hermaphrodites with losses in MS produced viable progeny, demonstrating functionality of the gonad. (The exceptional animal possessed oocytes and spermatozoa but did not produce progeny, apparently because oocytes could not exit the spermatheca.)

Based on animals suffering losses in P_1 or P_2, the earliest embryonic progenitors of the germ line, *let-60* activity is also not critically required for mitotic proliferation of the germ line. This proliferation occurs postembryonically over several days and provides a steady supply of germ nuclei for meiosis (15). However, as described below, germ nuclei lacking *let-60* activity are unable to exit the pachytene stage of meiosis during oogenesis of hermaphrodites.

Embryonic expression of *let-60* is needed for only one vital cell fate. The failure to observe animals with losses in ABp indicates that lethality results when this cell fails to inherit the array. However, a paradox is apparent: neither of the daughters of ABp (ABpl or ABpr) needs to inherit the array—only ABp itself does (Fig. 1). Although lethality could result from a combination of effects, seven animals had two independent losses within the ABp sublineage such that many of the descendants of both ABpl and ABpr lacked the array. Without exception, the array was retained in either ABplp or ABprp, indicating that lethality results only when *let-60* activity is absent from both of these sublineages, as would occur, of course, in ABp losses. Thus, the vital requirement for *let-60* is extremely restricted in the cell lineage and exhibits lineal plasticity.

Closer examination of 30 animals with losses in ABpl and of 24 animals with losses in ABplp revealed an abnormality: in each of these 54 animals, the nucleolus of the excretory duct cell, a cell (ABplpaaaapa) that descends from ABplp in wild-type animals (26), was never enlarged, demonstrating that this cell possessed the array and therefore could not have been derived from ABpl or ABplp in these animals (Fig. 2A). By contrast, examination of a strain, MH888, identical to MH809 except for wild-type chromosomal copies of *let-60* demonstrated that the nucleolus of the duct cell can be scored reliably: the nucleolus was clearly enlarged in 2 of 2 animals with losses in ABpl and in 38 of 38 animals that failed completely to inherit the array (Fig. 2B).

The alteration in the origin of the duct cell in animals with losses in ABpl or ABplp implicates *let-60* in establishment of this cell fate in normal development. Based on laser ablations, the duct cell is known to differentiate from one of a pair of cells that are initially equivalent in developmental potential (26). The second cell fate that derives from this pair is thought to be G1 (ABprpaaaapa in wild-type animals), a cell that functions as the excretory pore cell in embryos and as a neuroblast later in development. Since ablation of ABplpaaaap, normally the mother of the duct cell (Fig. 2D), in wild-type embryos presumably results in ABprpaaaapa adopting the duct fate (26), a loss of *let-60* activity in ABplp apparently results in a similar switch in cell fates.

The excretory duct cell is one of two vital components of an osmoregulatory system; it is thought to be a conduit for the excretion of excess fluid from the large excretory cell to the exterior of an animal (23). Because ablation of the nucleus of a fully differentiated duct cell in wild-type animals results in a swollen morphology and eventual death (23), an interpretation of the lethality associated with the failure of AB or ABp to inherit *let-60* activity is the complete inability to specify the duct cell fate. Indeed, there was no evidence for the presence of a duct in 17 of 17 swollen L1 larvae segregating from MH809 when they were examined by Nomarski microscopy. These animals clearly possessed a fully formed excretory cell, the other vital component of the system. Swelling is often first evident in a region of the excretory cell that corresponds to where it would be joined to the duct in wild-type animals.

Conversely, 16 of 29 animals homozygous for *let-60(n1046; G13E)*, which encodes an activated form of the protein with a substitution of glutamic acid for glycine at position 13 (3), appeared to have two duct cell nuclei (Fig. 2C), and many had a protrusion of the cuticle at its junction with the duct, indicating a weakness in the junction. The protrusion is evident in L1 larvae and is consistent with an absence of G1, a cell that forms part of the junction in embryos (26). It appears, therefore, that *let-60* is both necessary and sufficient for establishment of the duct cell fate. Furthermore, because a switch in the origin of the duct cell was the only abnormality evident when losses in ABpl were compared with those in ABpr, the only vital requirement for embryonic expression of *let-60* appears to be for this function.

Specific mosaicism is correlated with specific defects. Although genetic mosaic analysis permits a direct test of function within an animal and has been successfully applied to many genes in *C. elegans* (11), interpretations can be uncertain because of the potential for mRNA or protein to perdure in clones of cells not inheriting functional copies of a gene. In the case of *let-60*, the half-life of neither the mRNA nor the protein is known. However, confident interpretations are possible if specific defects are always associated with specific mosaicism. The data presented above indicate that the perdurability of *let-60* is not extensive: 54 of 54 animals with losses in ABpl or ABplp had a switch in origin of the duct cell. Nevertheless, mosaic animals were examined for correlations with, and for further insights into, the other defects associated with reduction-of-function alleles of *let-60*. Correlations have already been presented for mispositioning of the sex myoblasts in hermaphrodites (28).

let-60 is best known for being necessary and sufficient for postembryonic induction of the hermaphroditic vulva (see reference 27 and references therein). An expectation based on laser ablations and genetic analyses is a requirement for *let-60* in the vulval precursor cells. This exception has been completely met: seven of seven larvae observed at the L3 stage as lacking the array in all six vulval presursor cells as a consequence of two independent losses had a complete failure of induction. Based on hermaphrodites in which the vulval pre-

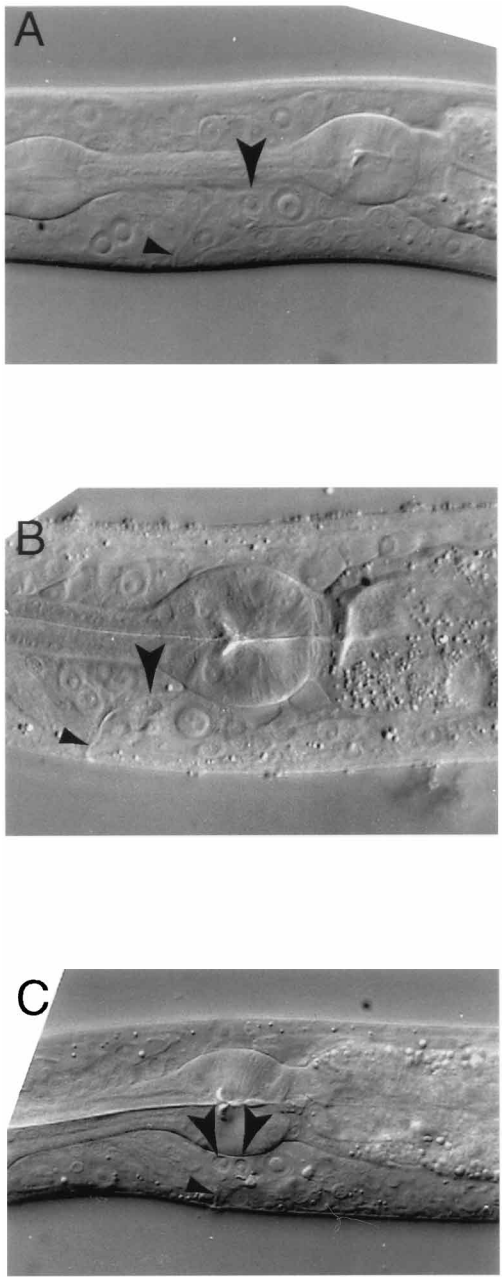

FIG. 2. The presence or absence of *let-60* activity can affect the origin of the excretory duct cell. (A) The nucleolus of the excretory duct cell nucleus (large arrowhead) is enlarged in an array-minus segregant from the control strain MH888. A photomicrograph of a Nomarski image encompassing part of the head and pharynx of an L3 larva is shown. The nucleus of the duct cell can be distinguished by its morphology and by its position just anterior (to the left in this micrograph) of the large nucleus of the excretory cell (23). The small arrowhead indicates part of the actual duct near its fusion with the cuticle. (B) The origin of the duct cell has changed in an L4 larva from MH809 with a loss of the array in ABpl. A small nucleolus is present in the nucleus (large arrowhead) of the duct cell. The small arrowhead is as in panel A. (C) Evidence for two duct cell nuclei in an animal homozygous for *let-60(n1046;G13E)*. Two nuclei with identical morphologies (large arrowheads) are just anterior to the nucleus of the excretory cell. A protrusion of the cuticle at its junction with the duct (small arrowhead) is also evident. (D) Fates and lineal relationship of the cells ABplpaaaapa and ABprpaaaapa in wild-type animals (adapted from reference 26). Many of the cells that descend from ABplp are the left lineal homologs of cells from ABprp (not all cells are shown). G1 and the duct cell constitute one of the exceptions to this left-right symmetry.

cursor cells were themselves mosaic, *let-60* activity was absolutely required for induction of the primary cell fate but not for the secondary fate (30) (see Materials and Methods), in complete agreement with mosaic analyses of *let-23* (16, 24). In contrast, losses affecting the somatic gonad, but not the vulval precursor cells, had no effect on induction. A cell in the somatic gonad is the source of the inductive signal in wild-type animals (see reference 27 for a review).

In males, mutations in *let-60* can affect differentiation of the copulatory spicules (4). Laser ablations and mutational analysis have suggested that a blast cell, B (ABprpppapa), receives an inductive signal from a second cell, F (ABplpppapp). *let-60*-mediated transduction of this signal within B would then specify it to produce the proper sublineage generating the spicules. The mosaic analysis is completely consistent with this model. Nine of nine males suffering losses in progenitors of B but not F had spicules with an abnormal, crumpled morphology, as for reduction-of-function mutations in *let-60*. [Three of the losses were in ABpr, two were in ABprp, and four were in ABprpp(p).] In contrast, six of six males with losses in progenitors of F but not B had morphologically normal spicules when examined with Nomarski optics. (Three of the losses were in ABpl, and three were in ABplp.) However, losses confined only to B itself were not examined, precluding a complete test of the model.

Reduction-of-function mutations in *let-60* can confer a failure of germ nuclei to exit the pachytene stage of meiosis during oogenesis but not during spermatogenesis (5). The mosaic analysis indicates that this pachytene arrest results from an absence of *let-60* activity in the germ line itself (Fig. 3). The complete inability to produce oocytes when the germ line lacks the array has precluded an assessment of whether a maternal contribution of *let-60* activity participates in early proliferation and differentiation.

Although effects on apoptosis or resistance to UV light or

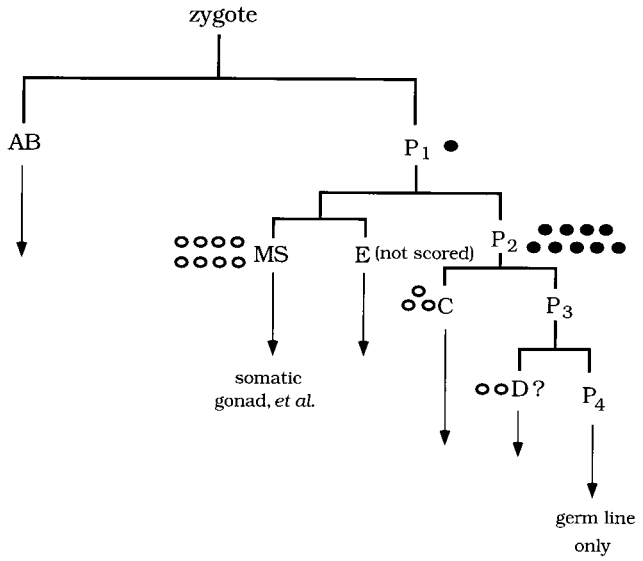

FIG. 3. *let-60* is required in the germ line of hermaphrodites for fertility, precluding the production of germ line clones. Individuals suffering losses of *kuEx72* in the early cells from the P_1 side of the lineage (26) are designated next to those cells by closed circles if exhibiting pachytene arrest and by open circles if not. Based on losses in P_2, the focus of the pachytene arrest was deduced to be P_4, the precursor of the germ line, since losses only in C did not result in this effect.

other stresses were not examined and although subtle effects on behavior or physiology could have been overlooked, no other defects were associated with animals mosaic for the likely null allele, suggesting that embryonic expression of *let-60* is largely required for specification of a only few cell fates. One final conclusion is evident from the data presented above: a positive correlation of cellular phenotype with cellular genotype proves that *let-60* functions in a cell-autonomous fashion, as expected for a gene encoding Ras.

DISCUSSION

Mosaic analysis of Ras function in *C. elegans* has revealed no overt role in cellular proliferation late in development. The main consequence of a lack of embryonic expression of *let-60* in various parts of the cell lineage is a cell-autonomous failure to specify a few cell fates, a striking observation given the involvement of *let-60* in transducing signals from at least two receptor tyrosine kinases and given the view that mammalian Ras proteins participate in several distinct signal transduction pathways in addition to the canonical one involving Raf, MEK, and MAP kinase (19). An alternative interpretation of the limited requirement shown here is the existence of a second gene for Ras that functions in those parts of the cell lineage that are not affected by an absence of *let-60* activity. However, evidence for a second gene is lacking. PCR of genomic DNA and cDNA libraries with probes based on specific regions of the *let-60* protein and other Ras proteins has resulted only in the reisolation of *let-60* and in the isolation of two genes encoding products similar to Rap1, a small G protein related to but distinct from Ras (29). Also, another gene has not been revealed in the genome sequencing project, which has determined nearly 60% of the predicted total number of genes (14) or in a collection, representing about 40% of the total number of genes, of expressed sequence tags (17). Another interpretation is perdurability of mRNA or protein, a possibility that can never be completely eliminated. However, the strong correlation of defects with specific mosaicism indicates that perdurability is not pervasive during development.

Because *let-60* is required downstream of at least two receptor tyrosine kinases, it was natural to assume that the lethality associated with reduction-of-function mutations was a result of a broad failure in differentiation or in general proliferation. Instead, the mosaic analysis indicates that it results from a failure to establish one cell fate, the excretory duct cell. A model for the effect of *let-60* activity on the origin of the duct cell is presented in Fig. 4. In wild-type animals, both ABplpaaaapa and ABprpaaaapa have the potential for activation of the *let-60* protein, which is required for induction of the duct cell fate in opposition to a default fate, G1. A signal from a third, unidentified cell activates the *let-60* protein in ABplpaaaapa, specifying it as the duct cell. ABplpaaaapa in turn inhibits ABprpaaaapa from adopting this fate, and it now differentiates as G1. The inheritance of potential Ras activity in ABpr but not in ABpl would preclude ABplpaaaapa not only from becoming the duct cell but also from inhibiting ABprpaaaapa. Freed of inhibition, this cell could respond to the inductive signal from the hypothetical third cell. The G13E form of Ras encoded by the *n1046* allele of *let-60* occasionally has sufficient gain of function to promote the duct cell fate in both cells, perhaps even independently of an inductive signal.

It should be stressed that the origin of the duct cell in animals with ABpl or ABplp losses is unknown. However, circumstantial evidence strongly suggests that ABplpaaaapa and ABprpaaaapa have indeed switched fates. In 16 of the animals suffering losses in ABplp (the other 8 animals were not examined), one and only one nucleus in the right, subventral part of the ring ganglion had an enlarged nucleolus. In wild-type animals, no descendants from ABplp are present there (26). Moreover, the position is consistent with that expected for RMHR, one of two neurons that are derived from G1 (the other neuron, RMHL, resides in the corresponding region on the left). Although the density of nuclei in this region makes an accurate assessment of position difficult, the simplest interpretation is that the cell with the G1 fate has descended from ABplp and not from ABprp.

let-60 has a second essential function, the production of oocytes (5). The mosaic analysis demonstrates that the gene activity is required in the germ line, a result in agreement with mosaic analysis of the gene for MAP kinase (5). The source of a presumed signal that allows germ nuclei to exit pachytene is unknown, nor has a receptor tyrosine kinase been implicated in receiving the signal. However, the requirement for both Ras and MAP kinase suggests that the canonical signal transduction pathway is employed in this process. As discussed below, a presumed absence of Ras in the germ line of *D. melanogaster* has no effect on oogenesis (13), indicating that the pathway, no matter what its nature, is not universally required for oogenesis. In any case, the requirement for *let-60* activity for the production of oocytes precludes an examination of whether maternally provided *let-60* function is required for differentiation or for general proliferation in the earliest stages of development.

One interpretation of a mosaic analysis of *let-23*, the gene whose product resembles an epidermal growth factor receptor, is that a maternal contribution of *let-60* mRNA and/or protein may be sufficient for at least one, and possibly several, steps in early development. Although animals homozygous for a null allele (*mn23*) of *let-23* die in the first larval stage with an appearance superficially like that of *let-60(sys101sy127)* homozygotes, animals require *let-23* activity in ABplp and in part of ABal for viability (16), both of which are at variance with the much more limited requirement deduced here for *let-60*. More-

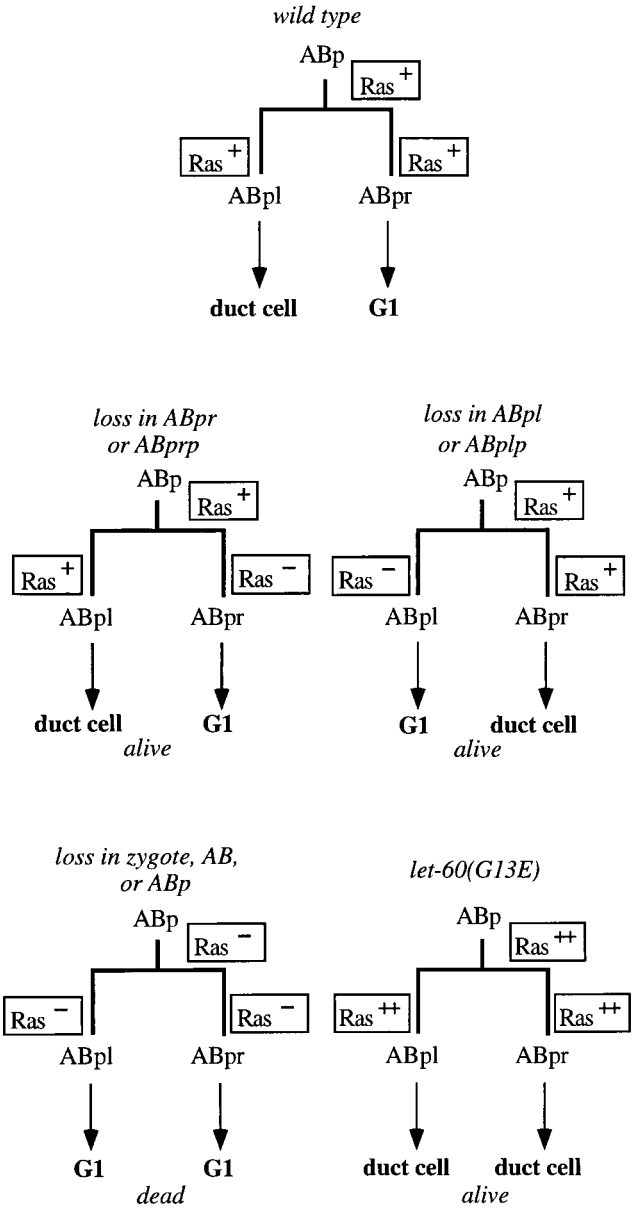

FIG. 4. Model for the effect of *let-60* activity on the origin of the duct cell. The inheritance of wild-type *let-60* DNA, and therefore potential Ras activity, is indicated as Ras^+. LET-60(G13E) is an activated form of the protein encoded by the *n1046* allele.

over, *let-60* appears to act genetically downstream of *let-23* in these sublineages, as animals doubly mutant for *let-23(mn23)* and *let-60(n1046;G13E)*, the activated allele, are viable (9). Perhaps maternally provided activity from the *let-60* gene, but not from *let-23*, is sufficient for vital functions in these sublineages. However, these experiments do not establish whether *let-60* is necessary in these sublineages; the ability of *let-60 (n1046)* to rescue the lethality conferred by *let-23(mn23)* may reflect a gain of a novel activity or the stimulation of a parallel signalling pathway. Furthermore, maternally provided activity from the *let-60* gene cannot be pervasive during embryogenesis, because the excretory system is established during this period (26). A definitive answer to the question awaits the isolation of temperature-sensitive mutations in *let-60* (the injection of antisense RNA into the gonad is problematical because of the requirement for *let-60* for oogenesis).

The inability to exclude a maternal contribution leaves unresolved whether *let-60* is required for general proliferation in the earliest stages of development. An experiment of a nature complementary to those presented here indicates that Ras is not required for the earliest proliferation of *D. melanogaster* embryos: animals presumably lacking both maternal and embryonic activity in *Ras1*, the only known gene for Ras in this organism (13), complete many rounds of cell division before arresting development as abnormal embryos (13). However, the cause of death and the nature of the overall development of these embryos remain unknown. Also, it might still be questionable whether the allele of *Ras1* used is null, because defects associated with the allele are weaker than those associated with a null allele of the gene for Raf, which is normally a downstream effector of Ras.

The failure to observe defects in general proliferation in animals mosaic for *let-60* may reflect a relatively simple mode of development for *C. elegans*. Consistent with this possibility, *let-60(n1046;G13E)*, the activated allele, does not confer tumorous states. Instead, its main effect is hyperinduction of certain cell fates (for example, vulval tissue [3, 9] or the excretory duct cell [see above]). Nevertheless, the evidence presented here was derived from living animals mosaic for a likely null allele rather than from cells in culture, from activated or dominant negative alleles, or from neutralizing antibodies, all of which may give misleading results. Also, the requirement for Ras was examined unambiguously in various parts of the cell lineage. Unlike the situation described above for *Drosophila*, where the cause of death is unknown, a correlation with the cell lineage has permitted insight into an essential requirement for Ras. It will be of interest to see the extent of the requirement for Ras for proliferation and differentiation in mice bearing gene knockouts.

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