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On the nature of undead cells in the nematode *Caenorhabditis elegans*

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SUMMARY

During the course of normal embryonic and post-embryonic development, 131 cells in a *Caenorhabditis elegans* hermaphrodite undergo programmed cell death. Loss of function mutations in either of the genes *ced-3* or *ced-4* abolish cell deaths, enabling these 'undead' cells to survive and be incorporated into the adult with no obvious deleterious consequences. Ultrastructural reconstructions have shown that undead cells exhibit many differentiated characteristics. Most of the reconstructed cells appeared to be neurons with all the characteristic features associated with such cells, such as processes, synaptic vesicles and pre-synaptic specializations. However, clear morphological differences were seen among the undead neurons, suggesting a diversity of cell type. One of the reconstructed cells was a rectal epithelial cell, which had displaced its lineal sister that normally functions in this role. Removal of the ability to undergo programmed cell death by mutation therefore reveals a diversity of cryptic differentiated states that are acquired by cells that normally are destined to die.

INTRODUCTION

Cell death is a common feature of many organisms in the animal kingdom. The question of why an organism should go to the trouble of producing more cells than it needs only to kill them off has long aroused the curiosity of biologists. In general, it is likely that there is not a single answer to this question, but rather that cell death during development is a strategy that is used for different purposes in different contexts. For example, a developing nervous system may be required to produce functional circuitry for a behaviour that is specific to a certain stage in the development of the organism. Cell death may be used to remove such circuitry when its function is no longer required and could be deleterious (Truman 1987). In a different context, it may be simpler, in terms of developmental strategies, to provide a blank region of tissue in which regulative morphogenetic fields can set up a pattern and then use cell death to sculpture this pattern, such as occurs in the formation of the vertebrate digits (Saunders 1966). Yet another example is the connection of motor neurons to muscles in the vertebrate. It seems likely that a comfortable excess of motor neurons is formed in the course of development and local competitive interactions operate to assign one motor neuron to each muscle fibre. Motor neurons that do not locate an uninnervated muscle fibre die, thus ensuring accurate matching of motor neuron number to muscle fibres without the need for a high precision developmental mechanism to achieve this (Cowan 1984).

The nematode *Caenorhabditis elegans* has been the subject of intensive developmental and genetic studies in recent years (Wood 1988). Many instances of programmed cell death occur both in embryonic and post-embryonic development. A dying cell is seen in a live animal as an increase in the refractility of a cell when it is viewed by differential interference contrast microscopy (Sulston & Horvitz 1977). Ultrastructural studies have shown that dying cells decrease in volume and are eliminated by phagocytosis by a neighbouring (usually epithelial) cell (Robertson & Thomson 1982). The whole process takes about 60 min.

Two general types of cell death have been described in *C. elegans*, murders and suicides (Horvitz *et al.* 1982). The linker cell of the developing vas deferens in the male dies when it contacts the U cells of the developing cloaca. Laser ablation studies have shown the U cells to be uniquely necessary for the death of the linker cell, which therefore can be considered to be murdered (Sulston & White 1980). In the case of nearly all the other cell deaths in *C. elegans* no killer cell has been identified so these deaths are taken to be suicides. Indeed, most occur around 30 minutes after the birth of the cell, giving the impression that these cells are destined to die at, or even before, their birth.

Although the reason for the occurrence of most of the cell deaths in *C. elegans* is not clear, in a couple of cases a purpose for a cell death can be deduced. In the case of the linker cell connecting to the cloaca mentioned above it seems likely that the death of the linker cells after being engulfed by the U cells is a strategy to enable the tubular internal epithelium of the vas deferens to connect to the external epithelium of the cloaca. The killing of the linker cell opens up the originally blind-ended developing vas deferens to the outside of the animal (chapter 4, Wood 1988). The second case involves some sexually dimorphic neurons. The embryonic cell lineages are not sexually dimorphic, producing in both sexes a pair of motor neurons that innervate the vulval muscles of the

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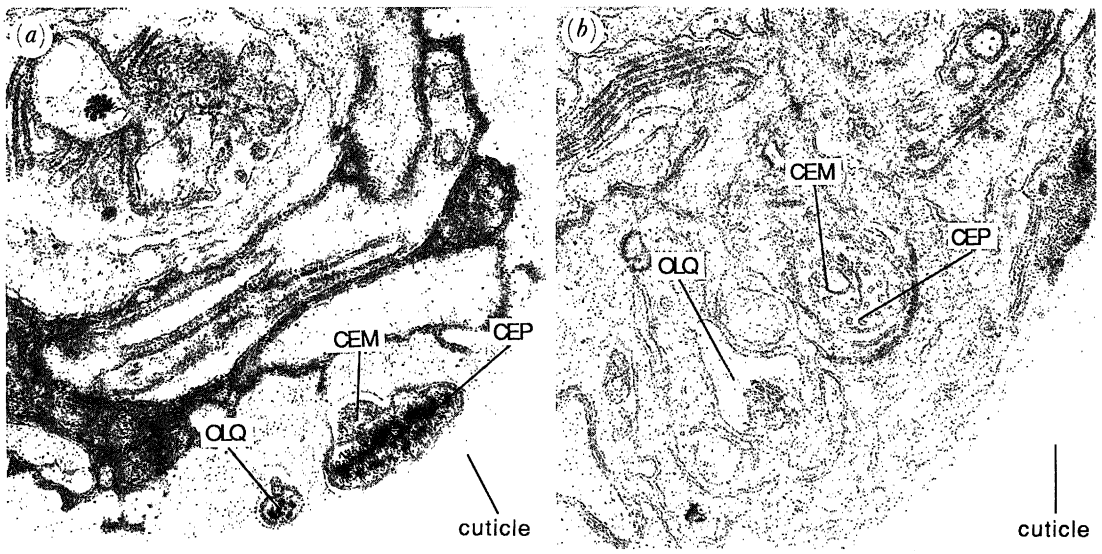

Figure 1. Two sections near the tip of the head showing the ending of a cephalic sensillum in a hermaphrodite *ced-3(n717)* animal. Panel (*a*) shows the neuron ending embedded in the cuticle and panel (*b*) shows the same sensillum more posteriorly. The dendrite of the CEP neuron can be seen to be paired with another neuron (CEM). This normally occurs only in the male. In wild-type hermaphrodites the CEM cells undergo programmed cell death and the CEP dendrite is unpaired as is the adjacent OLQ dendrite in this figure. (Magn. × 31 000).

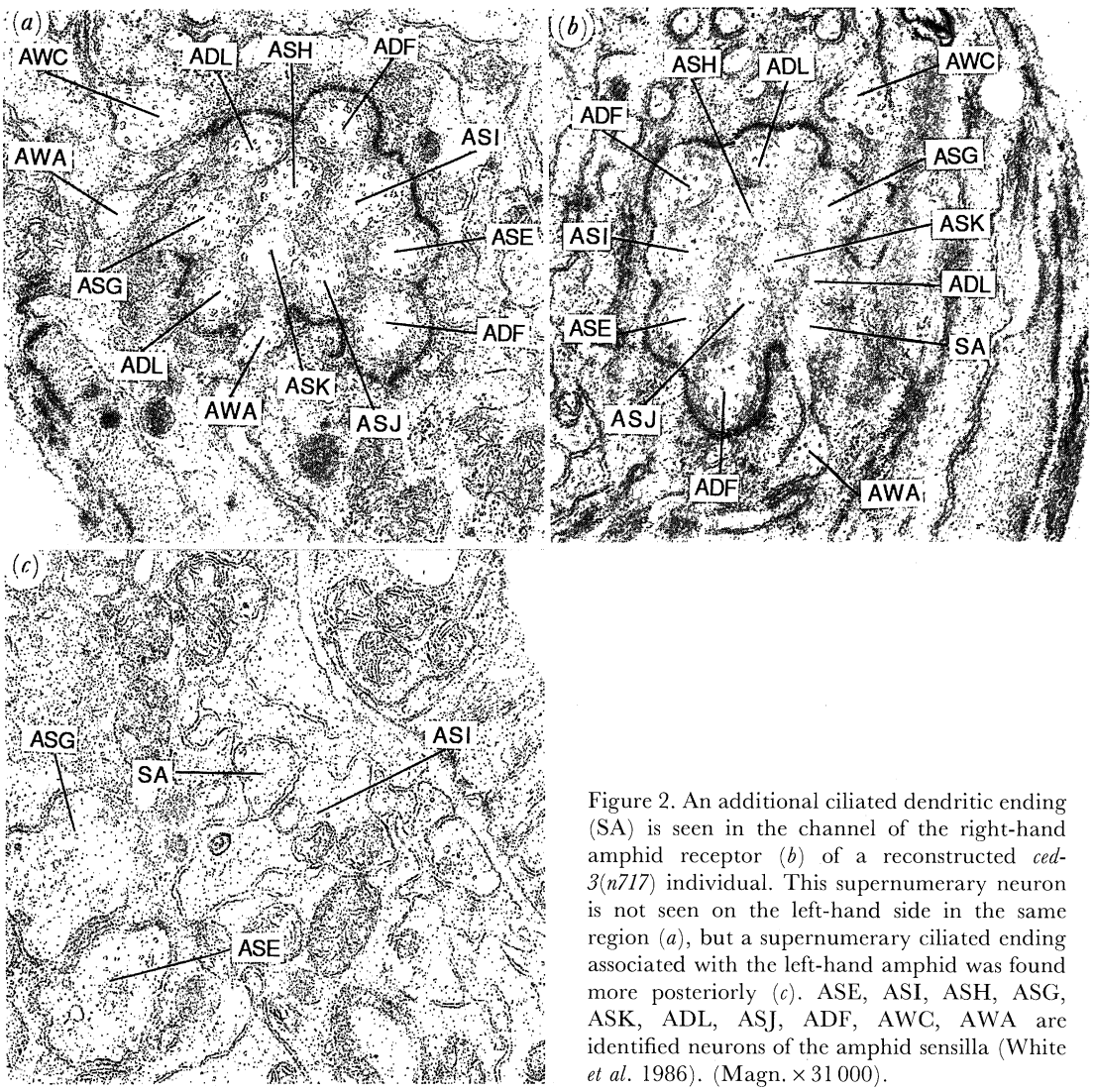

Figure 2. An additional ciliated dendritic ending (SA) is seen in the channel of the right-hand amphid receptor (*b*) of a reconstructed *ced-3(n717)* individual. This supernumerary neuron is not seen on the left-hand side in the same region (*a*), but a supernumerary ciliated ending associated with the left-hand amphid was found more posteriorly (*c*). ASE, ASI, ASH, ASG, ASK, ADL, ASJ, ADF, AWC, AWA are identified neurons of the amphid sensilla (White *et al.* 1986). (Magn. × 31 000).

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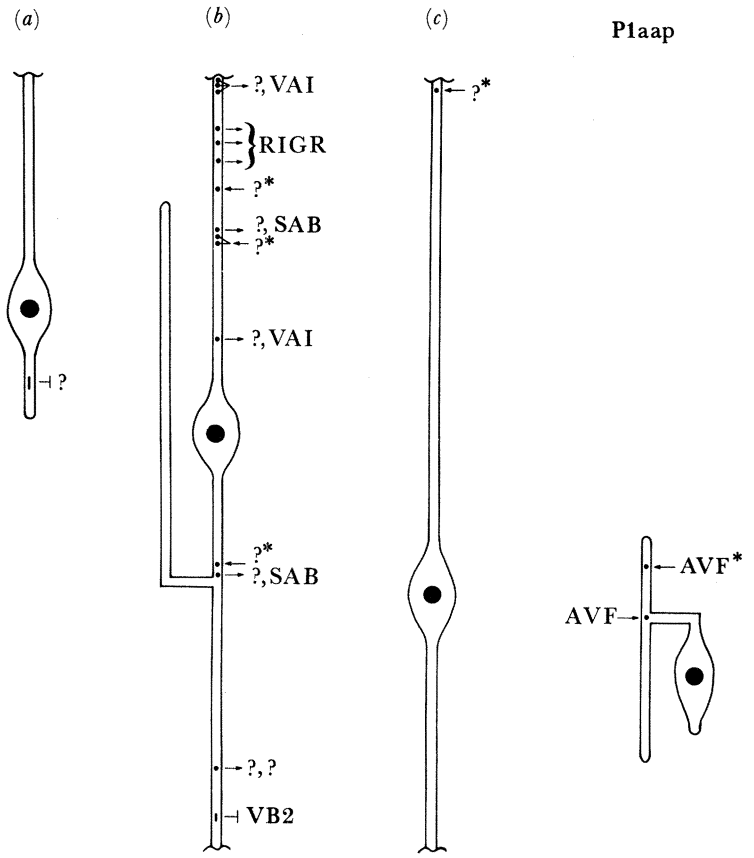

Figure 3. Plots of reconstructed identified undead neurons in the retrovesicular ganglion and anterior ventral cord. The lineal origins of (*a*), (*b*) or (*c*) could not be deduced, but the fourth cell was identified as being derived from the P1 ventral cord precursor. Arrows away from processes show synapses to other neurons; arrows pointing toward a process depict synapses from other neurons; vertical bars represent gap junctions and large solid circles represent the positions of the cell bodies. Identified neurons (RIGR etc.) are described in White *et al.* (1986). Unidentified synaptic partners are depicted with a '?'. An '*' indicates that there are additional post-synaptic elements at the synapse.

hermaphrodite (the HSNs) and a set of cephalic sensory receptor neurons only present in the male (the CEMs). In the course of late embryogenesis the HSNs undergo programmed cell death in the males and the CEMs do likewise in the hermaphrodites (Sulston *et al.* 1983). In this case it is clear that cell death is being used as a strategy for regulating the generation of sexually dimorphic structures.

Several genes have been identified as being functional components of the cell death mechanisms. Mutations in the gene *nuc-1* are deficient in a deoxyribonuclease causing a mass of undegraded DNA to persist after a cell dies (Sulston 1976). The genes *ced-1* and *ced-2* are necessary for the engulfment of dead cells. When these genes are mutated, cell death is initiated in the normal way but the cell corpses persist for some considerable time before degenerating (Hedgecock *et al.* 1983). The genes *ced-3* and *ced-4* have been shown to be necessary for the initiation of cell death. When either of these genes is mutated to a loss of function state, practically all programmed cell deaths, except the linker cell murder, are completely abolished (Ellis & Horvitz 1986). The surviving cells look quite healthy at the light microscope level of resolution. Surprisingly, animals in which all cell death has been suppressed by these mutations appear to be perfectly normal, both morphologically and behaviourally. In certain cases undead cells may function as normal neurons. Mutations in the gene *egl-1* cause a failure of egg-laying in hermaphrodites, because the HSN neurons which normally die in the male, also, inappropriately die in the hermaphrodite. The double mutant *egl-1*; *ced-3* is able to lay eggs normally because the HSN neurons no longer die, but survive to function normally (Ellis & Horvitz 1986). There is even evidence that an undead cell that never normally survives in either sex can replace the function of a neuron that has been eliminated by laser ablation (Avery & Horvitz 1987).

The differentiated state of undead cells that do not normally survive in any context in a wild-type animal is particularly interesting. In this study we have reconstructed several undead cells in *ced-3*(*n717*) mutants by serial section electron microscopy. The purpose of this work was to determine the nature of undead cells: to see whether they were all similar or exhibited a diversity of cell type, and to see whether they resembled any cell types seen in wild-type animals.

MATERIALS AND METHODS

Animals of the genotype *ced-3*(*n717*) were fixed, sectioned and stained for electron microscopy as described in White *et al.* (1986). Three reconstructions

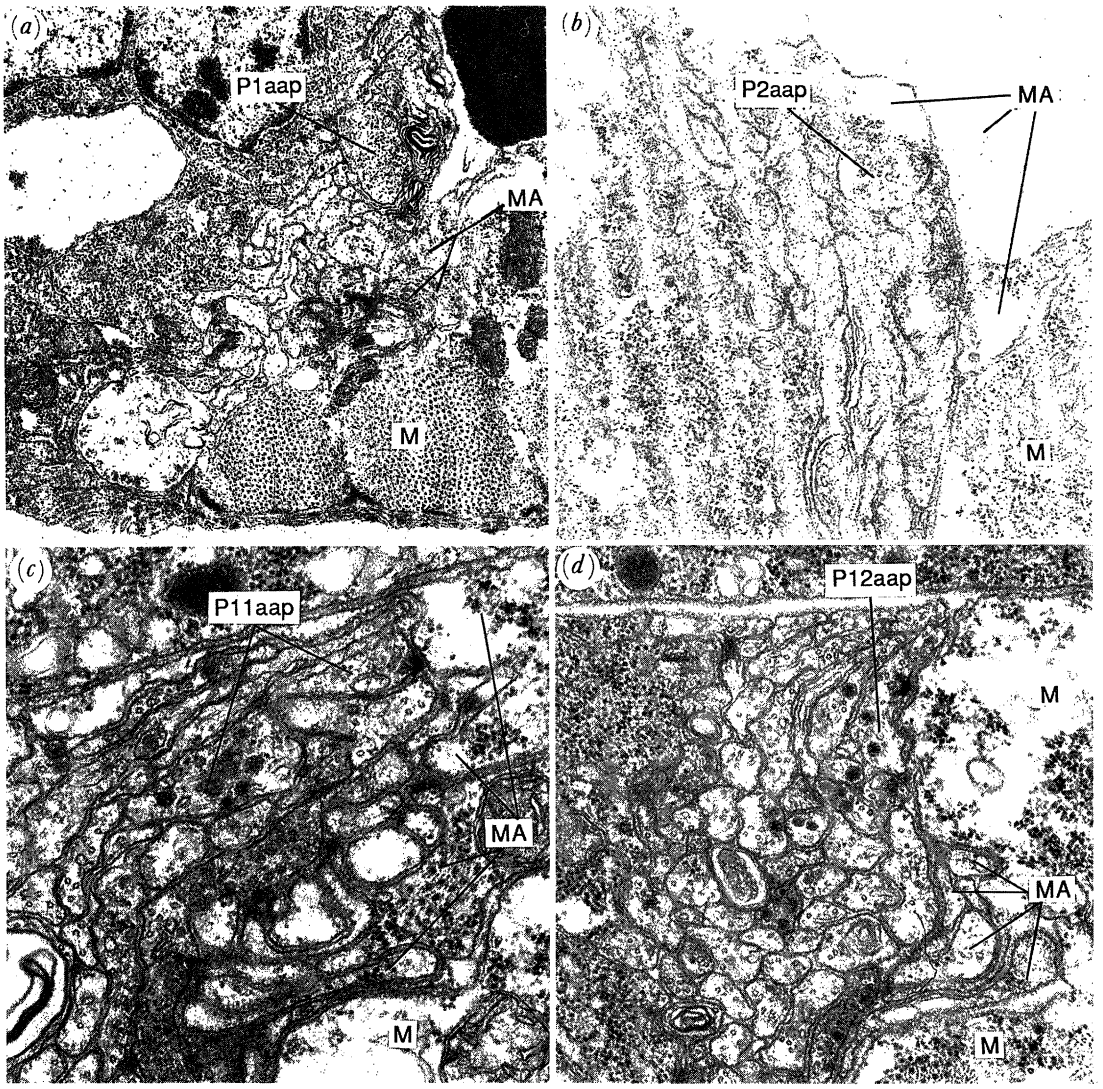

Figure 4. The undead P1aap cell looked rather sick. It made no synapses (figure 3) and had swirls of membranous material in its cytoplasm (*a*). The aap derivatives from P2 (*b*), P11 (*c*) and P12 (*d*) looked quite healthy and made neuromuscular junctions (NMJs). These NMJs looked reasonably normal with pre-synaptic specializations and clusterings of vesicles. However, dark-cored vesicles were seen in all the NMJs made by these cells. Such vesicles are never seen in the surviving aap derivatives of P3 and P8 in wild-type hermaphrodites which differentiate into VC motor neurons. However, similar vesicles are seen in the aapa derivatives of some of the P cells in the male (D. Albertson, unpublished observations). MA, muscle arm; M, muscle. (Magn. (*a*), 22500; (*b*), 35250; (*c*), 40500; (*d*) 47000).

were undertaken: one covering the sensory sensilla in the tip of the head, one covering the region of the ventral ganglion and anterior ventral cord, and one covering the pre-anal ganglion. Each reconstruction spanned a region of around 1000 sections. Every third available section was photographed and printed. In difficult regions extra fill-in pictures were taken. All cells within each series were reconstructed as described in White *et al.* (1986). From a knowledge of the detailed structure of the wild-type nervous system (White *et al.* 1986; Wood 1988), it was possible to identify all cells that do not usually die. These were found to be perfectly normal and could be readily identified. This made identification of the undead cells fairly easy as they appeared as supernumerary cells intercalated into a wild-type structure. In the case of the undead cells in the ventral cord it was possible to make what are probably accurate estimates of the lineal origins of the undead cells because of their position in the sequence of motor neurons in the ventral cord, and a knowledge of the lineages that produce these motor neurons (Sulston 1976).

RESULTS

Sensory endings in the tip of the head

Reconstruction of a *ced-3(n717)* individual in the tip of the head revealed several supernumerary processes in the bundles of processes that project to the sensory sensilla. Most of these processes petered out with undifferentiated endings. However, an extra ciliated process was seen to be associated with each of the four cephalic sensilla (figure 1). The ultrastructural appearance of these ciliated endings was very similar to those of the CEM neurons of the male (which normally die in the hermaphrodite). It therefore seems likely that these processes belong to the undead CEM cells in the *ced-3(n717)* hermaphrodite.

The amphids are a pair of chemoreceptors in the head (Ward *et al.* 1975; Ware *et al.* 1975). They have the same complement of cells in the male and hermaphrodite. An extra ciliated ending was found in the amphid channel of each of the amphid sensilla (figure 2) in the *ced-3(n717)* animal. By using ultrastructural and positional clues, it was possible to identify all the neurons normally present in the amphid channel and thereby identify the supernumerary neuron. On the right-hand side, the supernumerary neuron had its basal body in the same region as the other amphid channel neurons (figure 2*b*), but on the left-hand side, the basal body was situated considerably below the other channel neurons (figure 2*a*, *c*). This could represent a fixed left–right asymmetry, but as such asymmetries are very rare in the nervous system (White *et al.* 1986), it seems more likely that this represents a variability in the positioning of the basal bodies of these undead, supernumerary amphid neurons. This contrasts with the observation that the four undead CEM neurons were essentially identical, as are the four CEM ciliated endings in the male (unpublished observations).

Retro-vesicular ganglion (RVG) and anterior ventral cord

Four supernumerary cells were identified in the RVG of a *ced-3(n717)* individual; reconstructions of their processes are shown (figure 3). Three of these neurons (*a*, *b* and *c*) could not be assigned reliably to a lineage position. Cell a was a monopolar neuron that projected into the nerve ring and made few synapses in the RVG. Cell b was a bipolar cell that made several synapses to identified cells in the RVG; however, the combination of synaptic partners was unlike any other neuron in the wild-type hermaphrodite nervous system (White *et al.* 1986). Cell c was another bipolar cell but, unlike cell b it made practically no synapses in the reconstructed region.

During the course of post-embryonic development, a linear sequence of six epithelial cells on each side of the first-stage larva migrate into the ventral midline where they intercalate forming a single row of cells designated P1 to P12. The P cells then undergo a stereotyped pattern of cell divisions to produce a set of motor neurons and ventral epidermal cells (Sulston & Horvitz 1977). Programmed cell deaths occur in some of the derivatives of those P cells situated at the ends of the cord. The fourth undead cell in the RVG (P1aap in figure 3) and the anterior-most undead cell in the ventral cord were identified as being derivatives of the P1 and P2 ventral cord precursors, namely P1aap and P2aap (P1aap is the anterior, anterior, posterior great-grand-daughter of P1; for description of lineage nomenclature see Sulston *et al.* (1983)). Although P1 and P2 are initially bilaterally symmetrical cells with equivalent developmental potential (Sulston *et al.* 1983), the undead P1aap and P2aap cells were quite different morphologically. P1aap looked rather sick and had swirls of membranous material in its cytoplasm (figure 4*a*). It made no synapses, but received a couple of synapses from the identified interneuron AVF (figure 3). P2aap looked quite healthy and made several neuromuscular junctions (NMJs) (figure 4*b*). Occasional large, dark-cored vesicles were seen among the normal-looking synaptic vesicles. P2aap is sexually dimorphic, as it dies in hermaphrodites but survives in the males (Sulston & Horvitz 1977); however, the structure of this cell in wild-type males has not yet been determined.

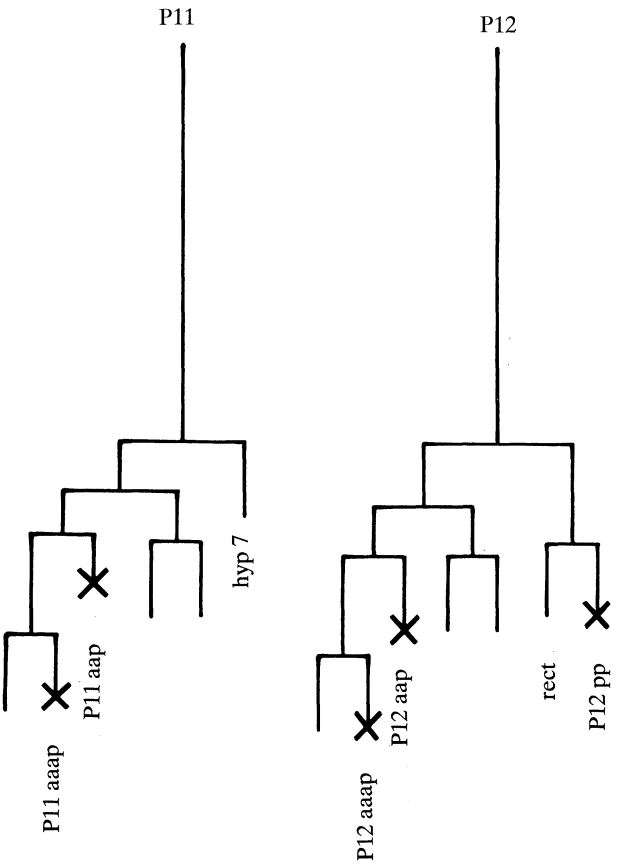

Figure 5. Lineages of the P11 and P12 ventral epithelial cells. The positions where cell deaths occur in wild-type animals are depicted with an X and the lineage identification of the dead cells is shown (note: a represents an anterior daughter of a division, and p the posterior daughter); rect, rectal epithelial cell.

Pre-anal ganglion (PAG) and posterior ventral cord

The wild-type lineages of P11 and P12 are shown in figure 5. These two cells are initially developmentally equivalent, bilaterally symmetric analogues; the one that ends up posterior after intercalation becomes P12. The posterior branch of the P11 lineage becomes an epidermal cell, in common with the equivalent cell from several of the other P lineages (Sulston & Horvitz 1977), whereas the posterior branch of P12 uniquely undergoes an extra division. The anterior daughter of this division becomes a rectal epithelial cell, while the posterior daughter dies.

In the reconstructed PAG of the *ced-3(n717)* animal all the undead cells could be assigned to their lineal

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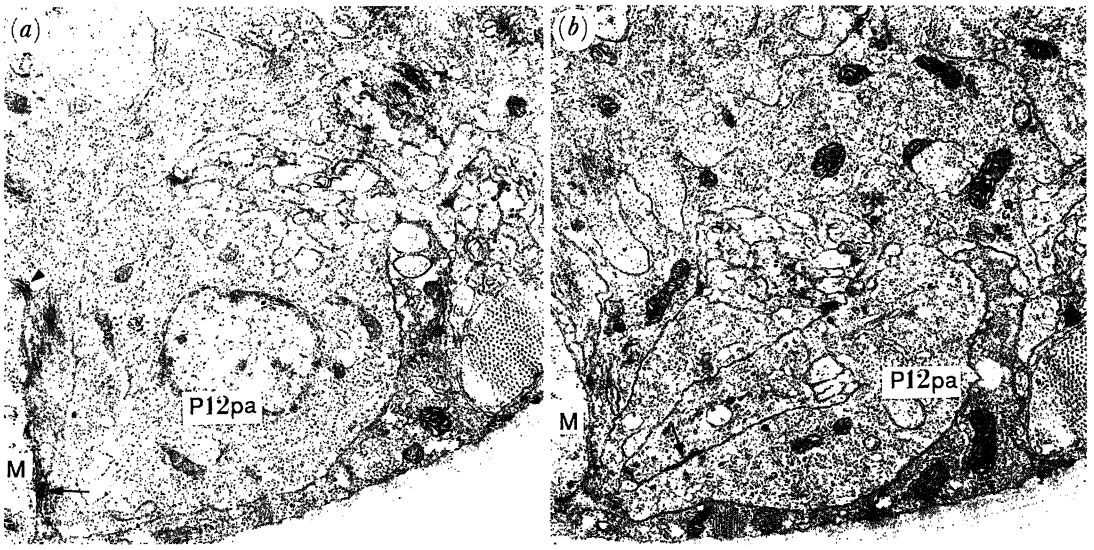

Figure 6. Micrographs showing P12pa in the preanal ganglion. This cell is normally a rectal epithelial cell but in a *ced-3(n717)* individual it has become a supernumerary epithelial cell. However, P12pa has retained some features of a rectal epithelial cell such as the hemidesmosome made to the rectal muscle (arrow in (*a*)). It also makes prominent desmosomal junctions to itself (arrow in (*b*)). P12pp, which normally dies in wild-type animals, became a normal-looking rectal epithelial cell in this animal (not shown). M, muscle. (Magn. × 15 000).

positions with reasonable levels of confidence. Surprisingly, the undead cell in the posterior branch of the P12 lineage (P12pp) became a rectal epithelial cell, whereas its anterior sister (P12pa), became a supernumerary epithelial cell (figure 6). The P12pa cell had several characteristic features of a rectal epithelial cell such as the presence of hemidesmosomal connections to a rectal muscle (figure 6*a*), and prominent desmosomal junctions (figure 6*b*). It therefore seems likely that P12pa was in fact a rectal epithelial cell, but it had been displaced from fulfilling this role by its undead sister which was also a rectal epithelial cell. Because of the undead cell's more posterior location on the lineage tree, it was born nearer to the rectum, apparently usurping its sister, the normal rectal epithelial cell.

The anterior (neuroblast) lineages of wild-type P11a and P12a cells are identical, each containing two cell deaths (figure 5). In the P cells where these deaths do not occur (P3–P8) the cells in the aaap position become VB motor neurons and the cells in the aap position become VC motor neurons in the hermaphrodite (Sulston 1976; White *et al.* 1986).

The undead P11aaap and P12aaap cells in the reconstructed *ced-3(n717)* were similar to each other (figure 7) and exhibited some similarities to VB motor neurons. They both had posteriorly directed axons, a characteristic feature of VB motor neurons. A few NMJs were seen on P11aaap, but although these structures had typical pre-synaptic specializations, there were few synaptic vesicles in the vicinity of these specializations (figure 8). The normal synaptic input for VB motor neurons is via gap junctions from AVB interneurons (White *et al.* 1986); these connections were not seen on either of the aaap cells although there were quite a few chemical synapses made onto P12aaap from unidentified processes (figure 7).

The aap derivatives of P11 and P12 die in the hermaphrodite, but survive in the male where their differentiated fates are quite different. The male P11aap goes through an extra round of division to produce two cells CA9 and CP9 (Sulston *et al.* 1980). CA9 innervates the male sex muscles and has dark-cored vesicles in its synaptic terminals. In contrast, the CP neurons do not have dark-cored vesicles but do make synapses onto a PDB neuron (D. Albertson, unpublished observations). The undead P11aap of the *ced-3(n717)* hermaphrodite had dark-cored vesicles, NMJs and synapses onto PDB (figures 7 & 4*c*), features of both the CA and CP neurons in the male. The P12aap cell in the male (PVX) is a highly branched neuron that has no dark-cored vesicles in its synaptic terminals in the PAG (D. Albertson, unpublished observations). The undead P12aap cell in the reconstructed *ced-3(n717)* animal was a neuron with a fairly simple branching structure. It made quite a few synapses (figure 7), but had dark-cored vesicles in its synaptic terminals (figure 4*d*) and so differed from the male-specific PVX neuron. There was, however, one similarity in synaptic specificity: in the *ced-3(n717)* animal P12aap synapsed onto AS11, a connection that is also made by PVX in wild-type males. Most of the synaptic partners of PVX, CA and CP are male-specific neurons that are not present in the hermaphrodite, so it is difficult to compare synaptic specificities because of the different neural environment in the male PAG.

The P11aap and P12aap cells of *ced-3(n717)* were rather different to their counterparts in the male. Some of the differences in synaptic connections can be explained by the fact that there are many extra neurons in the PAG of the male, produced by extra divisions, not by inhibiting cell deaths (Sulston & Horvitz 1977). These neurons make extensive synaptic connections to CA, CP and PVX in the male. However, some of the differences that were seen, such as the failure of P11aap to undergo an extra round of division

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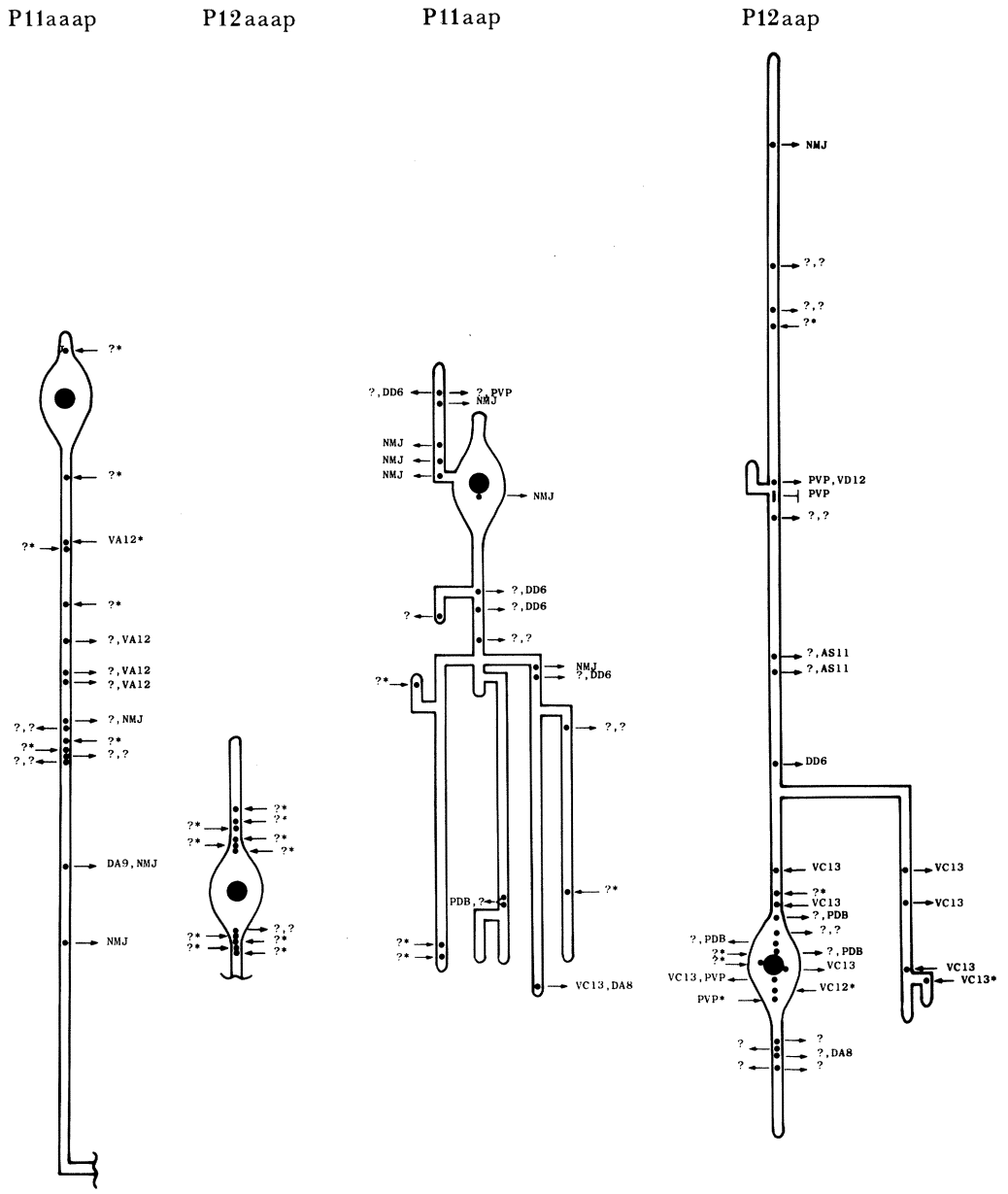

Figure 7. Plots of undead neurons in pre-anal ganglion with associated lineages (nomenclature as in figure 3). Cells produced in the aaap position in the P1 to P10 lineages differentiate into VB motor neurons. The undead aaap cells have some of the features of VB motor neurons, such as posteriorly directed axones and NMJs (only on P11aaap). The P11aap cell divides in the male to produce two neurons: CA and CP (Sulston *et al.* 1980). The undead P11aap in the hermaphrodite does not divide, but has some of the features of a CA neuron (dark-cored vesicles, NMJs) and one feature of a CP neuron (synapses onto PDB). The P12aap cell survives in the male; it does not divide, but becomes the neuron PVX. This neuron is highly branched and does not have dark-cored vesicles in its synaptic terminals, differing in these respects from the undead P12aap in the hermaphrodite. However, both these neurons synapse onto AS11.

and the presence of dark-cored vesicles in P12aap, probably did not arise from the absence of these neurons in the hermaphrodite

DISCUSSION

Removal of the ability to undergo programmed cell death by mutation has revealed that cells that normally die soon after birth do, in fact, have a cryptic ability to differentiate into normal-looking cells of diverse type. In some cases undead cells may even take over the function of other cells. The P12pp cell had apparently taken over the function of its sister which, in wild-type animals is a rectal epithelial cell. Also, it has been shown that an undead cell can sometimes replace the function of a pharyngeal motor neuron (M4) that has been eliminated by laser ablation (Avery & Horvitz 1987).

A characteristic feature of all the P cell lineages in wild-type animals is that lineal position usually corresponds to cell fate. For example, all the live aaap derivatives of the P cells become VB motor neurons. However the aaap derivatives of P11 and P12 in *ced-*

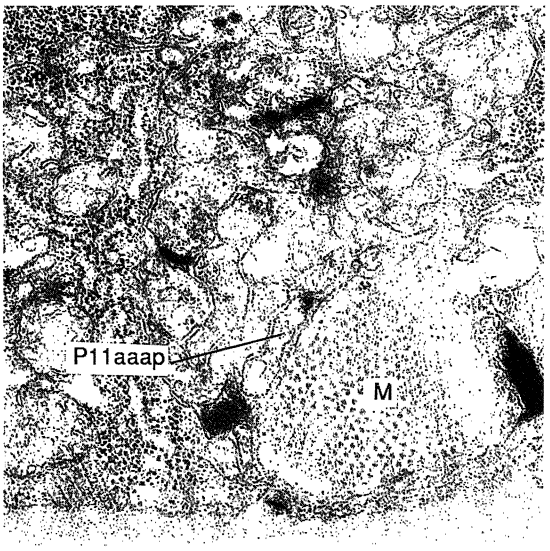

Figure 8. Micrograph showing a typical NMJ made by P11aaap. It has a distinct pre-synaptic specialization, but there are very few synaptic vesicles in the vicinity of this structure. M, muscle. (Magn. × 35 000).

3(n717) showed distinct differences from each other (figure 7). These differences could have arisen because of a basic difference in the differentiated state acquired by these cells. Alternatively, it could be that undead neurons exhibit considerable variability in the expression of certain characteristic traits. Evidence for this latter viewpoint comes from observations that have been made on other undead cells. Variations have been described in the levels of dopamine in undead V5paapp neurons (Ellis & Horvitz 1986) and in the ability of undead MSpaaaaap neurons to replace the function of a missing M4 motor neuron in the pharynx (Avery & Horvitz 1987). Little variation has been seen in certain undead neurons that are sexually dimorphic, such as the CEM receptor neurons of the male. However, these neurons are fully functional in one of the sexual forms of wild-type animals, unlike the undead neurons that exhibit variability, which are never normally produced in any context. It has been suggested that evolutionary drift in the cryptic differentiated characteristics of undead cells may provide a mechanism whereby the evolution of new cell types is sheltered by cell death (Avery & Horvitz 1987).

It is not clear whether undead cells are all equivalent, being born uncommitted and have acquired their diverse differentiated characteristics by local inductive interactions, or, alternatively, whether they have inherited their cryptic commitments. The behaviour of the undead cells in the P lineages gives some support to the latter view. P12aap and P12pp are born quite close together and therefore are presumably in similar inductive microenvironments. P12pp is an epithelial cell on the posterior branch of the P12 lineage; this same (Pp) posterior branch gives rise to epithelial cells in all the other P lineages. Conversely, all the other undead cells derived from P cells are neurons and come from the anterior (Pa) sublineages that produce exclusively neurons. It therefore seems likely that at least some of the cells that normally die in wild-type animals have cryptic commitments that are never realized before death intervenes.

The question of why an animal should produce cells in the course of development only to kill them off is often posed. In *C. elegans* one clear use of cell death is in the generation of sexual dimorphisms. However, the reason for producing cells that always die is not so clear. The precursors of the post-embryonically produced motor neurons of the ventral cord, the P cells, undergo similar, stereotyped lineages that produce VA, VB, VC, AS and VD motor neurons together with an epithelial cell. A few additional neuron types are produced by the P cells only at the ends of the cord (Sulston & Horvitz 1977; White *et al.* 1986). VA neurons have axons that are directed anteriorly, whereas those of VB are directed posteriorly. VB neurons situated at the posterior end of the cord would therefore have nowhere to place their axons. Similarly, VA neurons at the anterior end of the ventral cord would have nowhere to place their axons. Programmed cell death is used to eliminate the two posterior VB neurons but, interestingly, a different strategy is used in the anterior cord, whereby two AVF interneurons are produced in the lineal positions that would normally produce VA motor neurons (Wood 1988). Similar arguments may be used to justify the cell deaths that occur in the aap branches of P cells. The aap branches survive in P3–P8 in hermaphrodites, where they give rise to VC motor neurons that innervate vulval muscles. The vulva is situated near the middle of the ventral cord, and so it seems reasonable that vulval motor neurons should only be produced by the P cells in the proximity of the vulva and cell death used to eliminate VC cells produced at the ends of the cord where they cannot be effectively utilized. In this view, one use of cell death is as a strategy to modify developmental motifs so as to facilitate their use in different contexts.

Another use of cell death may be indicated by the death of P12pp. It is known that some regulative interaction at the posterior end of the cord diverts P12p from a probable default fate as a ventral epidermal cell to undergo an extra division only to kill off one of the daughters, the surviving daughter becoming a rectal epithelial cell. This apparently futile exercise may be necessary if the P12p cell has to undergo an additional cell division to switch its state from a ventral epidermal cell to a rectal epithelial cell, yet only one rectal epithelial cell can be used so the other is eliminated.

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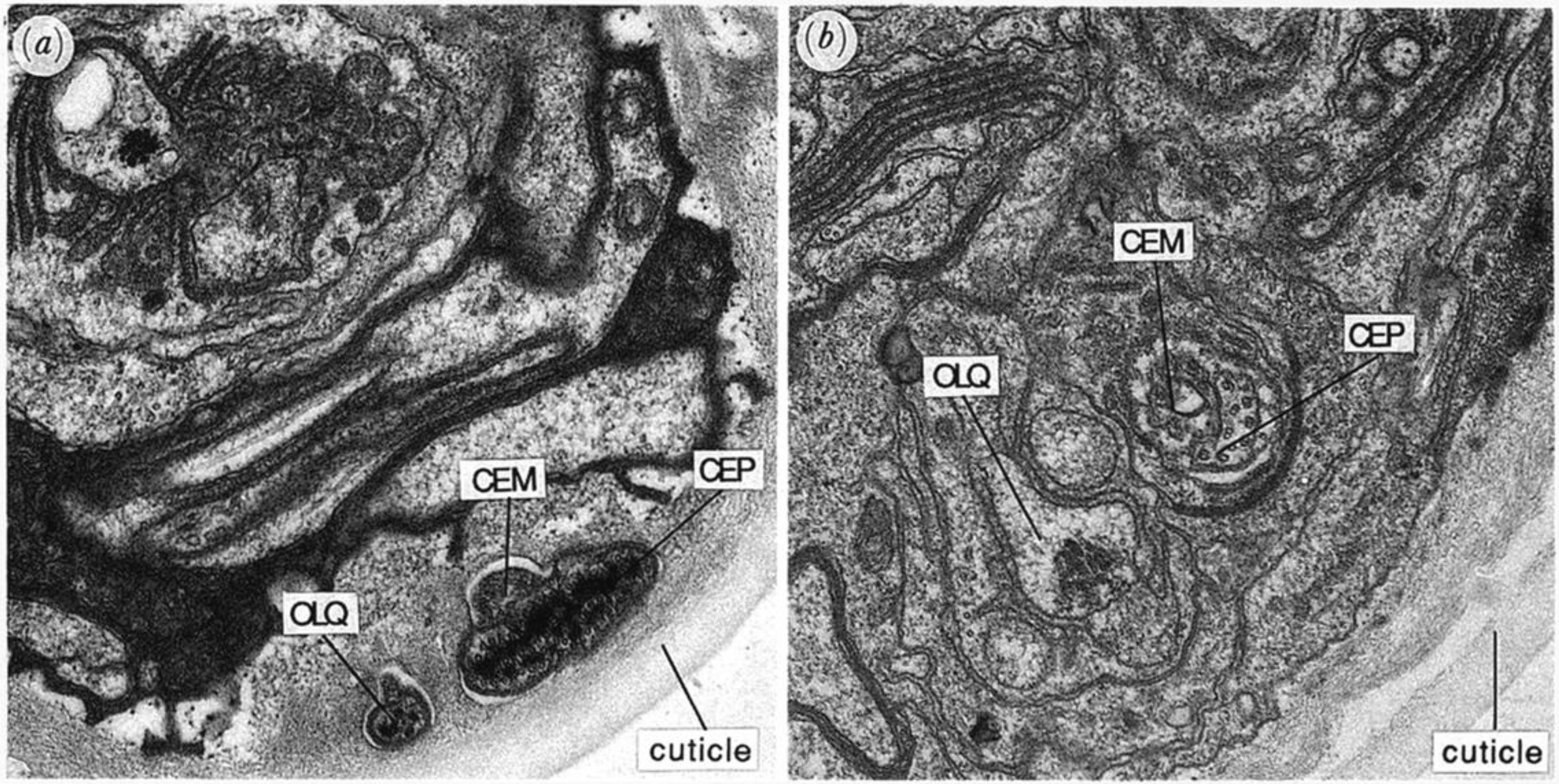
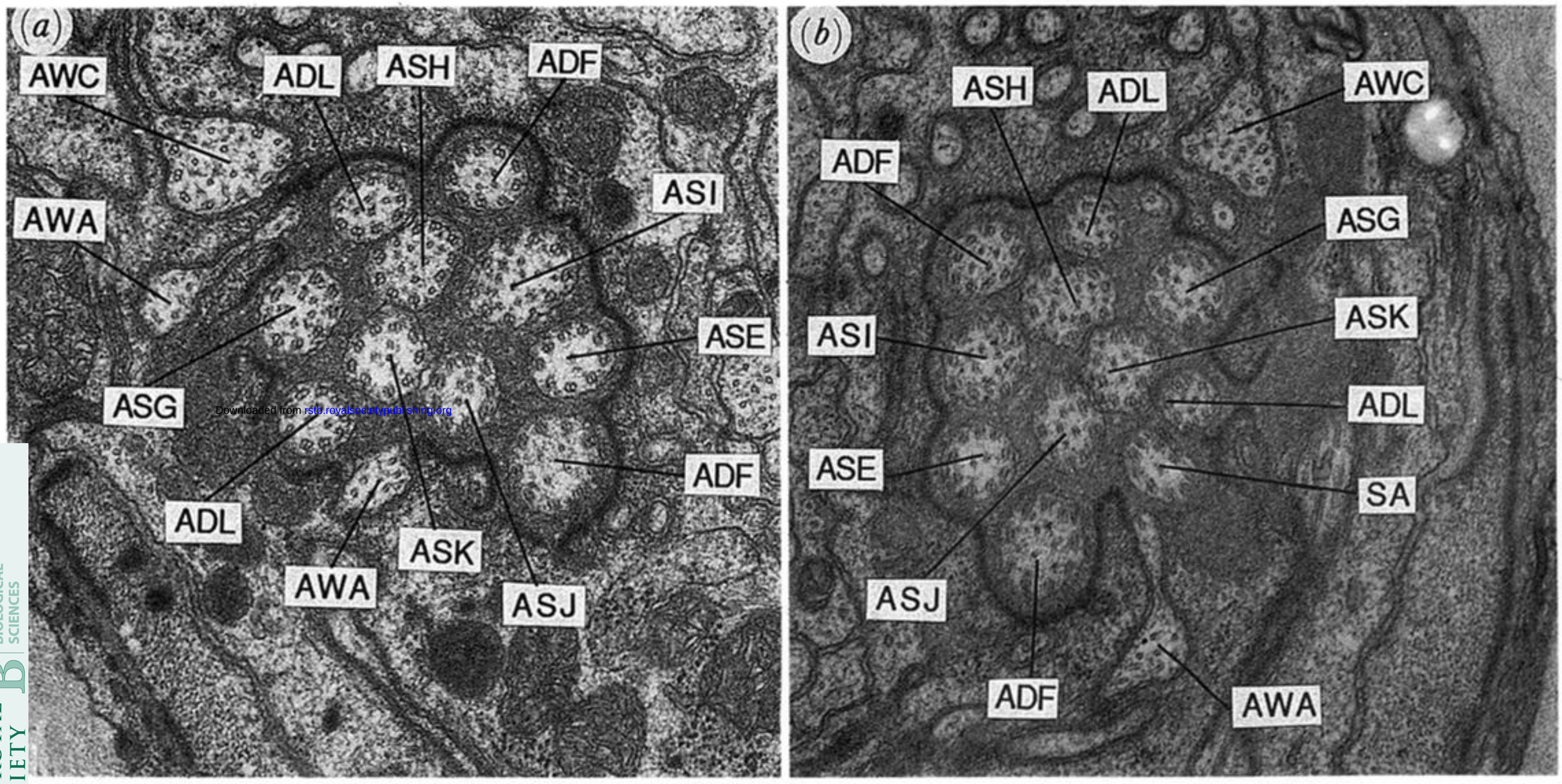


gure 1. Two sections near the tip of the head showing the ending of a cephalic sensillum in a hermaphrodite *ced-n717*) animal. Panel (*a*) shows the neuron ending embedded in the cuticle and panel (*b*) shows the same sensillum ore posteriorly. The dendrite of the CEP neuron can be seen to be paired with another neuron (CEM). This rmally occurs only in the male. In wild-type hermaphrodites the CEM cells undergo programmed cell death and the EP dendrite is unpaired as is the adjacent OLQ dendrite in this figure. (Magn. × 31 000).



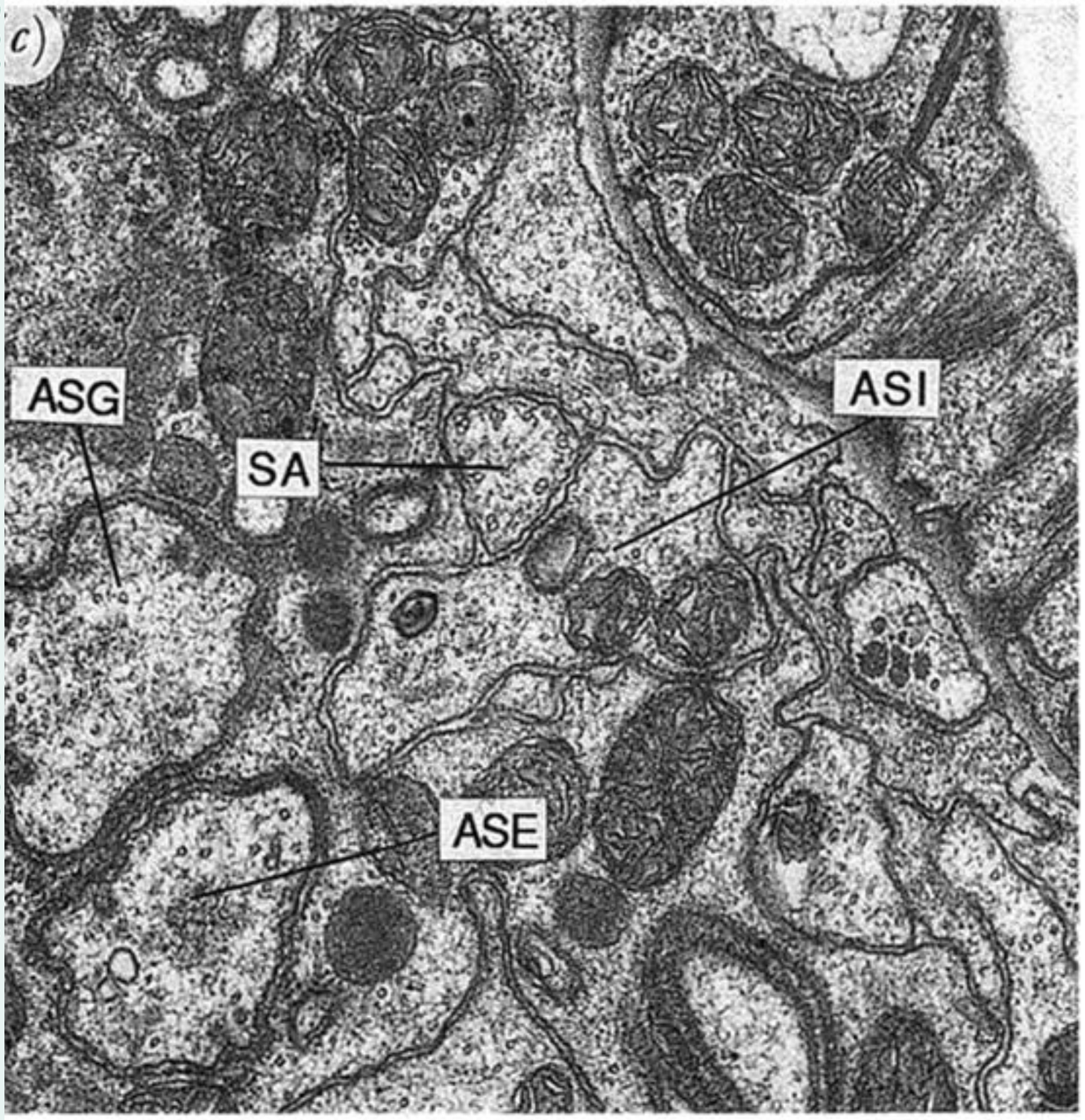


Figure 2. An additional ciliated dendritic ending (SA) is seen in the channel of the right-hand amphid receptor (*b*) of a reconstructed *ced-3(n717)* individual. This supernumerary neuron is not seen on the left-hand side in the same region (*a*), but a supernumerary ciliated ending associated with the left-hand amphid was found more posteriorly (*c*). ASE, ASI, ASH, ASG, ASK, ADL, ASJ, ADF, AWC, AWA are identified neurons of the amphid sensilla (White *et al.* 1986). (Magn. × 31 000).

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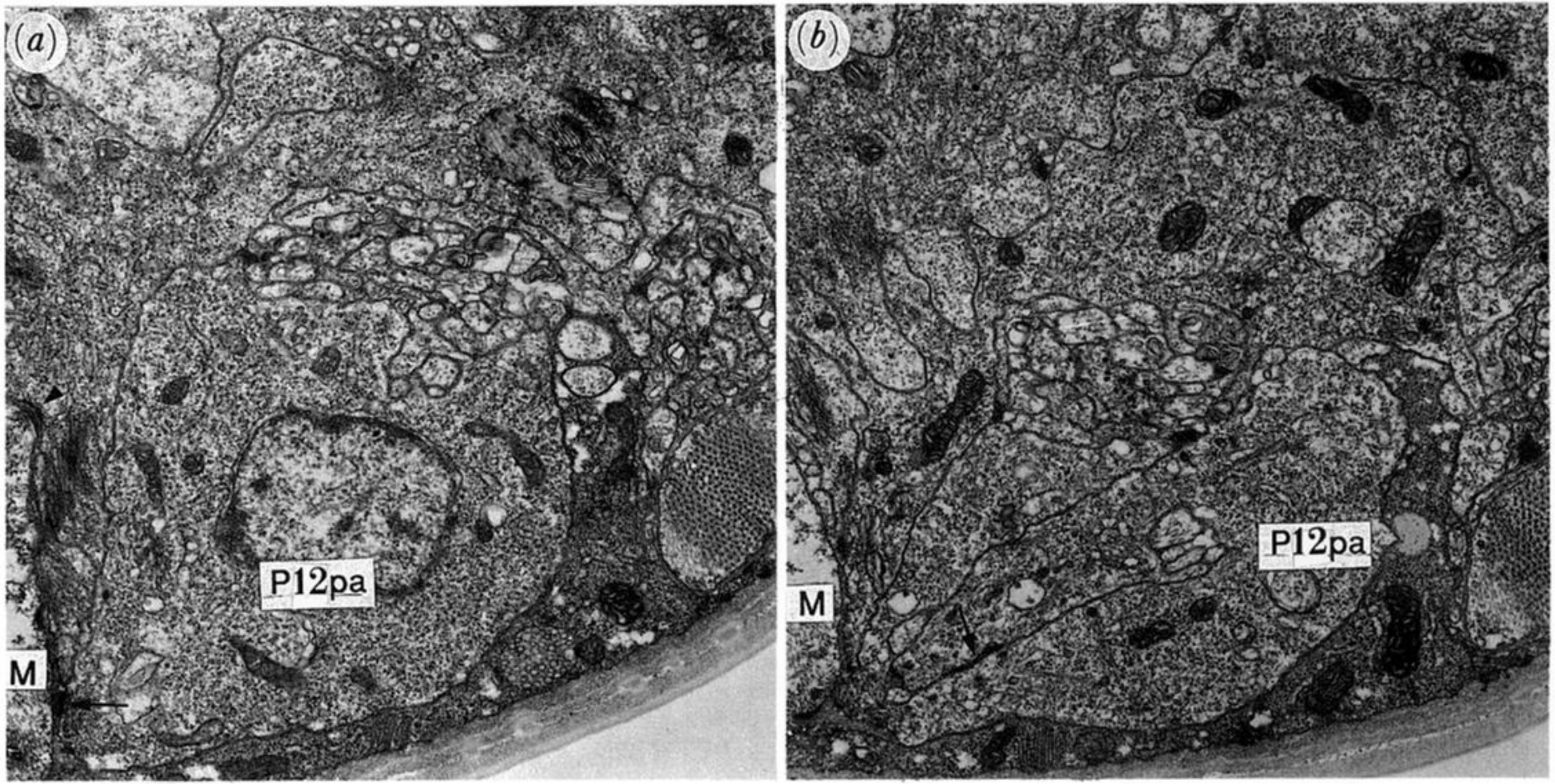
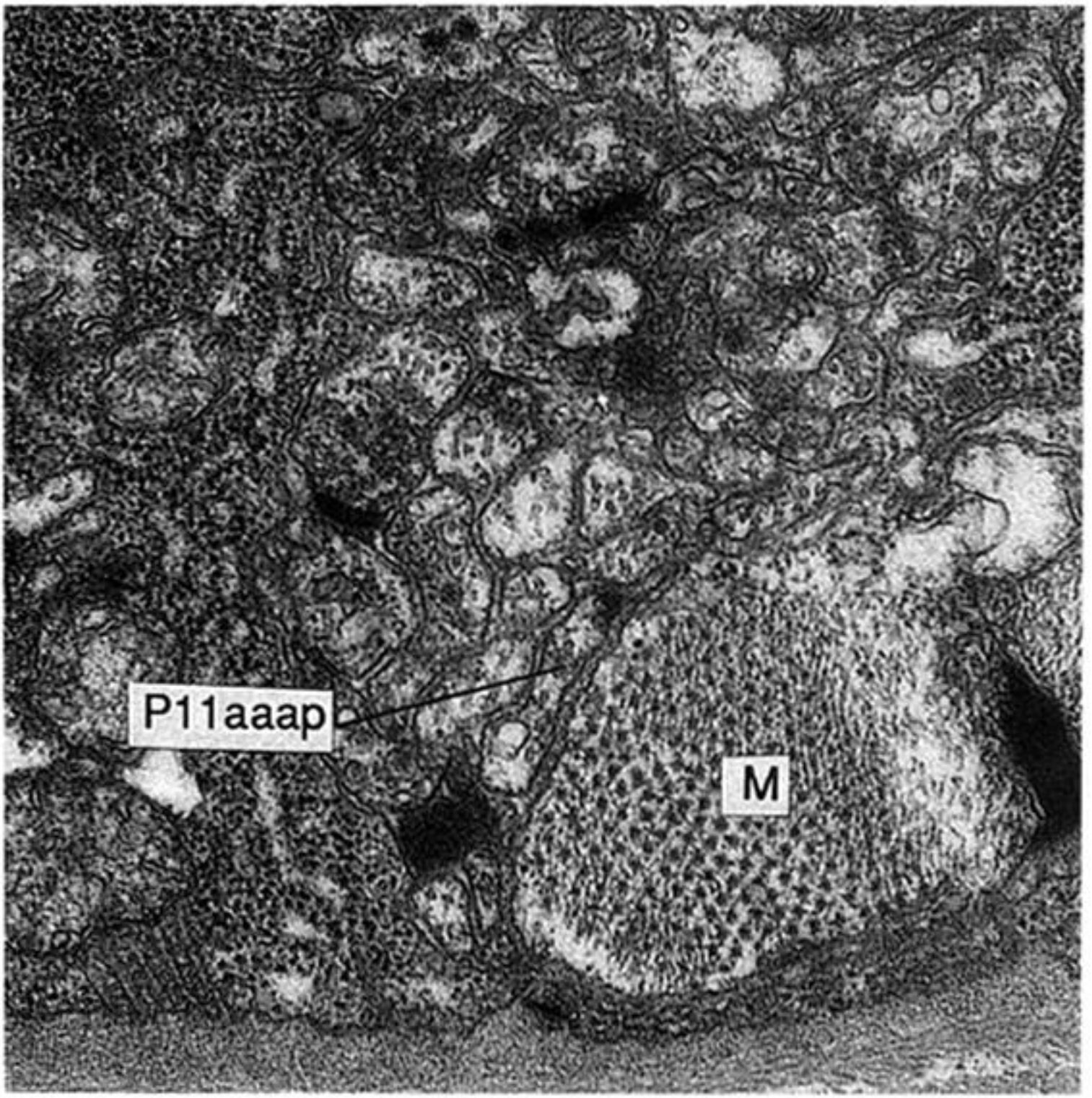
gure 4. The undead P1aap cell looked rather sick. It made no synapses (figure 3) and had swirls of membranous aterial in its cytoplasm (*a*). The aap derivatives from P2 (*b*), P11 (*c*) and P12 (*d*) looked quite healthy and made uromuscular junctions (NMJs). These NMJs looked reasonably normal with pre-synaptic specializations and usterings of vesicles. However, dark-cored vesicles were seen in all the NMJs made by these cells. Such vesicles are ver seen in the surviving aap derivatives of P3 and P8 in wild-type hermaphrodites which differentiate into VC otor neurons. However, similar vesicles are seen in the aapa derivatives of some of the P cells in the male (D. bertson, unpublished observations). MA, muscle arm; M, muscle. (Magn. (*a*), 22 500; (*b*), 35 250; (*c*), 40 500; (*d*) 000).



gure 6. Micrographs showing P12pa in the preanal ganglion. This cell is normally a rectal epithelial cell but in a *l-3(n717)* individual it has become a supernumerary epithelial cell. However, P12pa has retained some features of rectal epithelial cell such as the hemidesmosome made to the rectal muscle (arrow in (*a*)). It also makes prominent smosomal junctions to itself (arrow in (*b*)). P12pp, which normally dies in wild-type animals, became a normal-oking rectal epithelial cell in this animal (not shown). M, muscle. (Magn. × 15 000).



gure 8. Micrograph showing a typical NMJ made by
laaap. It has a distinct pre-synaptic specialization, but
ere are very few synaptic vesicles in the vicinity of this
ucture. M, muscle. (Magn. × 35 000).

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