PERSPECTIVE

The multifaceted *C. elegans* major sperm protein: an ephrin signaling antagonist in oocyte maturation

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In sexually reproducing animals, the ability to coordinate oocyte maturation and sperm availability has profound effects on reproductive success. In *Caenorhabditis elegans*, cell–cell signaling is crucial for promoting germline development, oocyte maturation, and ovulation. In this issue of *Genes & Development*, Miller et al. (2003) provide new insights into the role that sperm play in oocyte maturation and ovulation. Even before sperm and oocyte have the opportunity to meet, sperm establish their presence by signaling to oocytes and the somatic gonad. In turn, oocytes respond by resuming meiotic cell cycle progression and preparing for fertilization.

Meiotic arrest and oocyte maturation

The study of oocyte maturation has resulted in several important discoveries in cell biology, relevant to translational control, cell cycle regulation, and other areas (Stebbins-Boaz and Richter 1997; Nebreda and Ferby 2000). For example, the study of oocyte maturation in the frog *Rana pipiens* led to the landmark discovery of MPF (maturation promoting factor), which foreshadowed the identification of cyclins and their role in cell cycle regulation (Masui and Markert 1971). Less is known about the extracellular signaling mechanisms that control oocyte maturation, but these must also be important and are worthy of investigation.

In animals, oocytes undergo an arrest in cell cycle progression at the first meiotic prophase. Although this arrest is a universal characteristic of oocyte development, a wide variety of somatic and sperm-dependent mechanisms have evolved across the animal kingdom to alleviate this block and to promote oocyte maturation. In frogs, oocyte maturation is triggered by the somatic release of gonadotropins, which leads to the synthesis and release of progesterone from follicle cells. In turn, cAMP levels fall, and there is a rise in the accumulation of the translationally regulated Mos oncogene (Nebreda and Ferby 2000). An obvious change at this point is nuclear envelope breakdown (NEBD, or germinal vesicle breakdown), which is a widely conserved feature of maturation. Upon completion of maturation, vertebrate oocytes undergo a second arrest at metaphase, which is maintained until fertilization.

In many invertebrates, sperm are the source of a signal that promotes the resumption of meiosis in arrested oocytes (Masui 1985). One such animal is the nematode *Caenorhabditis elegans*. A number of studies have demonstrated that *C. elegans* sperm is responsible for promoting both oocyte maturation and ovulation (the release of an egg from the ovary in preparation for fertilization). The molecular identity of the sperm signal was recently revealed to be the *C. elegans* major sperm protein (MSP; Miller et al. 2001). In this issue of *Genes & Development*, Miller et al. (2003) report that the *C. elegans* ephrin receptor protein tyrosine kinase, VAB-1, is a receptor for MSP as well as for ephrins. They further provide data indicating that MSP antagonizes Eph/ephrin signaling in order to promote oocyte maturation and MAPK activation.

Morphological landmarks in *C. elegans* oocyte maturation and ovulation

The transparency of the worm and its ability to be immobilized with anesthetics have made it possible to identify morphological landmarks associated with the progression of a single oocyte from maturation to fertilization (Fig. 1; Ward and Carrel 1979; McCarter et al. 1997, 1999). These studies, combined with the use of mutants and laser ablation to eliminate gonadal cells and/or precursors, have shown the importance of sperm signaling in oocyte maturation and ovulation, and have led to the identification of soma to germ-line cell–cell interactions involved in promoting germ-line development (McCarter et al. 1997, 1999).

In wild-type hermaphrodites, the proximal-most oocyte (adjacent to spermatheca) undergoes maturation (Fig. 1). Oocyte maturation in *C. elegans* is characterized by NEBD, cortical rearrangement whereby the oocyte becomes more spherical, and nuclear changes eventually leading to the assembly of the meiotic spindle. During

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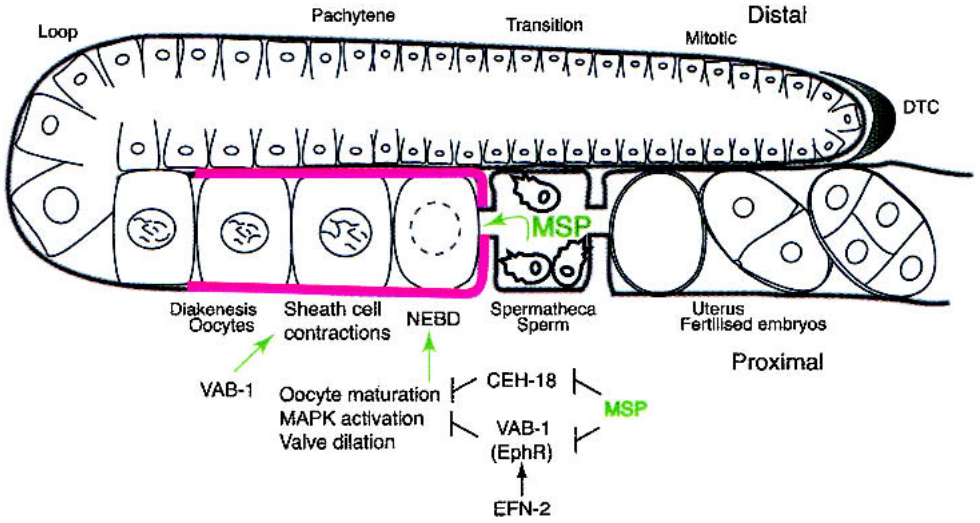

Figure 1. The *C. elegans* MSP controls multiple signaling pathways to promote oocyte maturation and ovulation. Germ cell development in the hermaphrodite is syncytial with distal to proximal polarity: mitotic proliferation, meiosis, cellularization, oocyte maturation, ovulation, and fertilization occur in an assembly-line fashion within a pair of U-shaped ovotestes (single arm shown). Although the gonad is syncytial, an individual germ nucleus and the plasma membranes incompletely surrounding its cytoplasm is referred to as a germ cell. The distal-most region of the gonad contains a pool of continuously proliferating mitotic germ cells, which are capped by the somatic distal tip cell (DTC). As germ cells move away from the DTC, they enter meiosis (transition zone) and undergo an extended period of pachytene. Germ cells specified to become oocytes exit pachytene in the loop region of the gonad, undergo cellularization, and begin swelling from increased cytoplasmic volume. Oocytes progress continuously through diakinesis in hermaphrodites when sperm are abundant; however, oocytes are arrested in diakinesis when sperm are absent. Just prior to ovulation (movement of the oocyte into the spermatheca) the proximal-most oocyte closest to the spermatheca undergoes NEBD and progresses toward metaphase. In addition, the cortex undergoes extensive rearrangement. Five pairs of sheath cells encase a large portion of the gonad. Ovulation is a cyclical event, occurring approximately every 23 min, and is aided by contractions from the proximal three pairs of sheath cells that form a myoepithelium surrounding the proximal-most oocytes (indicated by red outline; McCarter et al. 1997). Dilation of the distal spermathecal valve is coordinated with ovulation and allows the spermatheca to envelope the oocyte and to promote fertilization. The failure to dilate the valve traps oocytes in the somatic gonad (Iwasaki et al. 1996; Clandinin et al. 1998). The final reductional and equational divisions of meiosis, which result in the formation of haploid gametes and the extrusion of polar bodies, occur after fertilization. Embryonic development continues in the uterus. Sperm signaling through MSP antagonizes the inhibitory VAB-1 and CEH-18 pathways to promote oocyte maturation, MAPK activation, and spermathecal valve dilation. VAB-1 also positively modulates sheath cell contractions.

this period, a myoepithelial sheath, which surrounds proximal oocytes, contracts at a rate of 10–13 contractions per minute (basal rate). Following oocyte maturation, the contraction rate increases in intensity and in rate to ~19 contractions per minute. These contractions, combined with the dilation of the distal spermathecal valve, promote ovulation by allowing the oocyte to be enveloped by the spermatheca (McCarter et al. 1997, 1999). The importance of sheath cells is demonstrated by the finding that laser ablation of the most proximal two pairs of sheath cells causes defective ovulation (McCarter et al. 1997). It has also been shown by electron microscopy that proximal sheath cells are in cellular communication with oocytes through gap junctions (Hall et al. 1999).

Ovulation occurs at cyclical intervals of ~23 min when sperm are abundant; the increase in the rate of sheath cell contractions and dilation of the spermathecal valve are similarly cyclical and are dependent on the presence of maturing oocytes, but not sperm. Signaling from the oocyte to dilate the spermathecal valve is mediated through the EGF-like ligand LIN-3 and the receptor tyrosine kinase LET-23 (J. Mc-Carter, B. Bartlett, T. Dang, R. Hill, M. Lee, and T. Schedl, pers. comm.). It has been further shown that inositol triphosphate is responsible for mediating a RAS-independent response to LET-23 during this signaling event (Clandinin et al. 1998; Bui and Sternberg 2002). After ovulation, the oocyte undergoes fertilization in the spermatheca; subsequently the spermathecal–uterine valve dilates to permit movement of the fertilized egg from the spermatheca to the uterus, where the early cleavage stages of development occur.

The sperm signal

The role of sperm in promoting oocyte maturation and contraction of sheath cells has been documented with the aid of mutations that sexually transform hermaphrodites into females lacking sperm (McCarter et al. 1999). In the absence of sperm, oocytes from mutant females fail to mature and remain arrested in diakinesis for extended periods. The rate of sheath cell contractions in mutant females also falls to levels below wild-type basal

levels. The provision of mature sperm, immature spermatids, or even primary spermatocytes, but not necessarily the physical process of mating, restores oocyte maturation and sheath cell activity back to wild-type levels (McCarter et al. 1999).

The biochemical identification of major sperm protein

What is the molecular identity of the sperm signal, and how is this signal received and transduced? As a first step toward identifying the mystery molecule, Miller et al. (2001) developed a bioassay involving the microinjection of sperm-conditioned medium (SCM) into the uterus of females lacking sperm. It was found that SCM could substitute for sperm by providing an activity that stimulated both oocyte maturation and ovulation. The active component of SCM was further enriched through biochemical fractionation. Subsequently, a 14.1-kD peptide was shown by MALDI-TOF mass spectroscopy followed by tryptic peptide mapping and sequencing to correspond to the *C. elegans* major sperm protein (MSP; Miller et al. 2001). The identification of MSP as the sperm signal was unexpected because MSP already had a well-established, albeit unusual role as a cytoskeletal protein responsible for sperm motility (Italiano et al. 1996; L'Hernault 1997).

MSP, a protein with cytoskeletal and signaling properties

As implied by its name, MSP is the most abundant protein present in nematode sperm, comprising 15% of total protein (Klass and Hirsh 1981). MSP proteins are encoded by a multigene family, which precludes the ability to obtain a genetic mutant easily (Ward et al. 1988). MSP isoforms differ by only one to four amino acids. In contrast to the more familiar flagellated sperm, *C. elegans* sperm are amoeboid in shape and crawl by extending a lamellipod (Roberts and Stewart 1995). Surprisingly, this movement is not dependent on conventional motor proteins, nor is it actin-based, as sperm contain virtually no actin (Nelson et al. 1982). However, similar to actin, the dynamic assembly of MSP into fibrous networks has been shown to drive the movement of *C. elegans* sperm, although MSP bears no sequence similarity to actin. It is interesting to speculate that the acquisition of actin-like properties by MSP is an example of convergent evolution.

Is MSP really the sperm signal? The attribution of a specific biological function to a single activity enriched from whole-cell extracts is difficult to achieve with certainty. To provide further evidence that MSP is part of the sperm signaling machinery, two distinct MSP isoforms were expressed as HIS-tagged fusion proteins in bacteria and tested for their ability to mimic the effects of SCM (Miller et al. 2001). Each of the bacterially expressed MSP proteins proved capable of inducing oocyte maturation and ovulation in females lacking sperm. Moreover, depleting MSP by microinjecting anti-MSP antiserum suppressed ovulation by reducing the oocyte maturation rate. MSP was further shown to be composed of two mutually exclusive activities; an N-terminal activity (amino acids 1–106) promotes oocyte maturation, whereas a C-terminal activity (amino acids 106–126) stimulates sheath cell contractions. These results have led to the hypothesis that MSP activates distinct signaling pathways in oocytes and sheath cells (Miller et al. 2001).

In the new work, a fluoresceinated derivative of a 6xHis-tagged MSP (MSP-FITC) was shown to bind directly to oocytes and gonadal sheath cells, but not to male reproductive tissues (Miller et al. 2003). The binding of MSP-FITC is saturable and can be competed by cold MSP. It was also demonstrated that MSP-FITC retains the ability to promote oocyte maturation and sheath cell contractions in unmated females.

Activation of MAPK by MSP signaling

It is known that the MAPK pathway is activated during oocyte maturation (Miller et al. 2001; Page et al. 2001). Antibody staining using a MAP kinase monoclonal antibody (MAPK-YT) revealed that 2–3 of the most proximal oocytes were stained in females that were mated or injected with MSP. No staining was detected in *mpk-1* homozygous mutants or in unmated females, indicating that the antibody is specific for MPK-1 and that activation of the kinase is MSP-dependent. It remains to be determined whether there is an essential genetic requirement for MAPK activity in oocyte maturation.The *C. elegans* Aurora kinase ortholog AIR-2 is also recruited to the chromosomes of the most proximal oocyte prior to ovulation and fertilization in response to sperm (Schumacher et al. 1998); anti-AIR-2 staining is not detected in oocytes when sperm are absent. AIR-2 plays a role in both polar body extrusion and cytokinesis.

The MAPK cascade is also required during an earlier aspect of *C. elegans* germ-line development, which is distinct from oocyte maturation—the exit from pachytene in female germ cells (Church et al. 1995). Mutations in three sequential members of this pathway, *let-60/ras*, *mek-2* (MAPKK), and *mpk-1/sur-1* (MAPK) led to meiotic cell cycle arrest by preventing progression to the diplotene and diakinesis stages of meiosis. The analysis of genetic mosaics indicated that the activity of this pathway was required within the germ line; however, the components of the signaling mechanism, both ligand and receptor, are unknown. Laser ablation studies suggest that cells of the sheath or spermathecal lineages might play a role in transmitting this signal through cell–cell interactions (McCarter et al. 1999).

The *C. elegans* Eph RPTK, VAB-1, is a receptor for MSP

Preliminary identification of potential MSP receptors was achieved by using a combination of microarray data

to identify membrane-associated oocyte-enriched genes and RNAi (RNA mediated interference) to screen candidates (Reinke et al. 2000; Miller et al. 2003). Of the candidates tested, only *vab-1(RNAi)* resulted in a reduction in MSP-FITC binding.

The *C. elegans vab-1* gene encodes an Eph-related receptor protein tyrosine kinase (RPTK; George et al. 1998). In vertebrates, Eph receptors and membrane-bound ephrins mediate cell contact signaling that affects axon growth and guidance and morphogenesis by eliciting a variety of cytoskeletal responses (for review, see Kullander and Klein 2002). Eph receptors are divided into two subclasses based on their sequence and ligand-binding preferences. EphA receptors bind GPI-anchored A-ephrins, whereas EphB receptors bind B-ephrins, which have a membrane-spanning domain and a short cytoplasmic tail carrying multiple tyrosine residues. The binding of clustered, membrane-bound ephrin ligands activates autophosphorylation of tyrosines on the intracellular domain of the Eph receptor. However, Eph receptors display both kinase-dependent and -independent signaling activities (Henkemeyer et al. 1996). Eph receptors and B-ephrins are capable of mediating reverse signaling, whereby the extracellular domain of an Eph receptor functions as a ligand, which can be bound and intracellularly transduced by B-ephrins. More recently, evidence has been obtained indicating that GPI-anchored A-ephrins are also capable of bidirectional signaling during development of retinotectal and vomeronasal projections (Knoll and Drescher 2002).

The single *C. elegans* Eph receptor, VAB-1, is equally similar in sequence to EphA and EphB receptors (George et al. 1998). All four *C. elegans* ephrins are more similar in sequence to B-class ephrins, although they carry GPI modification signals that are characteristic of A-class ephrins (Wang et al. 1999; Chin-Sang et al. 2002).

The *C. elegans vab-1* gene was initially identified because mutations in *vab-1* produced variable abnormalities characterized by a notched head, embryonic and larval lethality, and defects in tail morphogenesis (Brenner 1974). Detailed characterization of the *vab-1* embryonic phenotype revealed that *vab-1* is involved in epidermal morphogenesis and is important for the completion of epidermal ventral enclosure of the embryo (George et al. 1998). Mosaic analysis has shown that *vab-1* functions nonautonomously during epidermal morphogenesis, because it is required in the neuroblasts underlying epidermal cells. The absence of *vab-1* activity also leads to defects in neuronal organization. Mutations that are predicted to eliminate kinase function reduce but do not eliminate *vab-1* activity. Hence, VAB-1 is predicted to have both kinase-dependent and -independent activities (George et al. 1998). In the case of MSP-FITC binding, *vab-1* alleles with mutant kinase activity remain capable of binding MSP-FITC (Miller et al. 2003). A recent study suggests that a LAR-like receptor tyrosine phosphatase PTP-3 also functions redundantly with VAB-1 and a subset of ephrins during epidermal morphogenesis (Harrington et al. 2002).

Interaction between VAB-1 and MSP

The identification of VAB-1 as the MSP receptor was a surprise because earlier studies gave little hint that the *C. elegans* Eph receptor or ephrin ligands might play roles in oocyte maturation. Evidence supporting the hypothesis that VAB-1 and MSP are likely to interact directly was obtained in two experiments (Miller et al. 2003). First, it was shown that MSP-FITC could bind specifically to COS-7 tissue culture cells expressing a VAB-1::DsRed fusion protein. Second, sensitive optical techniques made it possible to detect fluorescence from a VAB-1::GFP expression construct capable of rescuing *vab-1*-null animals on the membranes of oocytes and proximal gonadal sheath cells, sites of MSP binding.

Given that VAB-1 binds MSP, what role might VAB-1 play in oocyte maturation and sheath cell contractions during ovulation? A phenotypic reevaluation of *vab-1* mutant hermaphrodites revealed that *vab-1* is not essential for promoting oocyte maturation or sheath cell contractions, which is not surprising considering that such defects would have been detected in earlier studies. However, *vab-1* is required for the sperm-dependent increase in the gonadal sheath cell contraction rate (Miller et al. 2003). It was also observed that older *vab-1* mutant hermaphrodites lay significantly more unfertilized oocytes than wild-type hermaphrodites, indicating that oocytes from *vab-1* hermaphrodites continue to undergo maturation and ovulation despite the reduction or absence of sperm. The range in the number of oocytes in *vab-1* mutant hermaphrodites showing MAPK activation was also expanded; normally, 1–3 of the proximalmost oocytes stain with the MAPK-TY antibody, whereas 3–8 stained oocytes were detected in *vab-1* hermaphrodites. These data suggest that VAB-1 is a positive regulator of sheath cell contractions and a negative regulator of oocyte maturation and MAPK activation (Fig. 1). Hence, MSP appears to function by antagonizing the activity of VAB-1 in oocytes.

Parallel pathways modulate oocyte maturation and sheath cell contractions

Several lines of evidence indicate that VAB-1 is not the only receptor for MSP (Miller et al. 2003). First, MSP-FITC binding is reduced, but not eliminated in *vab-1*-null mutants. Second, the maturation rate of oocytes from unmated *vab-1* females is lower than that of wild-type hermaphrodites, although significantly higher than that of unmated wild-type females. Third, MSP microinjected into the uterus of unmated *vab-1* females remains capable of increasing the rate of oocyte maturation.

Miller et al. (2003) examined the possibility of a parallel role for sheath cell signaling in oocyte maturation by studying mutants of the POU-homeoprotein CEH-18, which is expressed in somatic sheath cells, but not oocytes (Rose et al. 1997). The maturation rate of oocytes in unmated *ceh-18*-null females was found to be significantly higher than that of unmated wild-type females,

indicating that CEH-18 normally inhibits oocyte maturation. Elimination of *ceh-18* activity did not disrupt MSP-FITC staining to oocytes. Taken together, these results suggested that CEH-18 defines a somatic gonad pathway that negatively regulates oocyte maturation.

Removal of both *ceh-18* and *vab-1* was accomplished by performing *vab-1(RNAi)* in unmated *ceh-18*-null females. The oocyte maturation rate in these animals was significantly higher than that observed in either unmated *ceh-18* or *vab-1* single-mutant females; mating did not significantly change the rate of oocyte maturation in *vab-1(RNAi); ceh-18*, females suggesting that oocyte maturation in this situation is independent of MSP. Similarly, in the absence of a sperm signal, VAB-1 and CEH-18 also appear to act together to inhibit MAPK activity in proximal oocytes. MAPK-YT stained 1–3 proximal oocytes in unmated *vab-1(RNAi); ceh-18* females, whereas staining was not detected in oocytes from unmated *vab-1* females.

Autonomy of VAB-1 activity in the germ line and somatic gonad

In lieu of performing conventional mosaic analysis, Miller et al. (2003) took advantage of a mutant named *rrf-1* that is defective for somatic RNAi, but competent for germ-line RNAi (Sijen et al. 2001). By analyzing the differential effects of removing *vab-1* activity in both soma and germ line by conventional RNAi or only in the germ line using *rrf-1*, it was demonstrated that VAB-1 functions cell-autonomously in the germ line to inhibit oocyte maturation and ovulation, and in somatic sheath cells to modulate contractions.

The role of ephrins

If MSP functions to antagonize Eph signaling through VAB-1, what role, if any, do the ephrins play in oocyte maturation and sheath cell contractions? In *C. elegans*, an animal with a fully sequenced genome, only a single Eph receptor (VAB-1) and four ephrin ligands (EFN-1, EFN-2, EFN-3, and EFN-4) have been identified (George et al. 1998; Chin-Sang et al. 1999, 2002; Wang et al. 1999). Prior to the report showing that VAB-1 is a receptor for MSP, animals carrying genetic mutations in each ephrin gene had been obtained and subjected to phenotypic analysis (Chin-Sang et al. 1999, 2002; Wang et al. 1999). Mutations in *efn-1/vab-2* showed a similar, but less penetrant range of early morphogenesis defects as *vab-1*, but mutations in *efn-2* or *efn-3* permitted almost normal embryonic development (Chin-Sang et al. 1999; Wang et al. 1999). Moreover, a triple knockout of *efn-1*, *efn-2*, and *efn-3* more closely resembled that of *vab-1* than any single mutant alone, suggesting that ephrins are partially redundant in their activities (Wang et al. 1999). It was also demonstrated that ephrins have additional nonoverlapping functions that require both the kinase-independent and kinase-dependent functions of VAB-1 (Wang et al. 1999).

Biochemical studies have revealed that all four ephrins have the potential to bind VAB-1, and that EFN-1, EFN-2, and EFN-3 are also capable of activating VAB-1 autophosphorylation (Chin-Sang et al. 1999; Wang et al. 1999). Mutations in *efn-4* are unusual because they strongly synergise with mutations in *vab-1*, suggesting that *efn-4* might function in a parallel pathway that is independent of *vab-1* (Chin-Sang et al. 2002). These studies did not specifically address whether any of the ephrins were involved in oocyte maturation or sheath cell contractions, although it had been observed that *efn-2* mutants produced smaller broods (Chin-Sang et al. 2002).

Miller et al. (2003) found that none of the ephrins was essential for oocyte maturation; however, similar to mutations in *vab-1*, *efn-2* hermaphrodites continued to lay unfertilized oocytes after sperm were depleted. Unmated *efn-2* females also had a higher rate of oocyte maturation than unmated females, but not as high as unmated *vab-1* females, suggesting that other ephrins might also function redundantly with *efn-2*. It was also shown that the role of VAB-1 in promoting sheath cell contractions is EFN-2-independent. RNAi analysis using *rrf-1* indicates that similar to *vab-1*, *efn-2* functions cell-autonomously in oocytes; however, EFN-2 has not yet been specifically localized to the oocyte surface (Wang et al. 1999).

Conservation of MSP and VAP

The data presented by Miller et al. (2003) support the argument that the interaction between MSP and the *C. elegans* Eph receptor is likely to be direct and that MSP functions to antagonize Eph signaling. This raises the question of whether MSP-like molecules also function to antagonize Eph signaling in other organisms. VAPs (VAMP-associated proteins) are proteins that share sequence similarity with MSP, although nematode MSPs are the only soluble members of this family (Miller et al. 2001). Collectively, VAPs are associated with a diverse range of functions and subcellular locations. The *Aplysia* VAP-33 was identified by its ability to interact with VAMP (vesicle-associated membrane protein) through yeast two-hybrid analysis and has been proposed to regulate neurotransmitter release (Skehel et al. 1995). In *Drosophila*, the VAP-33 homolog is enriched at neuromuscular junctions and controls bouton size and number (Pennetta et al. 2002). The presence of Eph receptors in neurons and neuromuscular junctions raises the possibility that VAPs might have an antagonistic effect on Eph signaling similar to that of MSP (Kullander and Klein 2002). It would be of interest to determine whether ectopic expression of an MSP-like molecule might have the potential to antagonize VAB-1 signaling during epidermal morphogenesis in *C. elegans*.

Outstanding questions

The discovery that MSP is the sperm signal in *C. elegans* and that the *C. elegans* Eph receptor, VAB-1, is a receptor

for MSP raises a number of interesting questions. First and foremost is how is MSP released from sperm? MSP is a soluble cytoplasmic protein that can polymerize to mediate sperm movement, but it lacks a signal secretion peptide. Hence, it is unlikely that MSP can be transported out of the cell through conventional mechanisms. Given the predilection of MSP to surprise, the finding that a specialized secretory pathway has evolved to either process or secrete MSP would not be unexpected.

Why might it be important to have a sperm-sensing mechanism that functions to inhibit oocyte maturation and ovulation when sperm are limited in number or absent? From an organismal standpoint, the generation of oocytes is metabolically expensive. When natural food resources are limited, the generation of oocytes that have little likelihood of being fertilized would be wasteful and disadvantageous. A far more efficient survival strategy would involve conserving metabolic expenditure by arresting oocyte maturation until sperm become available and procreation is once more possible. This strategy could be particularly important for gonochoristic nematode species that can only reproduce by mating. The just-in-time philosophy of inventory management has been successfully applied to car manufacturing; perhaps sperm-signaling mechanisms that promote oocyte maturation and ovulation in response to sperm availability should be viewed as prototypes of this efficacious strategy.

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