Development 136, 2211-2221 (2009) doi:10.1242/dev.034595

Somatic cAMP signaling regulates MSP-dependent oocyte growth and meiotic maturation in *C. elegans*

J. Amaranath Govindan*, Saravanapriah Nadarajan*, Seongseop Kim, Todd A. Starich and David Greenstein†

Soma-germline interactions control fertility at many levels, including stem cell proliferation, meiosis and gametogenesis, yet the nature of these fundamental signaling mechanisms and their potential evolutionary conservation are incompletely understood. In *C. elegans*, a sperm-sensing mechanism regulates oocyte meiotic maturation and ovulation, tightly coordinating sperm availability and fertilization. Sperm release the major sperm protein (MSP) signal to trigger meiotic resumption (meiotic maturation) and to promote contraction of the follicle-like gonadal sheath cells that surround oocytes. Using genetic mosaic analysis, we show that all known MSP-dependent meiotic maturation events in the germline require $G\alpha_s$-adenylate cyclase signaling in the gonadal sheath cells. We show that the MSP hormone promotes the sustained actomyosin-dependent cytoplasmic streaming that drives oocyte growth. Furthermore, we demonstrate that efficient oocyte production and cytoplasmic streaming require $G\alpha_s$-adenylate cyclase signaling in the gonadal sheath cells, thereby providing a somatic mechanism that coordinates oocyte growth and meiotic maturation with sperm availability. We present genetic evidence that MSP and $G\alpha_s$-adenylate cyclase signaling regulate oocyte growth and meiotic maturation in part by antagonizing gap-junctional communication between sheath cells and oocytes. In the absence of MSP or $G\alpha_s$-adenylate cyclase signaling, MSP binding sites are enriched and appear clustered on sheath cells. We discuss these results in the context of a model in which the sheath cells function as the major initial sensor of MSP, potentially via multiple classes of G-protein-coupled receptors. Our findings highlight a remarkable similarity between the regulation of meiotic resumption by soma-germline interactions in *C. elegans* and mammals.

KEY WORDS: MSP signaling, Meiosis, Meiotic maturation, Adenylate cyclase signaling, Oogenesis, Cytoplasmic streaming, *Caenorhabditis elegans*

INTRODUCTION

The *C. elegans* hermaphrodite gonad (Fig. 1A) is a paradigm for studying the role of soma-germline interactions. The somatic distal tip cell (DTC) caps the distal end of each gonad arm and maintains a population of proliferating germline stem cells; laser ablation of the DTC causes all germ cells to enter meiosis (Kimble and White, 1981). Laser ablation studies also show that cells of the somatic gonadal sheath and spermathecal lineages play multiple roles in germline development (McCarter et al., 1997). DTC signaling, which promotes the proliferation of the germline stem cell population and inhibits entry into the meiotic pathway, is among the best understood soma-germline interactions (Hansen and Schedl, 2006; Kimble and Crittenden, 2007).

Following exit from pachytene, germ cells differentiate as spermatocytes in the L4 stage and as oocytes in the adult stage. Actomyosin-dependent cytoplasmic streaming drives oocyte growth in the loop region of the gonad (Fig. 1A) (Wolke et al., 2007). Diakinesis-stage oocytes develop in the proximal gonad arm, with meiotic maturation occurring in an assembly-line fashion. Oocyte meiotic maturation is defined by the transition between diakinesis and metaphase of meiosis I and is accompanied by nuclear envelope breakdown (NEBD), rearrangement of the cortical cytoskeleton and meiotic spindle assembly. In most sexually reproducing animals, oocytes arrest during meiotic prophase I and then resume meiosis in response to hormonal signaling: for example, luteinizing hormone (LH) in mammals and MSP in *C. elegans*. When sperm are absent, as in mutant hermaphrodites that do not produce sperm (e.g. *fog* mutant females), oocytes arrest for prolonged periods until insemination (McCarter et al., 1999). The presence of sperm in the gonad also stimulates progression through pachytene and actomyosin-dependent streaming into growing oocytes (Jaramillo-Lambert et al., 2007; Wolke et al., 2007). Sperm secrete MSP by an unconventional vesicle budding mechanism to generate an extracellular MSP gradient (Kosinski et al., 2005). MSP is sufficient to trigger activation of mitogen activated protein kinase (MAPK) in proximal oocytes (Miller et al., 2001), which is required to initiate meiotic maturation (Lee et al., 2007; Arur et al., 2009). Oocytes form gap junctions with smooth muscle-like gonadal sheath cells that regulate meiotic maturation and contract to drive ovulation (Hall et al., 1999; Miller et al., 2003; Govindan et al., 2006).

Oocytes and sheath cells sense MSP through an oocyte MSP/EPH receptor (Miller et al., 2003) and unidentified receptors that are proposed to be G-protein-coupled receptors (GPCRs) (Govindan et al., 2006; Cheng et al., 2008). The VAB-1 MSP/EPH receptor negatively modulates oocyte meiotic maturation in the absence of sperm, and MSP counteracts this modulation (Miller et al., 2003; Cheng et al., 2008). Antagonistic G-protein pathways function in MSP signaling (Govindan et al., 2006; Harris et al., 2006) and regulate the trafficking of VAB-1 in the oocyte (Cheng et al., 2008).

Here we show that $G\alpha_s$-adenylate cyclase signaling in the gonadal sheath cells is required for all MSP meiotic maturation responses in the germline, including promoting the cytoplasmic streaming that drives oocyte growth. Thus, the gonadal sheath cells ensure that oocyte production and growth, as well as meiotic maturation, occur efficiently when sperm are present. In the accompanying paper (Nadarajan et al., 2009), we show that GLP-1/Notch signaling functions in the distal germline to regulate MSP-dependent

Department of Genetics, Cell Biology and Development, University of Minnesota, 6-160 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, USA.

*These authors contributed equally to this work
†Author for correspondence (e-mail: green959@umn.edu)

Accepted 22 April 2009

cytoplasmic streaming and oocyte growth. Together, these results suggest that MSP signaling organizes key steps by which germ cells generate zygotes when sperm are available for fertilization.

MATERIALS AND METHODS

C. elegans strains, genetics and phenotypic analysis

C. elegans strains used are available on request. Oocyte meiotic maturation rates (McCarter et al., 1999) and cytoplasmic streaming in the loop region (Wolke et al., 2007) were examined. MSP-142 (Baker et al., 2002) was injected (200 nM) into the uterus of unmated females (Miller et al., 2001). Statistical analyses used Student's *t*-test.

Sheath cell contraction rates were measured as previously described (McCarter et al., 1999), except 0.1% levamisole was used as an anesthetic and measurements were taken immediately. Identical results were obtained using 0.1% tricaine/0.01% tetramisole. Because we found that *egl-30* ($G\alpha_q$) function is necessary and sufficient to promote sheath contractions, we re-examined the role of *vab-1* in sheath contractions (Miller et al., 2003; Yin et al., 2004; Corrigan et al., 2005). Under our current mounting conditions, *vab-1(dx31)* and *vab-1(e2027)* null mutants exhibit normal sheath cell contractions. We determined that two factors probably contribute to previous measurements of low sheath contractions in *vab-1(dx31)*. First, *vab-1* mutants are more sensitive to the prolonged anesthetic mounting conditions previously used [6.0±3.3 contractions per minute (n=14) in *vab-1(dx31)* measured immediately after mounting, versus 2.7±2.5 contractions per minute (n=17) when measured 30 minutes after mounting, P<0.001]. Second, *vab-1* mutants that had a severe notched head phenotype exhibited lower sheath cell contraction rates even when measurements were made immediately after mounting (3.25±0.90 contractions per minute; n=8).

Mosaic analysis

gsa-1 genetic mosaics were sought using DG1922; pRP1505 (1 μg/ml) rescued *gsa-1(pk75)* (Korswagen et al., 1997) and pTG96 (*sur-5::gfp*; 75 μg/ml) provided a cell-autonomous marker (Yochem et al., 1998). *gsa-1* double mutant backgrounds were similarly analyzed. *acy-4* genetic mosaics were sought using a *sur-5::gfp* array (*tnEx37*) in which WRM061bE10 (75 μg/ml) rescued *acy-4(ok1806)*. Mosaics were characterized using DIC and GFP fluorescence microscopy; ~5000 and ~3000 animals were scored for *gsa-1* and *acy-4*, respectively. We assessed the genotype of the germline by progeny testing, except if both gonad arms were sterile. Somatic gonads mutant for *gsa-1* or *acy-4* invariably resulted in sterility of the affected arms, suggesting that perdurance is not an issue for these mosaics.

Immunofluorescence staining and MSP binding

A Zeiss motorized Axioplan 2 microscope with a 63× PlanApo (NA 1.4) objective lens and an apotome adaptor was employed for fluorescence microscopy. Fluorescence images were acquired with an AxioCam MRm camera and AxioVision acquisition software. MAPK activation (Miller et al., 2001), oocyte microtubule rearrangement (Harris et al., 2006), MSP binding (Miller et al., 2003) and extracellular MSP staining (Kosinski et al., 2005) were examined in dissected gonads.

Generation of rescuing ACY-4::GFP and GSA-1::GFP fusions

Construction of ACY-4::GFP and GSA-1::GFP fusions used recombineering (Warming et al., 2005). GFP (amplified from pPD95.77) was fused to the ACY-4 C-terminus in the WRM061bE10 fosmid (primer sequences used are available on request). Wild-type hermaphrodites were injected with the *acy-4::gfp* fosmid at 25 μg/ml and pRF4 plasmid at 50 μg/ml. Two extrachromosomal arrays (*tnEx42* and *tnEx43*) were crossed into *acy-4(ok1806)* and found to restore fertility. In a *fog-2(oz40)* female background, *tnEx42* and *tnEx43* did not exhibit elevated maturation rates. GFP was introduced into the first loop of GSA-1 using the WRM0623aH05 fosmid. *gsa-1(pk75)/hT2* hermaphrodites were injected with the *gsa-1*::*gfp* fosmid at 10 μg/ml and the pRF4 plasmid at 50 μg/ml. Homozygous *gsa-1(pk75)* animals expressing GSA-1::GFP were found to be rescued for fertility.

Generation of INX-14 and INX-22 antibodies

Antibodies to INX-14 and INX-22 were generated to the C-terminal 119 and 162 residues of INX-14 and INX-22, respectively, using fusions with maltose binding protein (MBP). MBP fusions were purified by SDS-PAGE and used to immunize rabbits and guinea pigs. Affinity purification used glutathione-S-transferase fusions. Antibodies were used to stain dissected and fixed gonads (Finney and Ruvkun, 1990). Specificity of the INX-14 and INX-22 antibodies was established by loss of staining following *inx-14(RNAi)* or *inx-22(RNAi)* by injection; markedly reduced staining was observed in *inx-22(tm1661)* gonads using the INX-22 antibodies.

RESULTS

$G\alpha_s$-adenylate cyclase signaling is required in the gonadal sheath cells for oocyte meiotic maturation

C. elegans possesses a single stimulatory G alpha ($G\alpha_s$) subunit, encoded by *gsa-1*, which is required for viability (Korswagen et al., 1997) and to promote meiotic maturation (Govindan et al., 2006). *gsa-1(RNAi)* in a wild-type background causes sterility due to a failure of oocytes to undergo meiotic maturation; however, fertility is observed following *gsa-1(RNAi)* in the *rrf-1(pk1417)* genetic background (Govindan et al., 2006) that is defective in somatic RNAi responses (Sijen et al., 2001). We conducted genetic mosaic analysis to address the cellular focus of action for $G\alpha_s$ signaling in oocyte meiotic maturation (Fig. 1B,C). The *gsa-1(pk75)* null allele was rescued by an extrachromosomal array carrying a cell-autonomous nuclear GFP marker. Mitotic instability of the array generates a clone of *gsa-1(0)* mutant cells within the lineage that can be recognized by the pattern of GFP expression. Loss of *gsa-1* function in the germline lineages (P2-P4; Fig. 1C) did not affect viability or fertility. The progeny of germline mosaic animals arrested as L1 larvae, recapitulating the *gsa-1(0)* zygotic phenotype. Thus, *gsa-1* is not required in the germline for oocyte meiotic maturation, and there is no maternal contribution of *gsa-1* to development.

Next, we sought array losses in the MS lineage, which generates the somatic cells of both gonad arms. Losses within the MS lineage cause sterility within a gonad arm because oocytes fail to undergo meiotic maturation despite the presence of sperm (Fig. 1B,C). The fertility of a gonad arm depends on the *gsa-1* genotype of the somatic cells within that particular gonad arm. For example, MSppp losses cause sterility in the anterior gonad arm but do not affect the fertility of the posterior arm. For both gonad arms to be sterile, two independent losses are needed, such as for example, losses affecting both precursor cells of the somatic gonad (Z1 and Z4). Consistent with these results, a rescuing *gsa-1(+)::gfp* transgene is expressed in the sheath and spermathecal cells (Fig. 1G).

The ten follicle-like sheath cells that encompass each gonad arm derive from two lineally distinct sheath-spermathecal (SS) precursor cells (Fig. 1C). Array losses that affect a single SS cell in a gonad arm resulted in fertility, suggesting that *gsa-1(+)* function in half of the gonadal sheath cells is sufficient. Sheath cells are connected by gap junctions (Hall et al., 1999) and might share second messengers, which could explain the sufficiency of *gsa-1(+)* function in a subset of sheath cells in a gonad arm. Alternatively, *gsa-1(+)* function might perdure within the SS lineage.

In the GTP-bound state, $G\alpha_s$ activates adenylate cyclase (ACY), which catalyzes the formation of cAMP. cAMP binds to the regulatory subunit of protein kinase A (encoded by *kin-2*), activating the catalytic subunit. In our prior analysis, we concluded that it was unclear which of the four *acy* genes participates in the regulation of meiotic maturation; however, we suggested an involvement of *acy-1* based on an analysis of a gain-of-function mutation (Govindan et al., 2006). Here, loss-of-function studies suggest that *acy-4* is required for meiotic maturation, and that *acy-1*, *acy-2* and *acy-3* are dispensable (Fig. 1E,F; see Table S1 in the supplementary material).

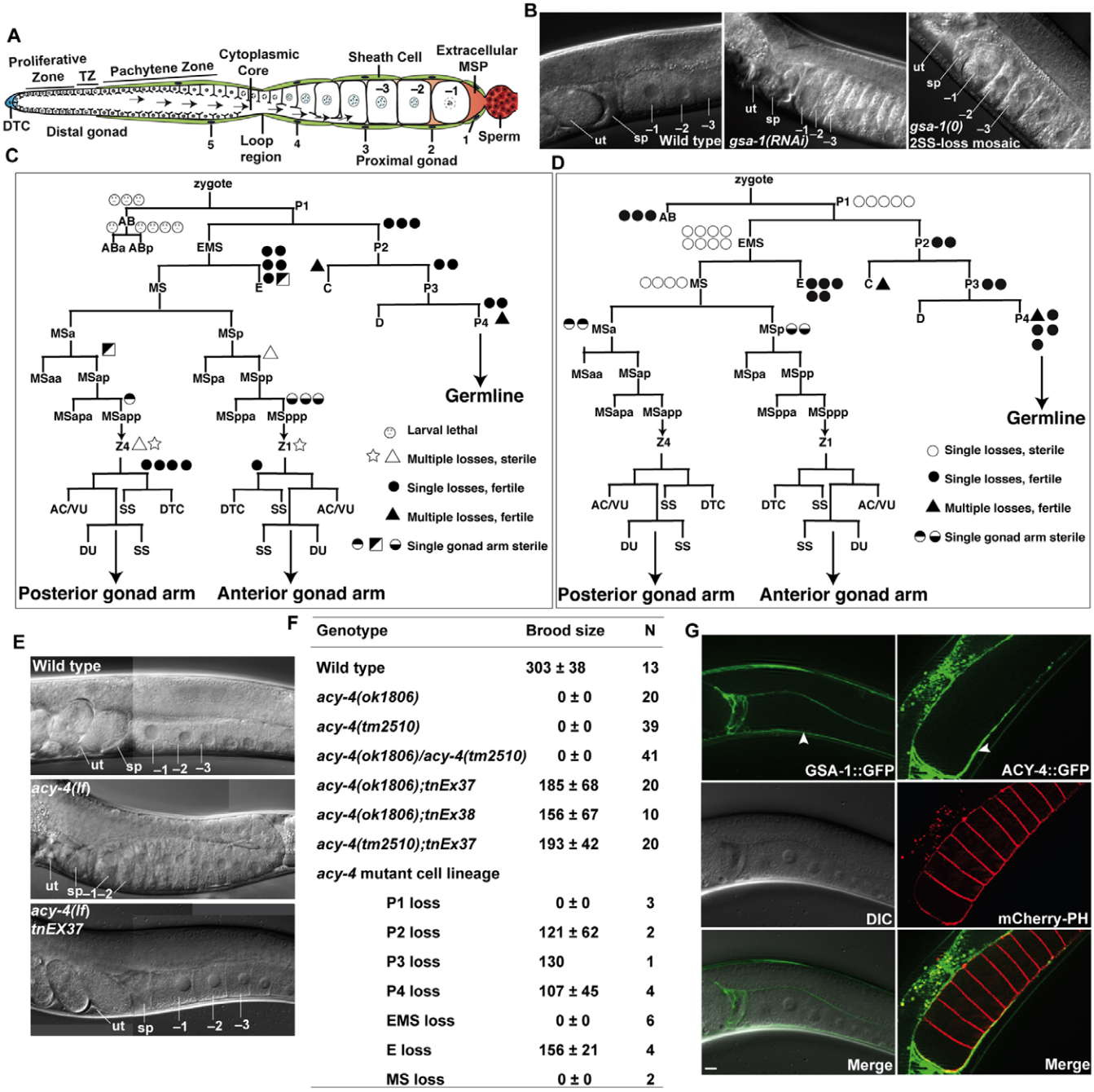


Fig. 1. Gα_s–ACY-4 signaling is required in the gonadal sheath cells for meiotic maturation. (**A**) Adult hermaphrodite gonad arm. Germ cells proliferate near the DTC and enter meiosis in the transition zone (TZ). The germline develops as a syncytium; germ cell nuclei share a common cytoplasmic core separated by incomplete membranes. Oocytes grow and cellularize in the proximal arm. The most proximal (–1) oocyte undergoes meiotic maturation in response to the MSP signal. Five pairs of sheath cells (1-5) surround the germline. (**B**) DIC images showing that loss of *gsa-1* in the sheath/spermathecal precursor (SS) cells causes oocytes to stack up in the gonad arm (right), as does *gsa-1(RNAi)* (middle); fertilized embryos are not found in the uterus (ut) or spermatheca (sp). (**C**) Mosaic analysis of *gsa-1* in meiotic maturation. Derivation of the somatic gonad and the germline, showing the points in the lineage where *gsa-1(+)* was lost and the resulting phenotypes. Circles represent single mosaic animals; polygons represent mosaics that had two independent losses. (**D**) Mosaic analysis of *acy-4* in meiotic maturation. (**E**) An *acy-4(lf)* mutation phenocopies loss of *gsa-1* in the somatic gonad (middle). Fertility is restored by an *acy-4(+)* array (*tnEx37 or tnEx38*) (bottom; compare with wild type, top). (**F**) Brood size measurements of *acy-4* mosaic animals. (**G**) GSA-1::GFP (left) and ACY-4::GFP (right) expression in gonadal sheath cells and spermathecal cells. The sheath cells (arrowhead) surround oocytes, which are labeled with the mCherry-PH membrane marker (red) for examination of ACY-4::GFP. Scale bar: 10 μm.

We examined deletions in *acy-4(ok1806)* and *acy-4(tm2510)* that remove domains required for catalytic activity, and are likely to constitute null alleles (see Fig. S1 in the supplementary material). *acy-4(ok1806)* and *acy-4(tm2510)* hermaphrodites are sterile due to a failure of oocytes to undergo meiotic maturation despite the presence of sperm (Fig. 1E,F). Because *acy-4(ok1806)* and *acy-*

4(tm2510) show indistinguishable phenotypes and fail to complement, we refer to these loss-of-function (lf) mutations as *acy-4(lf)*. The sterility of *acy-4(lf)* mutants is not due to defective sperm because mating with wild-type males does not restore fertility, and *acy-4(lf)* hermaphrodites produce normal numbers of sperm. Early germline development, including the proliferation of germline stem cells, and meiotic entry and progression, are apparently normal in *acy-4(lf)* mutant alleles; the development of the somatic gonad is also normal.

To investigate the cellular focus of action of *acy-4*, we conducted genetic mosaic analysis (Fig. 1D). We examined mosaics that had a wild-type germline and a mutant somatic gonad and vice versa. The mosaic analysis showed that *acy-4(+)* is required in the somatic gonad, but not in the germline, for meiotic maturation and fertility (Fig. 1D). Similar to the *gsa-1* genetic mosaic analysis, germline *acy-4* losses resulted in the production of broods of animals that recapitulated the *acy-4(lf)* phenotype and were sterile, showing that there is no essential maternal requirement for *acy-4* in development. Remarkably, we found one mosaic animal that had undergone multiple consecutive losses, such that the only *acy-4(+)* somatic cells in the animal were the gonadal sheath cells of the anterior arm and half of the spermathecal cells of the posterior arm (Fig. 2). In this mosaic, the anterior gonad arm was fertile, whereas the posterior gonad arm was sterile. Thus, *acy-4(+)* activity is required in the gonadal sheath cells, but not the spermathecal cells, for meiotic maturation. Moreover, expression of *acy-4(+)* only in the gonadal sheath cells of a gonad arm is sufficient to ensure the fertility of that gonad arm. In agreement with these findings, a rescuing *acy-4::gfp* fusion is expressed in gonadal sheath cells (Fig. 1G).

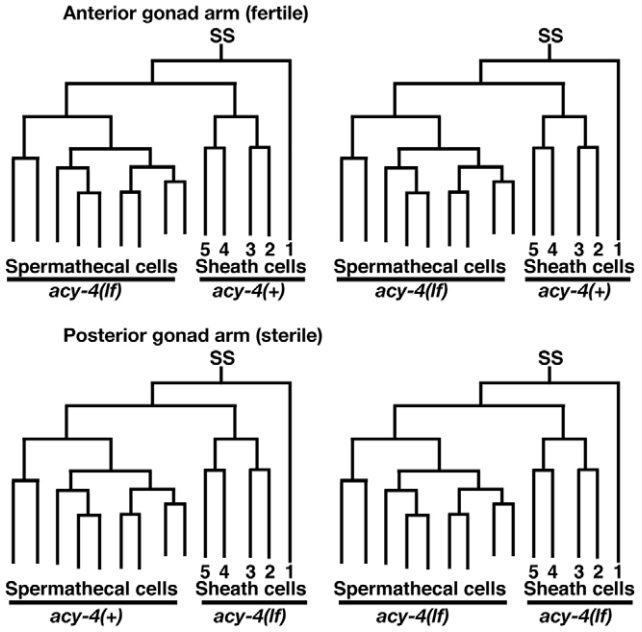


Fig. 2. *acy-4* functions in the gonadal sheath cells to promote meiotic maturation. Depiction of the sheath and spermathecal cell lineages of a mosaic animal in which the only *acy-4(+)* somatic cells were the gonadal sheath cells of the anterior arm and half of the spermathecal cells of the posterior arm. The anterior arm was fertile and the posterior arm was sterile. The germline was *acy-4(+)* as assessed by progeny testing. Because *acy-4* is not required in the germline, these mosaic results suggest that *acy-4(+)* function in the gonadal sheath cells is necessary and sufficient for oocyte meiotic maturation.

$G\alpha_s$–ACY-4 signaling is required for MSP-dependent meiotic maturation events

Time-lapse microscopy indicated that the hallmarks of meiotic maturation, nuclear envelope breakdown (NEBD) and cortical cytoskeletal rearrangement, do not occur in *acy-4(lf)* mutants. Quantitative analysis showed that *acy-4(lf)* mutants have an oocyte meiotic maturation rate of 0.0±0.0 (n=23) maturations per gonad arm per hour, compared with 2.93±0.52 (n=18) in wild-type hermaphrodites. As a result, the oocytes stack up in the gonad arm (Fig. 1E; see Table S2 in the supplementary material). A high-copy *acy-4(+)* array *(tnEx37)* was sufficient to trigger meiotic maturation in a female background: *fog-3(q443); acy-4(ok1806); tnEx37* females have a meiotic maturation rate of 1.47±0.38 maturations per gonad arm per hr (n=14), compared with 0±0 in array-minus siblings (n=15) and 2.93±0.52 (n=18) in wild-type hermaphrodites. This result suggests that when *acy-4* activity is heightened in the gonadal sheath cells by overexpression, meiotic maturation occurs without MSP. Taken together with the genetic mosaic analyses described above, these results show that *acy-4(+)* activity in the gonadal sheath cells is necessary and sufficient for oocyte meiotic maturation. The meiotic maturation defect in *gsa-1(RNAi)* and *acy-4(lf)* mutants is due to a signaling defect rather than an earlier developmental defect, because treatment of adults with the nonspecific phosphodiesterase inhibitors caffeine and isobutylmethylxanthine weakly restores fertility (see Fig. S2 in the supplementary material).

Since oocytes in *acy-4(lf)* mutants do not undergo meiotic maturation, we examined whether extracellular MSP is present and can bind to sheath cells and oocytes in *acy-4(lf)* mutants. Disruption of $G\alpha_s$–ACY-4 signaling after *gsa-1(RNAi)* or in *acy-4(lf)* backgrounds did not affect MSP release from sperm (Fig. 3A). Similarly, interfering with $G\alpha_s$–ACY-4 signaling did not reduce or eliminate MSP binding to the gonadal sheath cells and oocytes, as detected using a specific binding assay (Fig. 3B). When the binding assay was carried out in the presence of a 25-fold excess of unlabeled MSP, no binding was observed, indicating that the binding is specific. There was, however, a qualitative difference between MSP-FITC binding to the gonads of wild-type hermaphrodites, and *fog-2(oz40)* females, or *acy-4(lf)* or *gsa-1(RNAi)* hermaphrodites (Fig. 3B; see Fig. S3 in the supplementary material). Whereas MSP-FITC binds to oocytes and sheath cells in a uniform pattern in the wild type (Fig. 3B) (Miller et al., 2003), MSP-FITC labeled ring-like structures in *fog-2(oz40)* females or *acy-4(lf)* and *gsa-1(RNAi)* hermaphrodites (Fig. 3B, insets; see Fig. S3 in the supplementary material). These ring-like structures might correspond to clusters of MSP receptors that form at the sheath-oocyte interface (see Fig. S3 in the supplementary material). We quantified MSP-FITC binding to these ring-like structures in *fog-2(oz40)* and *acy-4(lf)* gonads and found that the binding is saturable (see Fig. S3 in the supplementary material), as it is in wild-type gonads (Miller et al., 2003). Importantly, MSP binding to the ring-like structures is observed in *vab-1(dx31); acy-4(lf)* double mutants (see Fig. S3 in the supplementary material), and thus must represent specific and saturable binding to unidentified receptors. The observation that these apparent receptor clusters form both in females, in which MSP is absent, and in *acy-4(lf)* mutants, is consistent with the possibility that $G\alpha_s$–ACY-4 signaling is required for a normal response to MSP.

Next, we examined molecular markers for MSP-dependent meiotic maturation signaling. MSP signaling triggers activation of MPK-1 MAP kinase (dpMPK-1) in proximal oocytes (Miller et al.,

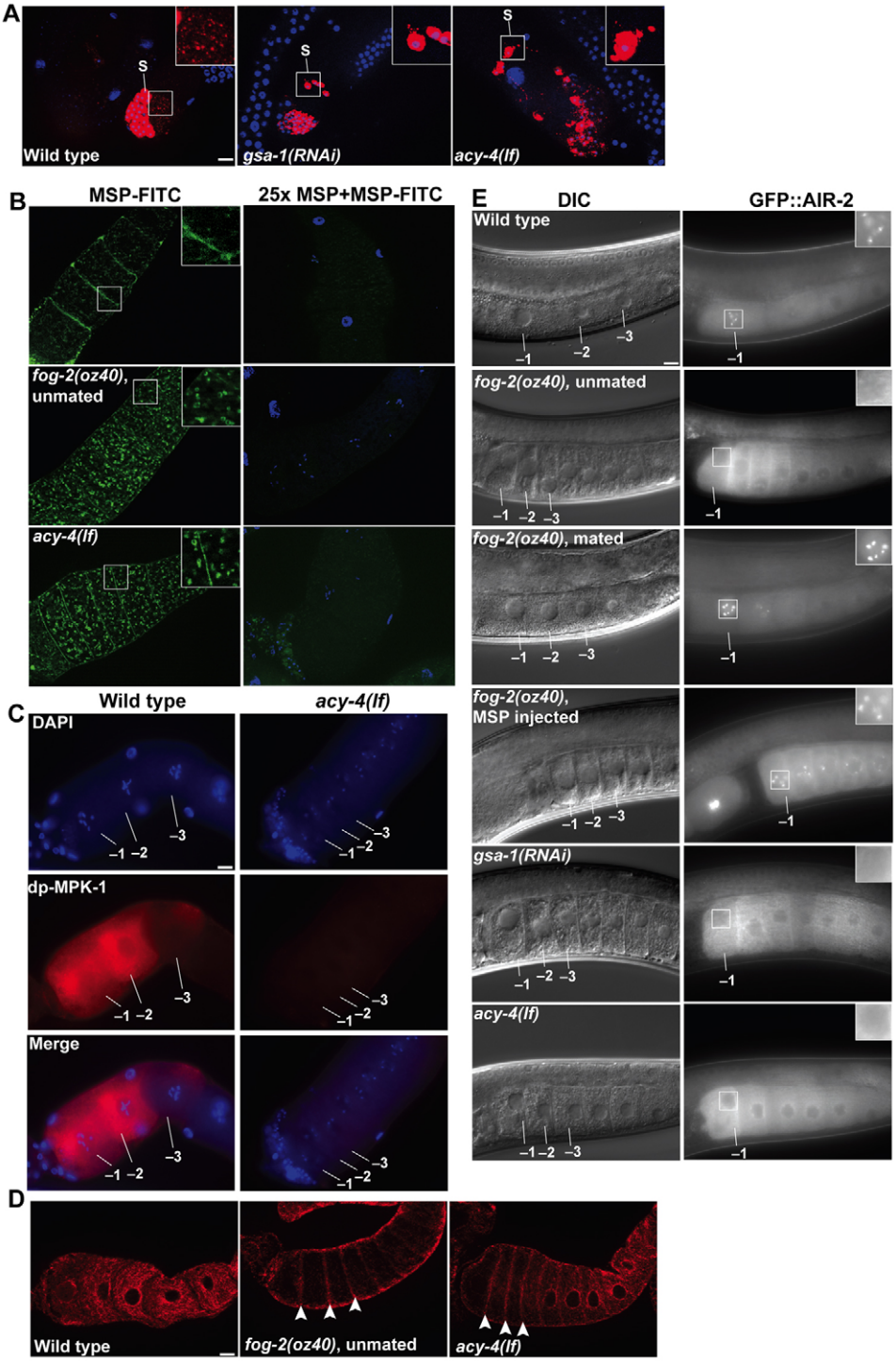
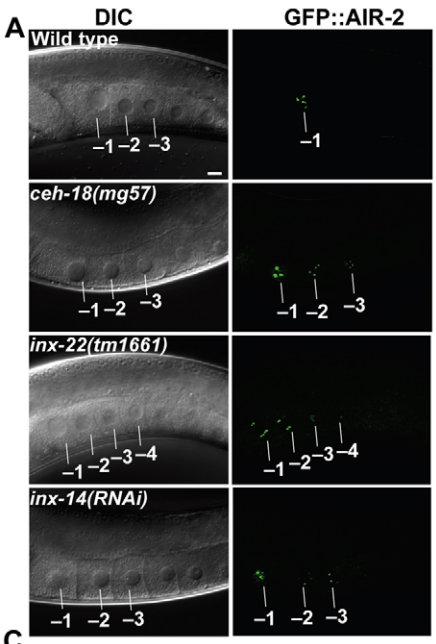
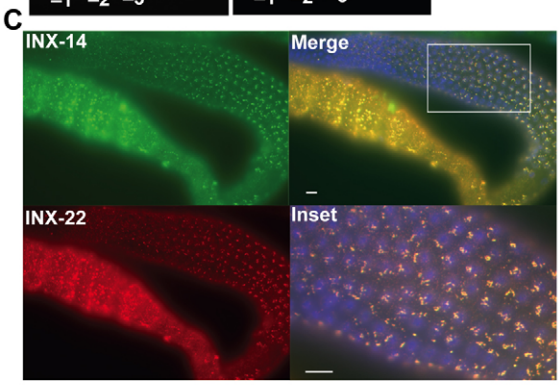


Fig. 3. $G\alpha_s$ and ACY-4 are required for multiple aspects of MSP signaling in the germline. (**A**) Detection of MSP (red) in the proximal gonad arm of hermaphrodites. Insets are magnified views showing punctate extracellular MSP released from sperm (s). *gsa-1* and *acy-4* are not required for MSP release (middle and right, respectively). (**B**) In situ binding of MSP-FITC (green) to dissected gonads in the absence (left) and presence (right) of a 25-fold excess of unlabeled MSP. MSP binds to *acy-4(lf)* mutant gonads (bottom), and the distribution of binding sites resembles that observed in unmated females (middle; see insets). Images were taken at the approximate position of the sheath-oocyte interface. Exposure times were 500 mseconds for wild-type and 200 mseconds for *acy-4(lf)* and *fog-2(oz40)*. (**C**) *acy-4* is required for MSP-dependent activation of MPK-1 in proximal oocytes (dp-MPK-1, red). dpMPK-1 staining is not observed in proximal oocytes (–1 to –3) of *acy-4(lf)* mutants at any stage (right). Weak dpMPK-1 staining is observed in the loop region and in pachytene-stage meiotic germ cells in young *acy-4(lf)* adults, similar to what has been reported for unmated young adult females (Lee et al., 2007). (**D**) *acy-4* is required for MSP-dependent cortical microtubule reorganization (microtubules, red). Cortical microtubules in proximal oocytes are indicated by arrowheads. Left, wild type; middle, *fog-2(oz40)* unmated; right, *acy-4(lf)*. (**E**) *gsa-1* and *acy-4* are required for MSP-dependent AIR-2 chromatin localization; insets show magnified widefield views. AIR-2::GFP (right) was detected using *ltIs14* (Audhya et al., 2005) and identical results were also obtained using *ojIs50* (Heallen et al., 2008). Genotypes and treatments are as indicated. Scale bars: 10 μm. DAPI-labeled DNA is blue.

2001), which is required to initiate meiotic maturation (Lee et al., 2007; Arur et al., 2009). Whereas wild-type hermaphrodites exhibited dpMPK-1 in proximal oocytes, *acy-4(lf)* mutant oocytes did not (Fig. 3C). MSP signaling also promotes reorganization of the oocyte microtubule cytoskeleton prior to NEBD by affecting microtubule localization and dynamics (Harris et al., 2006). Cortical microtubule reorganization is an early response to MSP and has been suggested to promote meiotic spindle assembly by facilitating the search and capture of microtubules by meiotic chromatin following NEBD (Harris et al., 2006). In the proximal oocytes of wild-type hermaphrodites, microtubules were dispersed throughout the cytoplasm in an interphase-like meshwork (Fig. 3D), whereas microtubules were cortically enriched in *fog-2(oz40)* females and *acy-4(lf)* mutant hermaphrodites (Fig. 3D). This result further shows that, in the absence of *acy-4(+)* activity in the gonadal sheath cells, oocytes arrest in diakinesis despite the presence of MSP.

Extracellular MSP displays a graded distribution in the gonads of hermaphrodites and mated females, such that the –1 oocyte receives the highest level of MSP and distally localized oocytes receive less (Kosinski et al., 2005). MAPK activation and microtubule reorganization in response to MSP are initiated in oocytes –1 to –4, depending on the amount of sperm present; however, NEBD only





B

Genotype	% of oocytes with chromatin-localized AIR-2::GFP (N) Oocyte position in the gonad arm –1	–2	–3	–4
Wild type	96% (72)	32% (72)	1% (71)	0% (58)
fog-2(oz40)	0% (60)	0% (60)	0% (60)	0% (60)
fog-2(oz40), mated	100% (28)	64% (28)	4% (28)	0% (28)
fog-2(oz40), MSP injected	100% (15)	100% (15)	100% (15)	100% (15)
gsa-1(RNAi)	0% (20)	0% (20)	0% (20)	0% (20)
acy-4(lf)	0% (50)	0% (50)	0% (50)	0% (50)
ceh-18(mg57)	100% (35)	91% (35)	28% (35)	23% (31)
inx-22(tm1661)	100% (68)	100% (68)	97% (68)	44% (68)
inx-14(RNAi)	100% (18)	94% (18)	61% (18)	17% (18)

D

Genotype	Fertile gonad arms/ total
gsa-1(pk75)	0/10
gsa-1(pk75); goa-1(sa734)	0/9
gsa-1(pk75) inx-22(tm1661)	6/7
gsa-1(RNAi); inx-22(tm1661)	20/20
gsa-1(pk75) ceh-18(mg57)	6/6
inx-22(tm1661); acy-4(lf)	0/20
acy-4(lf); ceh-18(mg57)	0/20
acy-4(lf); kin-2(ce179)	50/50
goa-1(sa734); acy-4(lf)	0/20
inx-14(RNAi); inx-22(tm1661); acy-4(lf)	0/20

Fig. 4. Gap junctions function downstream of $G\alpha_s$ and ACY-4. (**A**) Mutations in *ceh-18* and *inx-22*, and *inx-14(RNAi)*, extend the chromatin localization of AIR-2. Images of AIR-2::GFP (right) were taken with an apotome attachment. (**B**) Quantitation of MSP-dependent AIR-2 chromatin localization in proximal oocytes. (**C**) Detection of INX-14 (green) and INX-22 (red) in the germline using specific antibodies. Inset shows extensive colocalization of INX-14 and INX-22 throughout the germline (DAPI-labeled DNA is blue). Photomicrographs were taken at the approximate position of the oocyte-sheath interface. (**D**) Epistasis analysis between gap-junctional components and *gsa-1* and *acy-4*. *gsa-1(+)* function was removed from the somatic cells of individual gonad arms using mosaic analysis in double mutant backgrounds. In this test, *inx-22* and *ceh-18* suppress the meiotic maturation defect of *gsa-1(0)* but not *acy-4(lf)*. By contrast, a null mutation in *goa-1*, which encodes the $G\alpha_{o/i}$ negative regulator of meiotic maturation (Govindan et al., 2006), does not suppress the sterility caused by somatic loss of *gsa-1(+)* or *acy-4(lf)*. Thus, *goa-1* probably functions upstream of or in parallel to *acy-4*, which is consistent with the somatic role proposed for *goa-1* (Govindan et al., 2006). In addition, *acy-4* is epistatic to *goa-1* in a female background, as no meiotic maturation was observed in unmated *goa-1(sa734); fog-3(q443); acy-4(ok1806)* females, whereas derepression of meiotic maturation is observed in *goa-1(sa734)* females (Govindan et al., 2006). AIR-2::GFP is not extended to distal oocytes in *goa-1(sa734)* or *goa-1(RNAi)* hermaphrodites or females (see Table S3 in the supplementary material). Scale bars: 10 μm.

occurs in the –1 oocyte, which is in the correct position for ovulation and fertilization. Whereas an unknown mechanism spatially restricts meiotic maturation to the –1 oocyte (Yamamoto et al., 2006), early MSP-dependent meiotic maturation responses in more distal oocytes might serve to increase meiotic maturation rates when sperm are plentiful. We developed AIR-2::GFP as a marker for graded MSP responses. Using antibodies, it was previously shown that the chromatin localization of the AIR-2 Aurora B kinase is dependent on the presence of sperm; strong chromatin localization of AIR-2 was observed in the –1 oocyte and fainter staining was observed in the –2 oocyte (Schumacher et al., 1998). In a wild-type hermaphrodite background, AIR-2::GFP was localized to chromatin in a sperm-dependent manner; however, we also observed localization to chromosomes of the –2 and –3 oocytes at a reduced frequency (Fig. 3E; Fig. 4B). Specifically, whereas 96% of –1 oocytes had chromatin-localized AIR-2::GFP in wild-type hermaphrodites (n=72), the –2 and –3 oocytes exhibited chromatin-localized AIR-2::GFP in only 32% and 1% of cases, respectively (Fig. 4B). MSP was sufficient to induce chromatin localization of AIR-2::GFP to proximal oocytes of unmated females (Fig. 3E; Fig. 4B). Interestingly, all proximal oocytes (–1 to –4; n=15) exhibited chromatin-localized AIR-2::GFP following MSP injection into unmated females (Fig. 4B), unlike the proximal-to-distal graded distributions observed in hermaphrodites or mated females (Fig. 4B). By contrast, we found that AIR-2::GFP localized to the cytoplasm of all oocytes in *gsa-1(RNAi)* and *acy-4(lf)* mutant

hermaphrodites (Fig. 3E; Fig. 4B), resembling the localization observed in females. This result suggests that oocytes at any position are not able to sense MSP in the absence of *gsa-1(+)* or *acy-4(+)* function. Taken together, these data indicate that $G\alpha_s$–ACY-4 signaling is required in sheath cells for multiple MSP signaling responses in oocytes.

Gonadal sheath contractions require EGL-30 ($G\alpha_q$) but not $G\alpha_s$–ACY-4 signaling

Because $G\alpha_s$-adenylate cyclase signaling in gonadal sheath cells is required for MSP-dependent meiotic maturation responses in the germline, we asked whether *acy-4* and *gsa-1* are required for sheath cell contractions. MSP is sufficient to stimulate the basal contractions of the myoepithelial gonadal sheath cells (Miller et al., 2001); the basal sheath contractions occur in the interval between the preceding ovulation and the subsequent onset of NEBD and are less intense than ovulatory contractions (McCarter et al., 1999). Importantly, MSP was found to be a bipartite signal: the 21 highly conserved C-terminal amino acids of MSP was sufficient to stimulate sheath cell contraction, but not meiotic maturation (Miller et al., 2001). We observed normal sheath cell contractions in *gsa-1(RNAi)* and *acy-4(lf)* hermaphrodites (Fig. 5). This observation confirms that extracellular MSP is available to bind to sheath cells in *acy-4(lf)* mutants. Thus, the failure of oocytes to undergo meiotic maturation when the gonadal sheath cells lack *gsa-1(+)* or *acy-4(+)* function must be due to an inability to sense or respond to MSP.

Previous results suggested that *plc-3*, which encodes a phospholipase C, is required for sheath contractions (Yin et al., 2004). Because phospholipase C is a major effector of $G\alpha_q$ signaling (Neer, 1995), we considered the possibility that the MSP-dependent sheath cell contractions involve the function of *egl-30* ($G\alpha_q$) signaling. Consistent with this possibility, reduction-of-function (rf) *egl-30* mutations and *egl-30(RNAi)* lowered the sheath cell contraction rate (Fig. 5). By contrast, the *egl-30(tg26*gf*)* mutation was sufficient to stimulate sheath cell contractions in the absence of MSP, and *egl-30(tg26*gf*)* also increased the contraction rate in hermaphrodites (Fig. 5). EGL-30 ($G\alpha_q$) is likely to function in the gonadal sheath cells because normal sheath cell contraction rates were observed after *egl-30(RNAi)* in the somatic RNAi-defective *rrf-1(pk1417)* strain (Fig. 5). However, a high-copy *acy-4(+)* array (*tnEx37*) was sufficient to promote sheath cell contractions in females (Fig. 5). Since *acy-4(+)* is not required for sheath contractions in hermaphrodites, this finding might be indicative of a regulatory intersection between the *egl-30* and *gsa-1* pathways, as found in studies of locomotion (Schade et al., 2005).

$G\alpha_s$–ACY-4 signaling promotes meiotic maturation via gap junction proteins

Sheath cells form gap junctions with oocytes, and *inx-14* and *inx-22*, which encode innexin/pannexin gap junction proteins, negatively regulate meiotic maturation in the absence of MSP (Govindan et al., 2006; Whitten and Miller, 2007). INX-14 and INX-22 are expressed in the germline and colocalize at plaque-like structures (Fig. 4C), suggesting that they are components of sheath-oocyte gap junctions. Interestingly, we also observed INX-14 and INX-22 throughout the germline (Fig. 4C), beginning in the L2 stage (T.A.S. and D.G., unpublished). Consistent with this expression data, the *inx-14* deletion alleles *tm2593* and *tm2864* caused zygotic-sterile phenotypes owing to a defect in germline proliferation and gametogenesis (see Fig. S4 in the supplementary material). Genetic mosaic analysis of *inx-14* confirmed a germline focus of action (see Fig. S5 in the supplementary material).

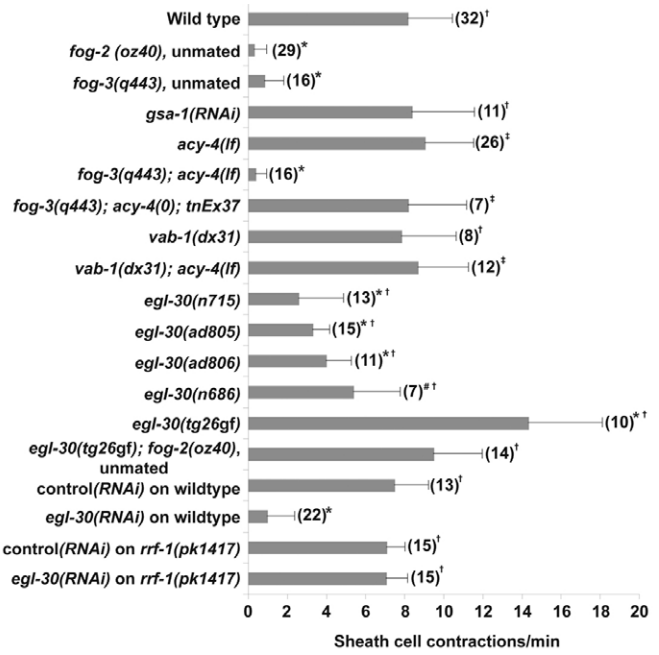


Fig. 5. EGL-30 ($G\alpha_q$) is necessary and sufficient to promote sheath cell contraction. Measurement of sheath cell contraction rates in mutant backgrounds and after RNAi injection. $G\alpha_s$-adenylate cyclase signaling is not required for sheath cell contractions. EGL-30 ($G\alpha_q$) signaling is necessary and sufficient for sheath cell contractions. Note that the effect of *egl-30(rf)* mutations on sheath cell contractions parallels the allelic series described for movement, feeding and egg-laying behaviors (Brundage et al., 1996). The number of gonad arms analyzed is indicated; *, $P<0.0005$ compared with wild type; †, $P<0.0005$ compared with *fog-2(oz40)*; ‡, $P<0.0005$ compared with *fog-3(q443)*; #, $P<0.001$ compared with wild type. Control RNAi was *gnrr-4*.

To test whether communication between sheath cells and oocytes negatively regulates meiotic progression in oocytes –2 to –4, we examined the effects of *inx-22* and *inx-14(RNAi)* on AIR-2::GFP localization (Fig. 4A,B). *inx-22(tm1661)* and *inx-14(RNAi)* increased the frequency with which AIR-2::GFP localizes to the chromosomes of the most proximal four oocytes. For example, in wild-type hermaphrodites, the –2 oocyte exhibited AIR-2::GFP chromatin localization in only 32% of cases (n=72), whereas the –2 oocyte almost always (94-100%) exhibited chromatin-localized AIR-2::GFP in *inx-22(tm1661)* or *inx-14(RNAi)* hermaphrodites (Fig. 4A,B). We obtained a similar result with a null mutation in *ceh-18* (Fig. 4B), which encodes a POU-class homeodomain protein required for proper sheath cell differentiation and function (Rose et al., 1997). *inx-22(RNAi)* and *inx-14(RNAi)* also extended AIR-2::GFP localization to distal oocytes in a female background (see Table S3 in the supplementary material). Thus, *inx-14* and *inx-22*, and by inference gap-junctional communication between sheath cells and oocytes, negatively regulate the MSP meiotic maturation response.

Since *inx-14*, *inx-22* and *ceh-18* are negative regulators of MSP-dependent meiotic maturation, and *gsa-1* and *acy-4* are positive regulators, we tested their epistatic interaction. Previously we showed that *ceh-18(mg57)* hermaphrodites are fertile after *gsa-1(RNAi)* despite exhibiting normal RNAi responses (Govindan et al., 2006). We also reported that depletion of both *inx-14* and *inx-22*

Table 1. Time-course analysis of actomyosin-dependent cytoplasmic streaming

Genotype*	DIC particle speed (μm/minute±s.d.)		
	16 hours (*n*)	20 hours (*n*)	24 hours (*n*)
Wild type	6.70±2.40 (10)	5.40±2.20 (14)	5.50±1.80 (11)
fog-2(oz40)	1.20±1.10 (11)	0.44±0.86 (16)	0±0 (8)
fog-2(oz40), mated 15 minutes	ND	ND	4.70±2.30 (7)†
fog-2(oz40), mated 1 hour	ND	ND	5.13±0.47 (6)
fog-3(q443); acy-4(ok1806); tnEx37[*acy-4(+)*]	ND	ND	12.90±3.0 (9)‡
fog-3(q443); acy-4(ok1806)	ND	ND	0±0 (5)
fog-2(oz40), buffer injected	ND	ND	0±0 (5)
fog-2(oz40), MSP injected, 1 hour	ND	ND	5.69±3.10 (7)
inx-22(tm1661); fog-2(oz40)	ND	4.88±1.6 (7)	4.50±0.45 (5)§
gsa-1(RNAi)	4.08±2.0 (9)	1.60±2.40 (11)	0±0 (8)
acy-4(ok1806)	3.20±2.08 (10)	0.55±0.90 (11)	0±0 (10)
oma-1(zu405te33); oma-2(te51)	1.40±1.40 (8)	1.35±1.20 (9)	1.50±1.60 (9)

*Unmated *fog-2* or *fog-3* females were analyzed to eliminate sperm from the gonad, unless mated with CB4855 males.
†Flow rates were also measured 15 minutes after MSP injection into *fog-2(oz40)* females. At the 15 minute timepoint, two out of seven animals exhibited flow rates of 2.35±1.20. The other five animals did not exhibit flows.
‡$P<0.0001$ compared to the wild type.
§Flow rates were taken at 30 hours post-L4. ND, not determined.

was required to allow meiotic maturation after *gsa-1(RNAi)*. After further backcrossing of the *inx-22(tm1661)* strain, we found that it exhibited meiotic maturation and fertility following *gsa-1(RNAi)*, despite showing normal germline and somatic RNAi responses (Fig. 4D). To assess epistasis without relying on RNAi, we analyzed genetic mosaics. We reduced *gsa-1(+)* function in the somatic gonad by genetic mosaic analysis in double mutant backgrounds (Fig. 4D). We found that mutations in *ceh-18* and *inx-22* suppressed the sterility defect caused by loss of *gsa-1(+)* function in the somatic gonad (Fig. 4D), suggesting that gap junction proteins function downstream of $G\alpha_s$ signaling. However, mutations in *ceh-18* and *inx-22* did not suppress the sterility caused by *acy-4(lf)*. Additional targets of $G\alpha_s$–ACY-4 signaling might therefore regulate meiotic maturation in parallel with gap junction proteins. Consistent with this view, a reduction-of-function mutation in *kin-2*, which encodes the regulatory subunit of protein kinase A (PKA), suppressed the *acy-4* requirement for fertility (Fig. 4D). Alternatively, there might be two pools of ACY-4, one functioning downstream of GSA-1 and negatively regulating INX-22 [giving suppression of *gsa-1(0)* by *inx-22(tm1661)*], and a second pool with an unknown upstream activator and an unknown downstream target [resulting in a failure of suppression of *acy-4(lf)* by *inx-22(tm1661)*]. Although we cannot exclude this possibility, the observation that somatic gonad-loss mosaics of *gsa-1(0)* phenocopy *acy-4(lf)* mutations makes this explanation less likely.

MSP promotes actomyosin-dependent cytoplasmic streaming and oocyte growth through $G\alpha_s$–ACY-4 signaling

Sperm stimulate oocyte production in addition to meiotic maturation. We observed that disrupting $G\alpha_s$–ACY-4 signaling causes hermaphrodites to produce markedly fewer oocytes compared with the wild type (see Table S4 in the supplementary material). Oocyte production and growth are driven by the transport of materials from the cytoplasmic core (see Fig. 1A). A link between sperm and actomyosin-dependent cytoplasmic streaming was previously suggested (Wolke et al., 2007). To investigate the dependence of cytoplasmic streaming on the presence of sperm, we conducted a time-course analysis (Table 1). In hermaphrodites, we observed constant flow rates in young adults between 16 and 24 hours post-L4. By contrast, unmated *fog-2(oz40)* females exhibited lower flow rates at 16 and 20 hours post-L4. Flows ceased by 24 hours post-L4, but were restored by mating. To determine whether MSP is sufficient to stimulate the flows, we injected MSP into the uterus of unmated *fog-2(oz40)* females at 24 hours post-L4 and measured the flows in the loop region an hour later. We found that MSP injection is sufficient to promote the flows (Table 1). Moreover, the response was rapid, occurring within 15 minutes of mating or injection (Table 1). This result further suggests that the flows are not a consequence of meiotic maturation because MSP injection causes flow to resume after 15 minutes, whereas meiotic maturation resumes more slowly (~25-45 minutes) after injection.

Next we asked whether somatic $G\alpha_s$–ACY-4 signaling is required for sustained cytoplasmic streaming. As was the case with unmated females, in *gsa-1(RNAi)* or *acy-4(lf)* hermaphrodites the flows ceased by 24 hours post-L4 (Table 1). Somatic ACY-4 activity appears to be sufficient to promote sustained cytoplasmic streaming in the absence of MSP, because we observed flows in females bearing a high-copy *acy-4(+)* array (*tnEx37*; Table 1). Remarkably, the flow rates in *acy-4(lf); tnEx37* females were significantly higher than in the wild type (12.9±3.0 compared with 5.5±1.8; $P<0.0001$), perhaps because of an increase in ACY-4 activity. These results suggest that $G\alpha_s$–ACY-4 signaling in sheath cells is necessary and sufficient for MSP-dependent cytoplasmic streaming in the germline.

Consistent with the idea that $G\alpha_s$–ACY-4 signaling promotes the flows by antagonizing innexin function, we observed that cytoplasmic streaming continues unabated in *inx-22(tm1661); fog-2(oz40)* females (Table 1). To test whether the cessation of cytoplasmic streaming in *gsa-1(RNAi)* and *acy-4(lf)* hermaphrodites is a consequence of the failure of oocytes to mature, we conducted a time-course analysis of the flow rates in *oma-1(zu405te33); oma-2(te51)* hermaphrodites, in which meiotic maturation does not occur (Detwiler et al., 2001). *oma-1(zu405te33); oma-2(te51)* hermaphrodites exhibited constant flow rates at all times analyzed, but these rates were lower than in wild type (Table 1). The sustained cytoplasmic flow in *oma-1; oma-2* hermaphrodites explains why their oocytes grow abnormally large. These observations suggest that meiotic maturation per se is not required for the flows to be sustained; however, these data do not address whether *oma*-1 and *oma-2* are required for normal flow rates, or whether normal flow rates have some dependence on continued meiotic maturation, as previously discussed (Lee et al., 2007).

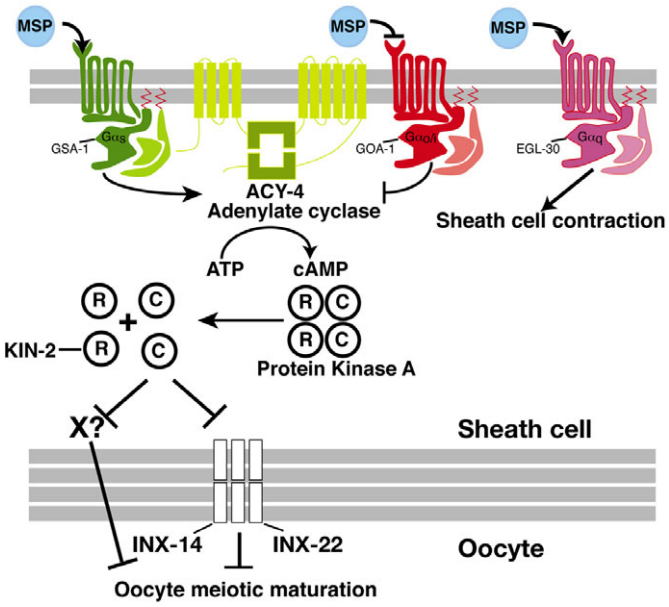


Fig. 6. A model for the control of oocyte meiotic maturation by Gα$_s$-adenylate cyclase signaling in the gonadal sheath cells. MSP is proposed to trigger Gα$_s$-adenylate cyclase signaling resulting in PKA activation. Based on genetic epistasis analysis, Gα$_s$-adenylate cyclase signaling is proposed to antagonize at least two pathways that inhibit meiotic maturation, one involving the germline innexins INX-14 and INX-22, and an uncharacterized pathway defined by mutations (X?) that suppress *acy-4(lf)* (S.K., J.A.G. and D.G., unpublished). See text for details.

DISCUSSION

The gonadal sheath cells control responsiveness to MSP

The results presented here show that the gonadal sheath cells function as the major determinant of all known germline responses to the MSP hormone. Our results show that Gα$_s$–ACY-4 signaling in the gonadal sheath cells is required for all described MSP responses in the germline occurring throughout the gonad tube, including the actomyosin-dependent cytoplasmic streaming that drives oocyte growth in the loop region of the gonad. In the accompanying article, we show that MSP rapidly induces the phosphorylation of the MLC-4 regulatory light chain of NMY-2 smooth muscle myosin as far as the proliferative zone (Nadarajan et al., 2009). Via MSP signaling, the gonad is rapidly converted into a reproductive mode when sperm are available for fertilization. Meiotic maturation, ovulation and fertilization occur repeatedly in an assembly-line fashion at a rate that matches the number of sperm present (Kosinski et al., 2005). In addition, oocyte production and the cytoplasmic streaming that drives oocyte growth and supplies maternal provisions for embryogenesis are stimulated. Thus, MSP signaling coordinates the growth and development of oocytes with meiotic progression and fertilization.

Innexins and soma-germline interactions in MSP signaling

Based on the examination of the extracellular MSP gradient in the gonad of hermaphrodites and mated females (Kosinski et al., 2005), it seems unlikely that MSP can reach the loop region where oocytes grow. The gonadal sheath cells cover the entire proximal gonad arm and most of the distal gonad arm, and form gap junctions with each other and with the germline, which provides a mechanism by which the gonadal sheath cells could communicate the presence of the MSP signal throughout the entire germline. Transmission electron microscopy and freeze-fracture analyses demonstrated that proximal sheath cells form gap junctions with oocytes (Hall et al., 1999). The observation that INX-14 and INX-22 colocalize within plaque-like structures at the sheath-oocyte interface is consistent with the possibility that these innexins are components of gap junctions between sheath cells and oocytes. *inx-14* and *inx-22* are negative regulators of meiotic maturation (Govindan et al., 2006; Whitten and Miller, 2007), oocyte microtubule reorganization (Harris et al., 2006) and recycling of the VAB-1 MSP/EPH receptor (Cheng et al., 2008) in the absence of sperm. Here we show that *inx-14* and *inx-22* also function as negative regulators of the MSP response in hermaphrodites and mated females. We also found that *inx-22* function is required for actomyosin-dependent cytoplasmic streaming to cease when MSP is absent. Genetic epistasis data suggest that Gα$_s$-adenylate cyclase signaling promotes meiotic progression in part by antagonizing *inx-14* and *inx-22* function. The genetic finding that *inx-14* and *inx-22* function in the germline is epistatic to *gsa-1* function in the gonadal sheath cells is consistent with the possibility that direct communication between these two cell types is an important aspect of the regulation of meiotic maturation. The mechanism by which Gα$_s$ antagonizes innexin function is unclear, but might involve gating. Equally unclear is the nature of the molecules that might move through these junctions, or the direction in which they pass, as previously discussed (Hall et al., 1999).

Models for Gα$_s$-adenlyate cyclase signaling in MSP sensing

We consider two models to explain the requirement of Gα$_s$-adenylate cyclase in the gonadal sheath cells for MSP responses in the germline. In the first model, Gα$_s$-adenylate cyclase signaling would affect the competence of oocytes to respond to MSP. In this model, Gα$_s$-adenylate cyclase signaling would not participate in the direct sensing of the MSP gradient, but would function in parallel to enable oocytes or sheath cells to respond by other pathways. This model is difficult to reconcile with multiple lines of experimental evidence. Most importantly, activation of Gα$_s$-adenylate cyclase signaling in the sheath cells by multiple means is sufficient to drive meiotic maturation in the absence of MSP. For example, overexpression of *acy-4* using a high-copy array causes meiotic maturation and elevated rates of cytoplasmic streaming in the absence of MSP (Table 1). Similarly, weak gain-of-function mutations in *gsa-1* or a reduction-of-function mutation in the *kin-2* regulatory subunit of cAMP-dependent PKA cause meiotic maturation in a female background (Govindan et al., 2006). The possibility that Gα$_s$-adenylate cyclase signaling has an earlier developmental role in the gonadal sheath cell lineages can be excluded by the finding that treatment of *acy-4(lf)* adults with phosphodiesterase inhibitors suppresses sterility. Likewise, our data exclude the possibility that MSP is unavailable to bind MSP receptors or that the receptors themselves are not expressed. Rather, we observed extracellular MSP in *gsa-1(RNAi)* and *acy-4(lf)* gonads, and in both cases clustered MSP binding sites were observed on sheath cells with a pattern and affinity identical to that observed in unmated females lacking MSP. Crucially, *gsa-1(RNAi)* and *acy-4(lf)* hermaphrodites exhibit gonadal sheath cell contractions, which require the 21 C-terminal amino acids of MSP and EGL-30 (Gα$_q$) signaling.

Based on this reasoning, we favor a second model in which the unidentified MSP receptors on gonadal sheath cells are GPCRs: $G\alpha_s$-coupled receptors would trigger meiotic maturation, $G\alpha_{o/i}$-coupled receptors would inhibit meiotic maturation in the absence of MSP and $G\alpha_q$-coupled receptors would promote sheath cell contraction (Fig. 6). In this model, the VAB-1 MSP/EPH receptor on oocytes would have a modulatory role. This model is consistent with the finding that the oocyte VAB-1 MSP/EPH receptor accumulates in recycling endosomes, in the absence of MSP, where it functions to inhibit meiotic maturation (Cheng et al., 2008). Importantly, $G\alpha_s$-adenylate cyclase signaling was shown to be required for the trafficking of VAB-1 to the oocyte plasma membrane from recycling endosomes when MSP is present (Cheng et al., 2008). This result is consistent with the view that the sheath cells control the MSP response and function as the initial MSP sensor. The identification of the sheath cell MSP receptors will represent a critical test of this model.

Meiotic resumption in *C. elegans* and humans

The regulation of meiotic resumption in *C. elegans* and humans is remarkably similar. The somatic cells of the follicle maintain meiotic arrest of mammalian oocytes (Pincus and Enzmann, 1935). In *C. elegans*, the gonadal sheath cells inhibit meiotic maturation when sperm are absent and promote meiotic resumption when sperm are present (Govindan et al., 2006; this work). In both systems, extracellular hormones (LH in mammals and MSP in *C. elegans*) trigger meiotic resumption through the conserved $G\alpha_s$-adenylate cyclase signaling pathway. Mural granulosa cells on the periphery of the follicle express the LH receptor, which is a GPCR. Cumulus granulosa cells form gap junctions with the oocyte using specialized extensions called transzonal projections, which penetrate the zona pellucida and reach the oocyte cell surface (Anderson and Albertini, 1976). Disruption of gap-junctional communication within ovarian follicles using inhibitors is sufficient to promote meiotic maturation (Piontkewitz and Dekel, 1993; Sela-Abramovich et al., 2006). LH signaling was recently shown to promote the closure of gap junctions between somatic cells of the follicle (Norris et al., 2008). Evidence was presented showing that closure of gap junctions within the follicle in response to LH, although sufficient for meiotic resumption, was not strictly necessary, suggesting the involvement of additional pathways (Norris et al., 2008). Similarly, the INX-14 and INX-22 gap junction proteins negatively regulate meiotic progression in both the presence and absence of MSP in *C. elegans*. Furthermore, an important part of the mechanism by which MSP promotes meiotic maturation involves the antagonistic action of $G\alpha_s$-adenylate cyclase signaling and the INX-14 and INX-22 innexins. A major difference between the systems is that $G\alpha_s$-adenylate cyclase signaling also has a function within the oocyte to maintain meiotic arrest in vertebrates and mammals (Maller and Krebs, 1977; Mehlmann et al., 2002; Mehlmann et al., 2004; Horner et al., 2003). In *C. elegans*, $G\alpha_s$–ACY-4 signaling functions exclusively in the gonadal sheath cells to promote meiotic maturation, as established by genetic mosaic analysis.

We are grateful to the *Caenorhabditis* Genetics Center, Anjon Audhya, Shohei Mitani, Karen Oegema, Jill Schumacher and Tim Schedl for providing strains and reagents. We especially thank Bob Herman, Tim Schedl and John Yochem for many helpful suggestions and experimental advice. Hua Cheng, Dave Hansen, Bob Herman, Jane Hubbard, Rindy Jaffe, Ann Rougvie, Caroline Spike and John Yochem provided comments on the manuscript. This work was supported by NIH grants GM65115 and GM57173 to D.G. Deposited in PMC for release after 12 months.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/136/13/2211/DC1

References

Anderson, E. and Albertini, D. F. (1976). Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J. Cell Biol.* **71**, 680-686.

Arur, S., Ohmachi, M., Nayak, S., Hayes, M., Miranda, A., Hay, A., Golden, A. and Schedl, T. (2009). Functional genomic identification of multiple ERK substrates in *Caenorhabditis elegans* germ cell development. *Proc. Natl. Acad. Sci. USA* **106**, 4776-4781.

Audhya, A., Hyndman, F., McLeod, I. X., Maddox, A. S., Yates, J. R., 3rd, Desai, A. and Oegema, K. (2005). A complex containing the Sm protein CAR-1 and the RNA helicase CGH-1 is required for embryonic cytokinesis in *Caenorhabditis elegans*. *J. Cell Biol.* **171**, 267-279.

Baker, A. M., Roberts, T. M. and Stewart, M. (2002). 2.6 Å resolution crystal structure of helices of the motile major sperm protein (MSP) of *Caenorhabditis elegans*. *J. Mol. Biol.* **319**, 491-499.

Brundage, L., Avery, L., Katz, A., Kim, U. J., Mendel, J. E., Sternberg, P. W. and Simon, M. I. (1996). Mutations in a *C. elegans* $G_q\alpha$ gene disrupt movement, egg laying, and viability. *Neuron* **16**, 999-1009.

Cheng, H., Govindan, J. A. and Greenstein, D. (2008). Regulated trafficking of the MSP/Eph receptor during oocyte meiotic maturation in *C. elegans*. *Curr. Biol.* **18**, 705-714.

Corrigan, C., Subramanian, R. and Miller, M. A. (2005). Eph and NMDA receptors control Ca2+/calmodulin-dependent protein kinase II activation during *C. elegans* oocyte meiotic maturation. *Development* **132**, 5225-5237.

Detwiler, M. R., Reuben, M., Li, X., Rogers, E. and Lin, R. (2001). Two zinc finger proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in *C. elegans*. *Dev. Cell* **1**, 187-199.

Finney, M. and Ruvkun, G. (1990). The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. *Cell* **63**, 895-905.

Govindan, J. A., Cheng, H., Harris, J. E. and Greenstein, D. (2006). $G\alpha_{o/i}$ and $G\alpha_s$ signaling function in parallel with the MSP/Eph receptor to control meiotic diapause in *C. elegans*. *Curr. Biol.* **16**, 1257-1268.

Hall, D. H., Winfrey, V. P., Blaeuer, G., Hoffman, L. H., Furuta, T., Rose, K. L., Hobert, O. and Greenstein, D. (1999). Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: relations between the germline and soma. *Dev. Biol.* **212**, 101-123.

Hansen, D. and Schedl, T. (2006). The regulatory network controlling the proliferation-meiotic entry decision in the *Caenorhabditis elegans* germline. *Curr. Top. Dev. Biol.* **76**, 185-215.

Harris, J. E., Govindan, J. A., Yamamoto, I., Schwartz, J., Kaverina, I. and Greenstein, D. (2006). Major sperm protein signaling promotes oocyte microtubule reorganization prior to fertilization in *Caenorhabditis elegans*. *Dev. Biol.* **299**, 105-121.

Heallen, T. R., Adams, H. P., Furuta, T., Verbrugghe, K. J. and Schumacher, J. M. (2008). An Afg2/Spaf-related Cdc48-like AAA ATPase regulates the stability and activity of the *C. elegans* Aurora B kinase AIR-2. *Dev. Cell* **15**, 603-616.

Horner, K., Livera, G., Hinckley, M., Trinh, K., Storm, D. and Conti, M. (2003). Rodent oocytes express an active adenylyl cyclase required for meiotic arrest. *Dev. Biol.* **258**, 385-396.

Jaramillo-Lambert, A., Ellefson, M., Villeneuve, A. M. and Engebrecht, J. (2007). Differential timing of S phases, X chromosome replication, and meiotic prophase in the *C. elegans* germline. *Dev. Biol.* **308**, 206-221.

Kimble, J. E. and White, J. G. (1981). On the control of germ cell development in *Caenorhabditis elegans*. *Dev. Biol.* **81**, 208-219.

Kimble, J. and Crittenden, S. L. (2007). Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in *Caenorhabditis elegans*. *Annu. Rev. Cell Dev. Biol.* **23**, 405-433.

Korswagen, H. C., Park, J.-H., Oshima, Y. and Plasterk, R. H. A. (1997). An activating mutation in *Caenorhabditis elegans* G_s protein induces neural degeneration. *Genes Dev.* **11**, 1493-1503.

Kosinski, M., McDonald, K., Schwartz, J., Yamamoto, I. and Greenstein, D. (2005). *C. elegans* sperm bud vesicles to deliver a meiotic maturation signal to distant oocytes. *Development* **132**, 3357-3369.

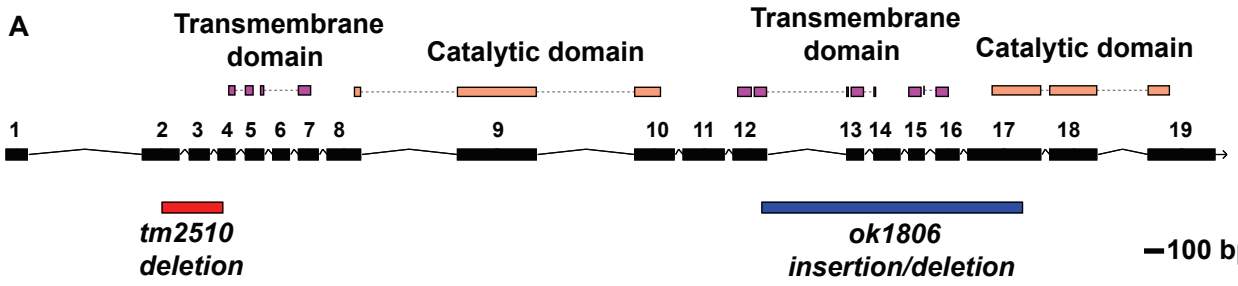
Lee, M.-H., Ohmachi, M., Arur, S., Nayak, S., Francis, R., Church, D., Lambie, E. and Schedl, T. (2007). Multiple functions and dynamic activation of MPK-1 ERK signaling in *C. elegans* germline development. *Genetics* **177**, 2039-2062.

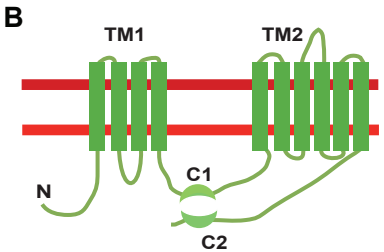
Maller, J. and Krebs, E. (1977). Progesterone-stimulated induction of meiotic cell division in *Xenopus* oocytes. Induction by regulatory subunit and inhibition by catalytic subunit of adenosine 3′,5′-monophosphate-dependent protein kinase. *J. Biol. Chem.* **252**, 1712-1718.

McCarter, J., Bartlett, B., Dang, T. and Schedl, T. (1997). Soma-germ cell interactions in *Caenorhabditis elegans*: multiple events of hermaphrodite germline development require the somatic sheath and spermathecal lineages. *Dev. Biol.* **181**, 121-143.

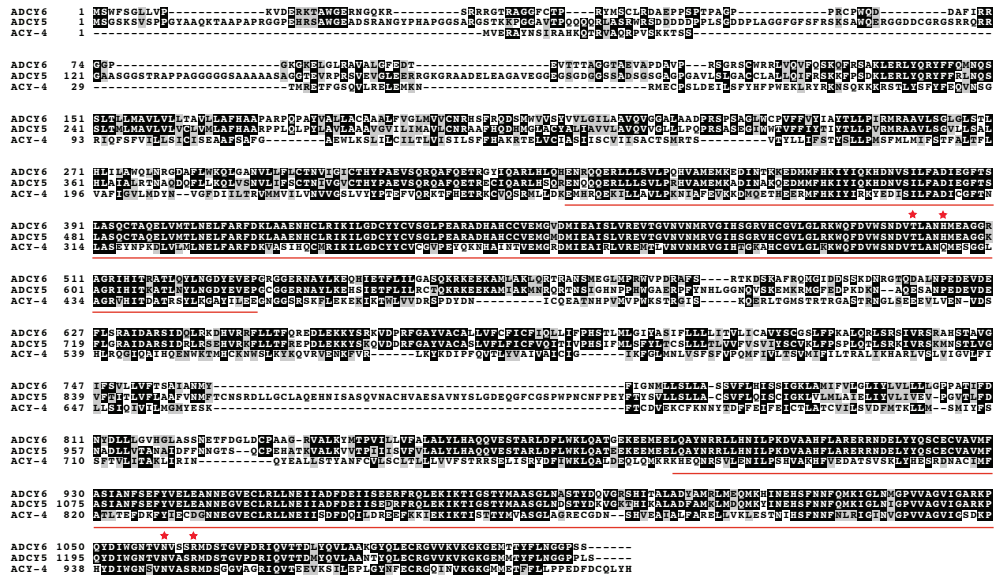
McCarter, J., Bartlett, B., Dang, T. and Schedl, T. (1999). On the control of oocyte meiotic maturation and ovulation in *C. elegans*. *Dev. Biol.* **205**, 111-128.

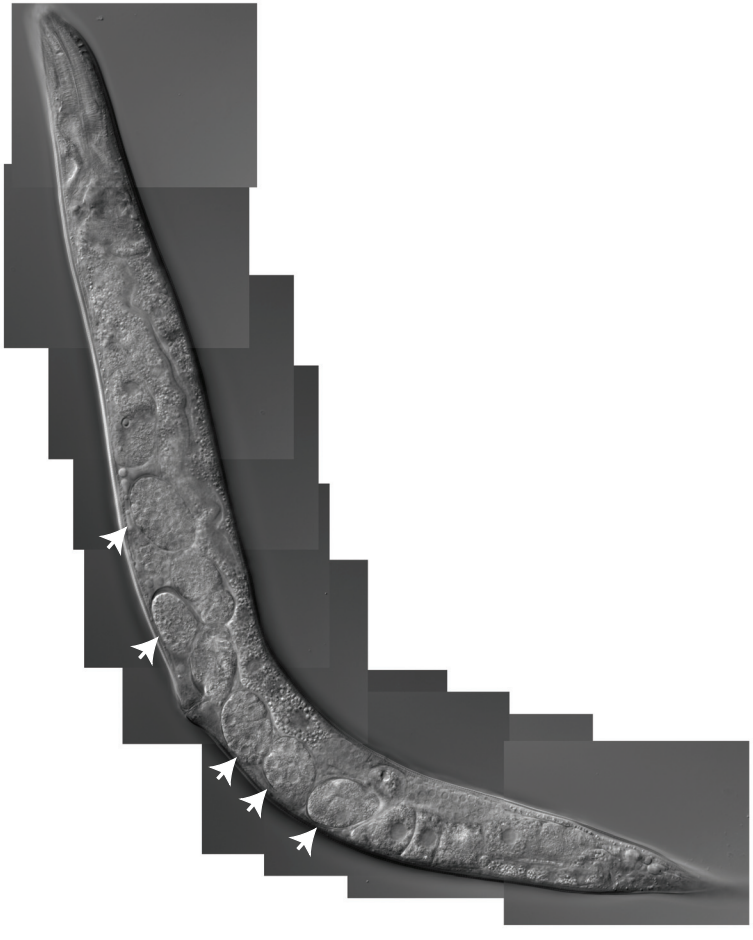
Mehlmann, L. M., Jones, T. L. and Jaffe, L. A. (2002). Meiotic arrest in the mouse follicle maintained by a G_s protein in the oocyte. *Science* **297**, 1343-1345.
Mehlmann, L. M., Saeki, Y., Tanaka, S., Brennan, T. J., Evsikov, A. V., Pendola, F. L., Knowles, B. B., Eppig, J. J. and Jaffe, L. A. (2004). The G_s-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. *Science* **306**, 1947-1950.
Miller, M. A., Nguyen, V. Q., Lee, M. H., Kosinski, M., Schedl, T., Caprioli, R. M. and Greenstein, D. (2001). A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. *Science* **291**, 2144-2147.
Miller, M. A., Ruest, P. J., Kosinski, M., Hanks, S. K. and Greenstein, D. (2003). An Eph receptor sperm-sensing control mechanism for oocyte meiotic maturation in *Caenorhabditis elegans*. *Genes Dev.* **17**, 187-200.
Nadarajan, S., Govindan, J. A., McGovern, M., Hubbard, E. J. A. and Greenstein, D. (2009). MSP and GLP-1/Notch signaling coordinately regulate actomyosin-dependent cytoplasmic streaming and oocyte growth in *C. elegans*. *Development* **136**, 2223-2234.
Neer, E. J. (1995). Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* **80**, 249-257.
Norris, R. P., Freudzon, M., Mehlmann, L. M., Cowan, A. E., Simon, A. M., Paul, D. L., Lampe, P. D. and Jaffe, L. A. (2008). Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption. *Development* **135**, 3229-3238.
Pincus, G. and Enzmann, E. V. (1935). The comparative behavior of mammalian eggs *in vivo* and *in vitro*. I. The activation of ovarian eggs. *J. Exp. Med.* **62**, 655-675.
Piontkewitz, Y. and Dekel, N. (1993). Heptanol, an alkanol that blocks gap junctions, induces oocyte maturation. *Endocr. J.* **1**, 365-372.
Rose, K. L., Winfrey, V. P., Hoffman, L. H., Hall, D. H., Furuta, T. and Greenstein, D. (1997). The POU gene *ceh-18* promotes gonadal sheath cell differentiation and function required for meiotic maturation and ovulation in *Caenorhabditis elegans*. *Dev. Biol.* **192**, 59-77.
Schade, M. A., Reynolds, N. K., Dollins, C. M. and Miller, K. G. (2005). Mutations that rescue the paralysis of *Caenorhabditis elegans ric-8* (synembryn) mutants activate the G alpha(s) pathway and define a third major branch of the synaptic signaling network. *Genetics* **169**, 631-649.
Schumacher, J. M., Golden, A. and Donovan, P. J. (1998). AIR-2: an Aurora/Ip11-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *C. elegans* embryos. *J. Cell Biol.* **143**, 1635-1646.
Sela-Abramovich, S., Edry, I., Galiani, D., Nevo, N. and Dekel, N. (2006). Disruption of gap junctional communication within the ovarian follicle induces oocyte maturation. *Endocrinology* **147**, 2280-2286.
Sijen, T., Fleenor, J., Simmer, F., Thijssen, K. L., Parrish, S., Timmons, L., Plasterk, R. H. and Fire, A. (2001). On the role of RNA amplification in dsRNA-triggered gene silencing. *Cell* **107**, 465-476.
Warming, S., Costantino, N., Court, D. L., Jenkins, N. A. and Copeland, N. G. (2005). Simple and highly efficient BAC recombineering using galK selection. *Nucleic Acids Res.* **33**, e36.
Whitten, S. J. and Miller, M. A. (2007). The role of gap junctions in *Caenorhabditis elegans* oocyte maturation and fertilization. *Dev. Biol.* **301**, 432-446.
Wolke, U., Jezuit, E. A. and Priess, J. R. (2007). Actin-dependent cytoplasmic streaming in *C. elegans* oogenesis. *Development* **134**, 2227-2236.
Yamamoto, I., Kosinski, M. E. and Greenstein, D. (2006). Start me up: Cell signaling and the journey from oocyte to embryo in *C. elegans*. *Dev. Dyn.* **235**, 571-585.
Yin, X., Gower, N. J., Baylis, H. A. and Strange, K. (2004). Inositol 1,4,5-trisphosphate signaling regulates rhythmic contractile activity of myoepithelial sheath cells in *Caenorhabditis elegans*. *Mol. Biol. Cell* **15**, 3938-3949.
Yochem, J., Gu, T. and Han, M. (1998). A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. *Genetics* **149**, 1323-1334.

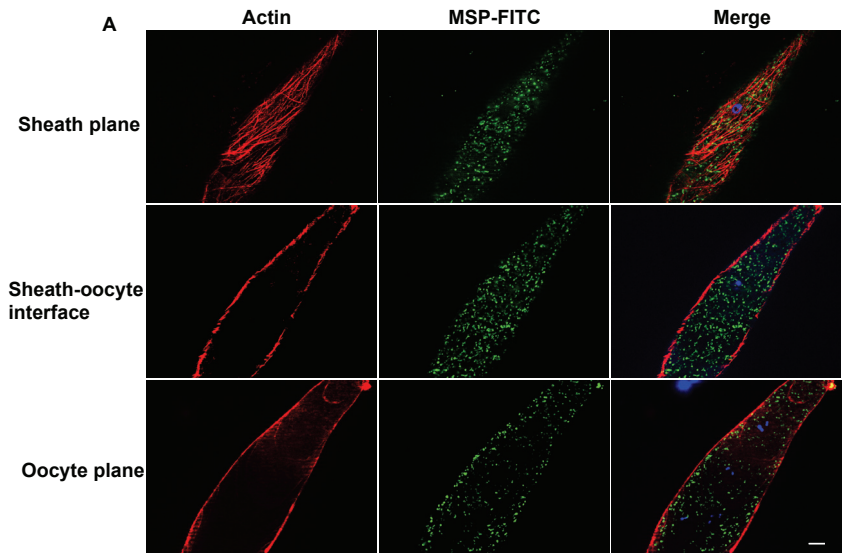

A
Transmembrane domain
Catalytic domain
Transmembrane domain
Catalytic domain
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
tm2510 deletion
ok1806 insertion/deletion
100 bp

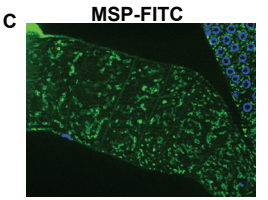

B
TM1
TM2
N
C1
C2

C

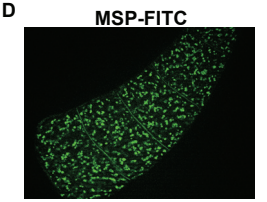

ADCY6
ADCY5
ACY-4



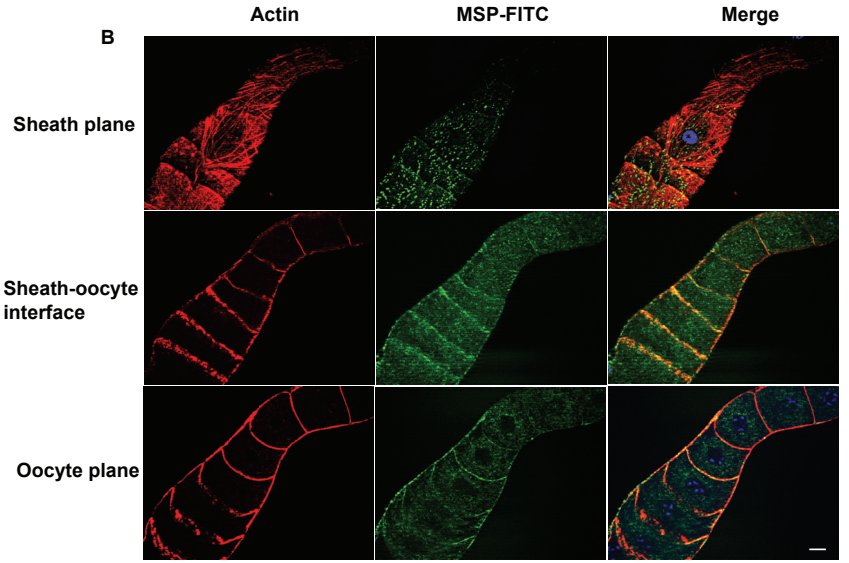


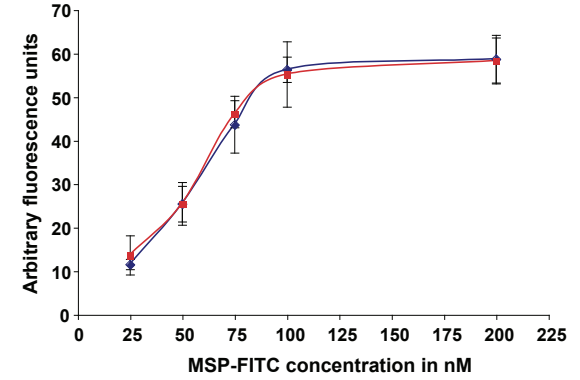


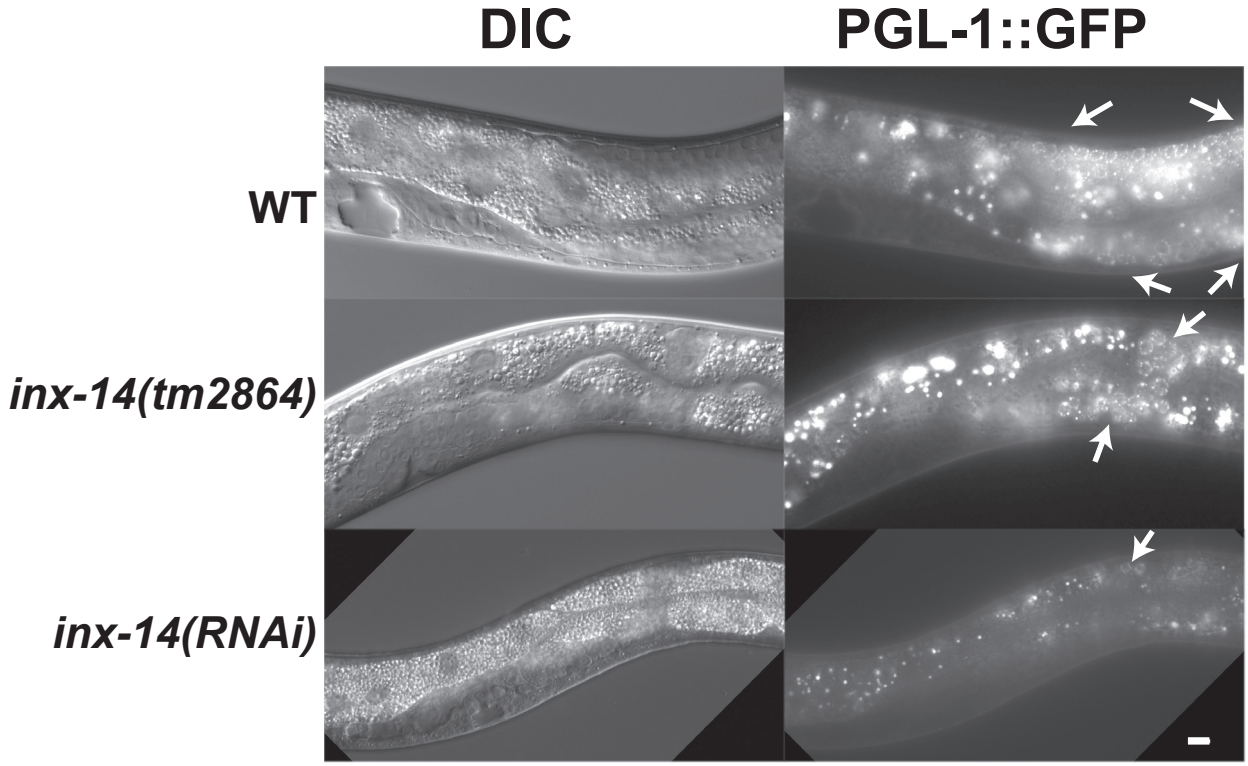
vab-1(dx31); acy-4(lf)



gsa-1(RNAi) on wildtype






DIC
PGL-1::GFP
WT
inx-14(tm2864)
inx-14(RNAi)

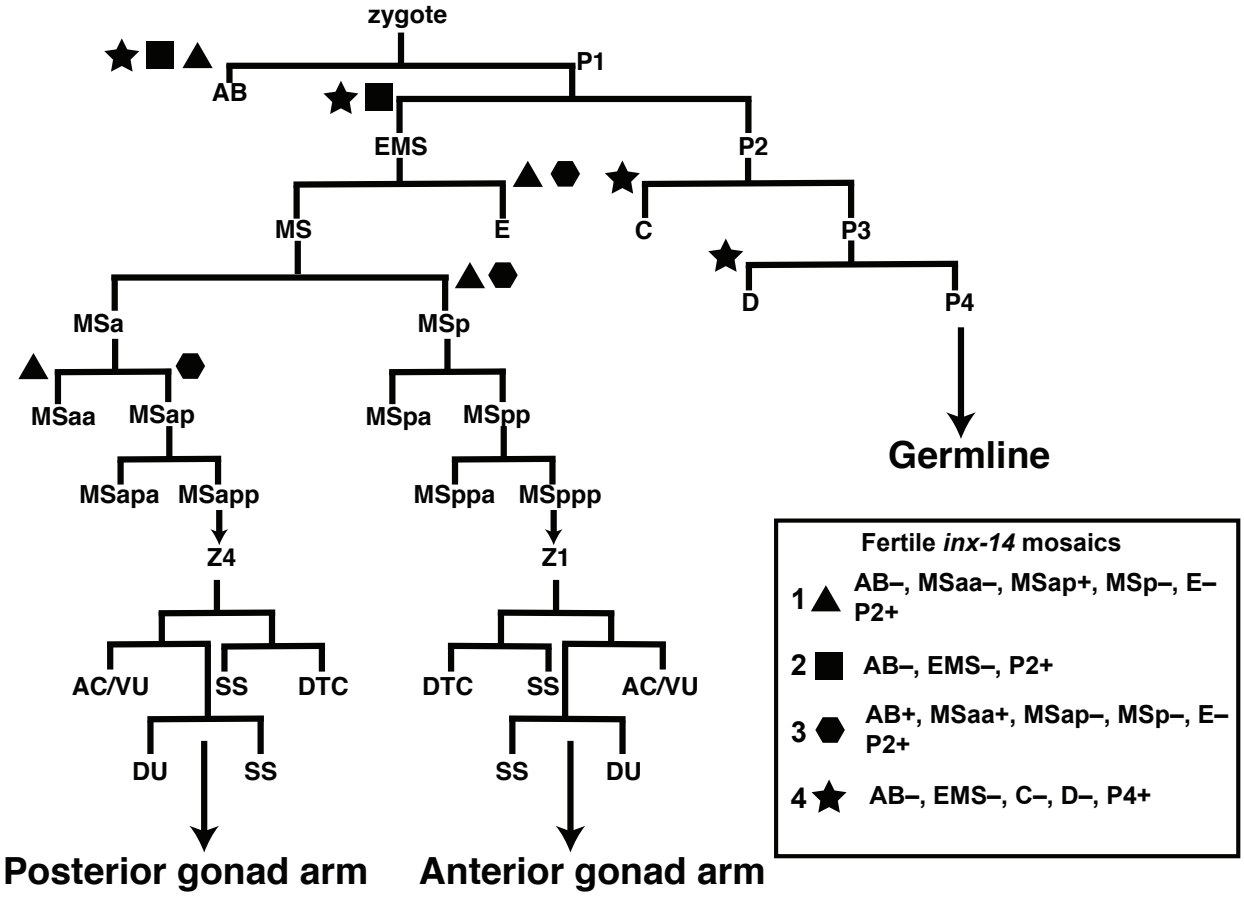

zygote
P1
AB
EMS
P2
MS
E
C
P3
D
P4
MSa
MSp
MSaa
MSap
MSpa
MSpp
Germline
MSapa
MSapp
MSppa
MSppp
Z4
Z1
AC/VU
SS
DTC
DTC
SS
AC/VU
DU
SS
SS
DU
Posterior gonad arm
Anterior gonad arm
Fertile inx-14 mosaics
1 AB–, MSaa–, MSap+, MSp–, E– P2+
2 AB–, EMS–, P2+
3 AB+, MSaa+, MSap–, MSp–, E– P2+
4 AB–, EMS–, C–, D–, P4+

Table S1. RNAi of *acy-1*, *acy-2* or *acy-3* does not block fertility

RNAi treatment of wild-type hermaphrodites	% Fertility (*n*)
Control RNAi*	100% (20)
acy-1(RNAi)	100% (26)†
acy-2(RNAi)	100% (20)†
acy-3(RNAi)	100% (32)†
gsa-1(RNAi)‡	0% (20)

Control RNAi was L4440. RNAi was used for *acy-1* and *acy-2* because deletion mutations are lethal.

†No apparent defects in meiotic maturation or ovulation were observed by DIC microscopy. The identity of the clones was verified by DNA sequencing.

‡In our prior analysis, we concluded that it was unclear which of the four *acy* genes participates in the regulation of meiotic maturation (Govindan et al., 2006). We noted, however, that some *acy-1(ce2*gf*)* gain-of-function (gf) females exhibited slightly de-repressed meiotic maturation rates and thus incorporated an involvement of *acy-1* in our model (Govindan et al., 2006). The finding that *acy-4* is required for oocyte meiotic maturation and fertility is sufficient to explain the requirement for *gsa-1*. We cannot eliminate the possibility that *acy-1*, *acy-2* or *acy-3* might augment the essential function of *gsa-1* and *acy-4*. A weak involvement of *acy-1*, *acy-2* or *acy-3* might explain the weak suppression of *acy-4(lf)* infertility by phosphodiesterase inhibitors (see Fig. S2 in the supplementary material)

Table S2. Low frequency of meiotic maturation in *acy-4(lf)* mutants

	Gonad arms containing endomitotic oocytes			
Genotype	1-day adult (*n*)	2-day adult (*n*)	3-day adult (*n*)	4-day adult (*n*)
Wild type	0% (30)	0% (22)	0% (12)	0% (10)
acy-4(ok1806)	0% (34)	13% (38)	22% (30)	23% (25)

Meiotic maturation was examined in *acy-4(lf)* mutants over longer periods. The sterility defect of *acy-4(lf)* mutants is not due to a failure in ovulation because no endomitotic oocytes, which result from defective ovulation, were observed in 1-day adults. Instead, the *acy-4(lf)* oocytes of 1-day-old adults contain six bivalents within the nucleus that are in diakinesis of prophase I. With time, stochastic meiotic maturation occurs at low frequency in *acy-4(lf)* mutants, as it does in females; however, ovulation is also blocked, and a few endomitotic oocytes accumulate in the gonad arm at later times. The few oocytes that are ovulated in unmated females become endomitotic in the uterus.

Table S3. Analysis of AIR-2::GFP localization in female backgrounds

Gene (RNAi)*	Extended AIR-2::GFP localization in unmated *fog-2(oz40)* females†
Control‡	0
goa-1#	–
gpb-1	–
inx-22	+
inx-14	+
par-5	–
kin-2	+
rpt-3	–
arf-1.1	–
ptc-1	–
vab-1	–
ran-1	–
phi-11	–
dab-1	–
vav-1	–
pkc-1	–
pqn-19	–
gsa-1	0

*RNAi of negative regulators of meiotic maturation (Govindan et al., 2006) was conducted on *fog-2(oz40)* females containing *ltIs14[pie-1p-air-2::gfp]*. All analysis in this table was done in the absence of sperm. For the results of MSP injections into *fog-2(oz40)* females on AIR-2::GFP localization see main text.

†The localizaton of AIR-2::GFP was characterized as having an 'extended' pattern (+) if all proximal oocytes (–1 to –3) in a gonad arm exhibited chromatin-localized AIR-2::GFP. Most negative regulators only exhibited AIR-2::GFP localization to the –1 oocyte (–). At least ten gonad arms were analyzed.

‡The control was L4440 empty vector RNAi; no chromatin localization of AIR-2::GFP was observed (0).

#*goa-1(RNAi)* in a *ltIs14* hermaphrodite background does not display extended AIR-2::GFP.

Table S4. $G\alpha_s$–ACY-4 signaling promotes oocyte production

	Number of oocytes produced (per gonad arm)*		
Genotype	16 hours (*n*)	24 hours (*n*)	36 hours (*n*)
Wild type	24±6 (15)	56±9 (14)	97±16 (15)
fog-2(oz40), unmated	14±2 (39)	18±1 (29)	18±2 (30)
gsa-1(RNAi)	8±2 (30)	10±2 (27)	10±2 (26)
acy-4(ok1806)	8±2 (15)	10±2 (39)	10±3 (36)
oma-1(zu405te33); oma-2(te51)	10±2 (36)	13±2 (41)	14±2 (41)

*Cumulative totals of all oocytes produced per gonad arm 16 hours, 24 hours and 36 hours after the mid-L4 stage.