INVESTIGATION

SACY-1 DEAD-Box Helicase Links the Somatic Control of Oocyte Meiotic Maturation to the Sperm-to-Oocyte Switch and Gamete Maintenance in Caenorhabditis elegans

Seongseop Kim, J. Amaranath Govindan, Zheng Jin Tu, and David Greenstein
Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT

In sexually reproducing animals, oocytes arrest at diplotene or diakinesis and resume meiosis (meiotic maturation) in response to hormones. In Caenorhabditis elegans, major sperm protein triggers meiotic resumption through a mechanism involving somatic Gαs–adenylate cyclase signaling and soma-to-germline gap-junctional communication. Using genetic mosaic analysis, we show that the major effector of Gαs–adenylate cyclase signaling, protein kinase A (PKA), is required in gonadal sheath cells for oocyte meiotic maturation and dispensable in the germ line. This result rules out a model in which cyclic nucleotides must transit through sheath-oocyte gap junctions to activate PKA in the germ line, as proposed in vertebrate systems. We conducted a genetic screen to identify regulators of oocyte meiotic maturation functioning downstream of Gαs–adenylate cyclase–PKA signaling. We molecularly identified 10 regulatory loci, which include essential and nonessential factors. sacy-1, which encodes a highly conserved DEAD-box helicase, is an essential germline factor that negatively regulates meiotic maturation. SACY-1 is a multifunctional protein that establishes a mechanistic link connecting the somatic control of meiotic maturation to germline sex determination and gamete maintenance. Modulatory factors include multiple subunits of a CoREST-like complex and the TWK-1 two-pore potassium channel. These factors are not absolutely required for meiotic maturation or its negative regulation in the absence of sperm, but function cumulatively to enable somatic control of meiotic maturation. This work provides insights into the genetic control of meiotic maturation signaling in C. elegans, and the conserved factors identified here might inform analysis in other systems through either homology or analogy.

IN sexually reproducing animals, cells of the germ line form gametes, which unite at fertilization and establish a heritable link between generations. Meiosis halves the number of chromosomes contributed by each gamete thereby ensuring the embryo inherits two full sets of chromosomes. Meiosis and germline sex determination are closely coordinated to ensure fertility (reviewed by Kimble and Crittenden 2007; Ewen and Koopman 2010; Murray et al. 2010). During development germ cells must adopt a sexual fate so as to differentiate either as sperm or oocytes. Sex-specific timing in the meiotic process is commonly observed: spermatocytes proceed through the meiotic divisions in an uninterrupted fashion, whereas oocytes almost invariably arrest once, and sometimes twice, depending on the species. Oocyte meiotic maturation is defined by the transition between diakinesis and metaphase of meiosis I and is accompanied by nuclear envelope breakdown, rearrangement of the cortical cytoskeleton, and meiotic spindle assembly (Masui and Clarke 1979). While the timing of the meiotic divisions with respect to fertilization varies among species, maturation promoting factor (MPF), CDK1/cyclin B, is a universal regulator of oocyte meiotic cell cycle progression (reviewed by Ferrell et al. 2009; Von Stetina and Orr-Weaver 2011). By contrast, the mechanisms by which the meiotic maturation process is regulated and integrated within the oogenic program are comparatively less well understood. Maternal age-related defects in the oocyte meiotic maturation process represent the largest single source of birth defects and infertility in
developed countries, which motivates studies in both mammalian and invertebrate model systems (reviewed by Hassold and Hunt 2009).

The nematode *Caenorhabditis elegans* has emerged as a useful model for studying the regulation of oocyte meiotic maturation by intercellular signaling (reviewed by Han et al. 2010; Von Stetina and Orr-Weaver 2011; Kim et al. 2013). *C. elegans* hermaphrodites possess two U-shaped gonad arms that produce sperm during the last larval stage and oocytes during adulthood (Figure 1A). Oocyte meiotic maturation, ovulation, and fertilization occur iteratively in an assembly line-like fashion approximately every 23 min (McCarter et al. 1999). By contrast, strong loss-of-function (lf) mutations in sex-determination genes that feminize the germ line such that exclusively oocytes are produced, cause meiotic maturation to occur infrequently (approximately every 8 hr) and thus oocytes stack up in the gonad arm (McCarter et al. 1999). Mating of these females to males restores normal rates of meiotic maturation, ovulation, and fertilization (McCarter et al. 1999). These experiments (McCarter et al. 1999) and related observations (Ward and Carrel 1979) provided evidence that sperm produce a diffusible signal that promotes oocyte meiotic maturation and oocyte production. Subsequently, a biochemical purification of the maturation-inducing signal from sperm revealed that the major sperm proteins (MSPs) are sufficient to promote oocyte meiotic maturation and gonadal sheath cell contraction (Miller et al. 2001). MSPs appear to be released from sperm using an unconventional vesicle budding mechanism (Kosinski et al. 2005). MSPs were originally shown to function as the chief cytoskeletal element in the actin-independent amoeboid locomotion of nematode sperm (Italiano et al. 1996; Miao et al. 2003; reviewed by Roberts and Stewart 2012). MSPs were proposed to function as central elements of a sperm-sensing mechanism that couples meiotic maturation and fertilization rates to sperm availability, thereby ensuring efficient progeny production and utilization of resources (Miller et al. 2003).

MSP triggers multiple molecular readouts of meiotic maturation, including activation of the MPK-1 mitogen-activated protein kinase (MAPK) (Miller et al. 2001), which is required for normal meiotic maturation (Lee et al. 2007). MSP also promotes the reorganization of the oocyte microtubule cytoskeleton (Harris et al. 2006), the localization of the AIR-2 Aurora B protein kinase to chromatin (Govindan et al. 2009), and the remodeling of oocyte ribonucleoprotein particles (Jud et al. 2008). In addition, MSP promotes the actomyosin-dependent cytoplasmic flows that drive oocyte growth and require the continued presence of sperm (Wolke et al. 2007; Nadarajan et al. 2009). In this regard, MSP might function in part by promoting the phosphorylation of the MLC-4 regulatory light chain of NMY-2 nonmuscle myosin (Nadarajan et al. 2009). Thus far, all described outcomes of MSP signaling in the germ line require Gα–acylcytlate cyclase activity in the gonadal sheath cells that surround oocytes (Govindan et al. 2009). Genetic mosaic analysis established that genotypically wild-type oocytes, which are surrounded by gonadal sheath cells that lack Gα or adenylate cyclase activity, behave as if they do not receive the MSP signal (Govindan et al. 2009). Further, activation of Gα–adenylate cyclase signaling in the gonadal sheath cells is sufficient to drive meiotic maturation at robust rates in the absence of sperm (Govindan et al. 2006, 2009).

A key question is how Gα–adenylate cyclase signaling in gonadal sheath cells promotes oocyte meiotic maturation in response to the MSP signal. Part of the answer to this question is that the gonadal sheath cells form gap junctions with oocytes (Hall et al. 1999), and these gap junctions function to inhibit meiotic maturation when sperm are absent (Govindan et al. 2006, 2009; Whitten and Miller 2007; T. Starich and D. Greenstein, unpublished results). A loss-of-function mutation in the inx-22 gene, which encodes the gap junction protein INX-22, suppresses gsa-1(lf) sterility (gsa-1 encodes Gα; Govindan et al. 2009). Here we use genetic analysis to delineate the molecular mechanisms by which somatic Gα–adenylate cyclase signaling promotes oocyte meiotic maturation. We use genetic mosaic analysis to show that the protein kinase A (PKA) target of Gα– adenylate cyclase signaling is required in gonadal sheath cells for oocyte meiotic maturation. Not only is PKA activity in the germ line dispensable for meiotic maturation, but PKA does not function in the germ line as a negative regulator of MPF activation, as observed in vertebrate systems (Maller and Krebs 1977; Lincoln et al. 2002; Han et al. 2005; Pirino et al. 2009; Oh et al. 2010). This genetic result rules out a model in which cyclic nucleotides must move through sheath-oocyte gap junctions to regulate meiotic maturation via PKA activity in the *C. elegans* germ line, as has been proposed in vertebrate systems (Anderson and Albertini 1976; Sela-Abramovich et al. 2006; Norris et al. 2008, 2009).

acy-4 encodes the adenylate cyclase that is required in gonadal sheath cells for oocyte meiotic maturation (Govindan et al. 2009). To identify new regulators of oocyte meiotic maturation that function downstream of somatic Gα–ACY-4–PKA signaling, we conducted a genetic screen for suppressor of acy-4(lf) sterility (Sacy) mutations. We characterized 66 Sacy mutations in at least 17 genes. By using whole-genome sequencing and other positional cloning tools, together with an analysis of previously isolated mutations, we molecularly identified 10 Sacy loci. The centerpiece of our analysis is sacy-1, which encodes a highly conserved DEAD-box helicase that functions in the germ line downstream of PKA signaling. Genetic analysis reveals that SACY-1 mediates multiple functions necessary for *C. elegans* reproduction: it is a strong negative regulator of oocyte meiotic maturation; it is a component of the germline sex-determination system, functioning in the hermaphrodite sperm-to-oocyte switch; and it is required to prevent necrotic cell death of gametes. Thus, sacy-1 links the somatic control of meiotic maturation to germline sex determination and the maintenance of oocyte quality. In addition, our genetic screen identified multiple components of a CoREST-
like complex and the \( \text{TWK-1} \) two-pore domain potassium channel, which we show function in the germline and somatic gonad, respectively. Genetic evidence suggests that CoREST and \( \text{TWK-1} \) likely function cumulatively to regulate meiotic maturation. This work provides a foundation for unraveling the genetic control of meiotic maturation signaling in \( \text{C. elegans} \). The insights gained may prove informative in the analysis of systems less amenable to forward genetic approaches.

**Materials and Methods**

**Strains**

\( \text{C. elegans} \) were cultured using standard methods at 20\(^\circ\) (Brenner 1974), except as otherwise noted. \( \text{OP50-1} \), a streptomycin-resistant \( \text{OP50} \) derivative, was used for routine strain maintenance and nematode growth medium (NGM) contained 200 \( \mu \text{g/ml} \) streptomycin sulfate added before autoclaving. Streptomycin was omitted for all experiments using \( \text{HT115(DE3)} \), and their respective controls. Alleles generated in this study are described in Table 1, and the molecular changes identified are listed in the Supporting Information, Table S1. In addition, the following mutations were used: LG I: \( \text{pde-6(ok3410)} \), \( \text{gsa-1(pk75)} \), \( \text{fog-1(e2121)} \), \( \text{dpy-5(e61)} \), \( \text{tom-1(ok188)} \), \( \text{tom-1(ok2437)} \), \( \text{tom-1(tm4724)} \), \( \text{let-605(h312)} \), \( \text{dpy-20(e1282)} \), and \( \text{ced-3(n717)} \); LG II: \( \text{tra-2(e2020)} \) and \( \text{rrf-3(pk1426)} \); LG III: \( \text{unc-32(e189)} \), \( \text{unc-119(ed3)} \), \( \text{unc-32(e189)} \), \( \text{unc-119(ed3)} \), \( \text{unc-33(mn407)} \), \( \text{unc-24(e138)} \), \( \text{oma-1(zu405te33)} \), \( \text{spr-2(ar211)} \), \( \text{spr-2(tm4802)} \), \( \text{fem-3(e1996)} \), \( \text{fem-3(q20)} \), \( \text{him-8(tm611)} \), \( \text{dpy-20(e1282)} \), and \( \text{ced-3(n717)} \); LG IV: \( \text{unc-46(e177)} \), \( \text{acy-4} \).
(ok1806), unc-68(e540), oma-2(te51), spr-1(gk734), unc-23(e25), fog-2(oza40), and fog-2(q71); LGX: spr-3(by108) and spr-3(ok2525). The following rearrangements, deficiencies, duplications, and extrachromosomal arrays were used: hT2[dpy-18(k662); bli-4(e937)] (I); III, hT2[bli-4(e937) let-2(q782) qb48] (I); III), tn18[gb51] (V), V, dpy-20(1); f0, qD16, tnEx31[gsa-1(+); sur-5:gfpl, tnEx37[acy-4(+); sur-5:gfpl, tnEx109[kin-1(+); sur-5:gfpl, tnEx131[acy-1:gfpl rol-6(su1006d)], tnEx133[acy-2:gfpl rol-6(su1006d)], tnEx134[acy-3:gfpl rol-6(su1006d)], tnEx159[GFPl: sacy-1 unc-119(+)], tnEx175[twtk-1:gfpl rol-6(su1006d)], tnEx180[twwk-1(+); sur-5:gfpl], tnEx181[twtk-1:gfpl str-1:gfpl], tnEx188[twwk-1(DC284):gfpl]. The genotypes of strains used in this study are listed in Table S2.

Isolation of suppressor of acy-4(II) sterility (Sacy) mutations

L4-stage acy-4(ok1806); tnEx37 animals were mutagenized with 50 mM methyl methanesulfonate (EMS) (Brenner 1974). GFP + F1 animals were cultured individually and fertile GFP - animals were sought in the F2 generation. Approximately 20,000 haploid genomes were screened and 63 suppressors had sufficient brood sizes to be analyzed further. Brood sizes are expressed as mean ± SD. All Sacy mutations were outcrossed to the parental strain and were recessive. Mutations analyzed in detail were outcrossed at least five times, or as otherwise noted. The polymerase chain reaction verified that all suppressor strains retained the acy-4 (ok1806) deletion, and did not contain a wild-type copy of the acy-4 gene or gfpl sequences (oligonucleotides used in this study are listed in Table S3). pde-6 alleles were identified as EMS-induced mutations in N2 that suppressed sterility following gsa-1(RNAi). The three pde-6 alleles failed to complement for this property, but exhibited normal RNAi responses with unc-22 and pos-1 triggers.

Genetic mapping and molecular identification of Sacy mutations

Assignment to linkage groups used SNP mapping (Davis et al. 2005) with crosses to DG2574, which was generated by introgressing the acy-4(ok1806) mutation, balanced by tnEx37, into the CB4856 Hawaiian background using 10 backcrosses. It proved difficult to fine map many of the Sacy mutations in the Hawaiian background, possibly because complex genetic interactions between Bristol and Hawaiian loci modified the penetrance of acy-4(II) (S. Kim, J. A. Govindan, and D. Greenstein, unpublished results). In addition, more than half of Sacy mutations localize to LGI and thus the documented incompatibility between Bristol and Hawaiian strains caused by the peel-1/zeel-1 system (Seidel et al. 2008) might have distorted the mapping results. Therefore, we utilized a strategy combining complementation testing, whole-genome sequencing, and transgenic rescue. Complementation tests were conducted between Sacy mutations mapping to the same linkage group. Briefly, transheterozygotes sacy(a)/sacy(b); acy-4(ok1806); tnEx37 were constructed and the fraction of GFP - fertile progeny was measured and compared to the parental strains. Because Sacy mutations might exhibit nonallelic noncomplementation, these assignments are viewed as provisional unless validated
by sequencing of multiple alleles, transgenic rescue, or suppression of acy-4(If) sterility by other available alleles.

Whole-genome sequencing for mutant identification was conducted on Illumina GAIIx and HiSeq2000 instruments according to the manufacturer’s instructions. The average depth of coverage was ~49-fold. Data were analyzed using MAQGene (Bigelow et al. 2009). Candidate Sacy mutations in independently isolated alleles were identified and confirmed by Sanger sequencing. Phylogenetic analysis was conducted as described (Dereeper et al. 2008).

Transgenic rescue and expression studies
Transgenic animals expressing translational gfp fusions were generated using recombineering (Warming et al. 2005; Tursun et al. 2009) and either microinjection (Stinchcomb et al. 1985) or biolistic transformation (Praitis et al. 2001). To create C-terminal TWK-1::GFP fusions, fosmid WRM0616aE06 was used. To generate a C-terminal truncation of TWK-1::GFP (TWK-1::C284::GFP), Escherichia coli GalK was first inserted before Thr284, and then GalK and the C terminus of TWK-1 (residues 284–451) were deleted using recombineering. The C-terminal truncated twk-1::gfp and controls were directly injected into twk-1(tm1397); unc-13(c51); acy-4(ok1806)/nT1[qls51] animals, and several transgenic lines expressing the str-1::gfp co-injection marker were established. The transgene arrays were tested for rescuing twk-1(+)( function in twk-1 (tm1397); acy-4(ok1806) animals by restoring acy-4(If) sterility. Fosmid WRM0640aH10 was used to generate an N-terminal GFP::SACY-1 fusion. Cre-mediated recombination was used to introduce the unc-119 gene (Zhang et al. 2008) into the fosmid for biolistic transformation. A gfp::sacy-1::expressing extrachromosomal array (tnEx159) was crossed into sacy-1 mutant backgrounds and tested for rescue. To generate C-terminal GFP fusions to ACY-1, ACY-2, and ACY-3, we used fosmids WRM067dg12, WRM0638bh07, and WRM0618cf11, respectively. Fusion constructs were injected into the wild type with rol-6(su1006d) as co-injection marker. Transgenes were then crossed into the spr-5(by134) mutant background.

Genetic mosaic analysis
Genetic mosaic analysis for kin-1 was performed using a rescuing kin-1(+) extrachromosomal array, tnEx109[kin-1(+)(sur-5::gfp) marker, carrying the cell autonomous sur-5::gfp marker (Yochem et al. 1998). kin-1(ok338), sacy-1(tm1385) kin-1 (ok338), spr-5(by134) kin-1(ok338), and twk-1(tm1397) kin-1(ok338) animals bearing tnEx109 were used for the analysis. To identify genetic mosaics with array losses in the somatic gonad, young adult hermaphrodites were examined on a Zeiss Axioskop using DIC and fluorescence microscopy with a 100× Plan-Neofluar (numerical aperture, N.A. 1.4) objective lens. To determine the point of array loss in animals exhibiting mosaic expression of the sur-5::gfp marker, the following cells were routinely examined: distal tip cell (DTC), gonadal sheath, spermatheca, coelomocytes, the head mesodermal cell (HMC), body wall muscles, hyp11, intestine, excretory cell, B, F, K, DVA, DVC, and the germ line (the presence of GFP+ progeny could only be scored if at least one gonad arm was fertile). To identify germline-loss mosaics, L4 hermaphrodites were cultured individually. Animals producing entire broods of GFP+ progeny were further examined by fluorescence microscopy. Unfertilized oocytes laid by kin-1(ok338) germline mosaics were counted over the first 6 days of adulthood. For twk-1, genetic mosaics were identified in similar fashion using twk-1(tm1397); acy-4(ok1806)/nT1[qls51]; tnEx180[twk-1(+)(sur-5::gfp)] as the parent strain. twk-1(tm1397) somatic gonad mosaics were fertile in the affected gonad arm, but germline mosaics could not be sought. For genetic mosaic analysis of the sacy-1(tm5503) gamete degeneration phenotype, we used sacy-1(tm5503); unc-119(ed3); tnEx159[gfp::sacy-1 unc-119(+)]. Coordinated (non-Unc) animals showing the sacy-1(tm5503) gamete degeneration phenotype were sought and analyzed by DIC and fluorescence microscopy.

RNA interference
All RNA interference (RNAi) experiments were conducted at 22° using injection (Fire et al. 1998) or feeding (Timmons and Fire 1998), as modified (Govindan et al. 2006).

Immunohistochemistry and microscopy
TWK-1::GFP was detected in dissected and fixed (Finney and Ruvkun 1990) gonads using mouse monoclonal anti-GFP antibodies (Abcam; 1:500). Rhodamine phalloidin (Sigma; 1:200) was used to detect actin. MSP and RME-2 were detected in dissected gonads as described (Kosinski et al. 2005), using mouse monoclonal anti-MSP antibody 4AS (1:300) and rabbit anti–RME-2 antibody (Grant and Hirsh 1999; kindly provided by B. Grant, Rutgers University, 1:50). Secondary antibodies were Alexa 488-conjugated goat antirabbit (Life Technologies, 1:500) and Alexa 488-conjugated goat antimouse (Jackson ImmunoResearch, 1:500), and Alexa 488-conjugated goat antirabbit (Life Technologies, 1:500). Acridine orange was used to stain apoptotic germ cells (Gumienny et al. 1999) and necrotic gametes in ced-3 (n717) and ced-4(n1162) mutant backgrounds. Adult hermaphrodites (18–24 hr post-L4) were cultured for 1 hr on 8 ml of OP50–1–seeded NGM to which 0.5 ml of M9 containing acridine orange (20 μg/ml) was added. After staining, worms were transferred to fresh medium for 1 hr and then analyzed by fluorescence microscopy. Prior to microscopy, worms were kept in the dark. DIC and fluorescent images were acquired on a Zeiss motorized Axioplan 2 microscope with either 40× Plan-NeoFluar (N.A. 1.3) or 63× Plan-Apochromat (N.A. 1.4) objective lenses using an AxioCam MRm camera and AxioVision software.

Results
kin-1 is required in the gonadal sheath cells for oocyte meiotic maturation
Ga-ACY-4 signaling is required in the gonadal sheath cells for oocyte meiotic maturation (Govindan et al. 2009). cAMP-
dependent PKA is a canonical downstream effector of Goalpha-adenylate cyclase signaling. In C. elegans, the catalytic and regulatory subunits of PKA are encoded by kin-1 and kin-2, respectively (Gross et al. 1990; Lu et al. 1990). The KIN-2 regulatory subunit functions by binding and inactivating the catalytic subunit in the absence of cAMP; binding of cAMP to KIN-2 alleviates its inhibition of KIN-1 activity (reviewed by Taylor et al. 1990). In a female genetic background, a kin-2 reduction-of-function (rf) mutation or kin-(2(RNAi) derepress oocyte meiotic maturation and MAPK activation in oocytes in the absence of sperm (Govindan et al. 2006). Further, kin-2 (ce179rf) suppresses the sterility caused by a strong loss-of-function mutation in acy-4 (Govindan et al. 2009). kin-2 (RNAi) experiments using the rrf-1 genetic background, which is sensitive to RNAi in the germ line but resistant in the somatic gonad (Sijen et al. 2001; Kumsta and Hansen 2012), suggested that kin-2 functions in the soma to inhibit meiotic maturation in the absence of sperm (Govindan et al. 2006). Because cyclic nucleotides have been suggested to move through gap junctions to regulate PKA activity in oocytes in mammalian systems (Sela-Abramovich et al. 2006; Norris et al. 2009), we sought to assess directly the involvement and focus of action of the kinase in meiotic maturation in C. elegans using genetic mosaic analysis.

We conducted genetic mosaic analysis using the kin-1 (ok338) deletion allele. The kin-1(ok338) allele deletes conserved subregions III–VI of the catalytic domain (Hanks et al. 1988) and introduces a frameshift before subregion VII, which generates multiple stop codons in all known isoforms. kin-1(ok338) exhibits a larval lethal phenotype apparently identical to that caused by an absence of Goalpha in the strong loss-of-function gsa-1(pk75) allele (Korswagen et al. 1997). We rescued kin-1(ok338) lethality using an extrachromosomal array bearing a wild-type copy of the gene linked to a cell-autonomous nuclear GFP marker. Approximately 98% of kin-1(ok338); tnEx109[kin-1(+);sur-5:GFP] animals reaching the L4 stage are fertile (n = 595) and segregate GFP-expressing fertile animals, non-GFP-expressing arrested larvae, and genetic mosaics. Loss of kin-1(+) function in germ-line lineages (P3 and P4; Figure 1C) did not affect viability or fertility. Thus, kin-1 is not required in the germ line for meiotic maturation. The progeny of germline mosaic animals (n = 26) arrested as L1 larvae, recapitulating the kin-1(ok338) zygotic phenotype.

To assess whether kin-1 might function in the germ line to inhibit meiotic maturation, as in vertebrate systems (Maller and Krebs 1977; Mehlmann 2005), we asked whether oocytes continue to undergo meiotic maturation and ovulation upon the depletion of sperm through self-fertilization in germline mosaics. We observed that kin-1(ok338) germline mosaics produced a total of 10 ± 14 unfertilized oocytes (n = 15) as compared to 14 ± 21 unfertilized oocytes (n = 18) for nonmosaic siblings (P > 0.5, Student’s t-test). Thus, kin-1(+) function is dispensable in the germ line both for meiotic maturation and also for its inhibition when sperm are absent or limiting.

Next, we sought array losses in the MS lineage, which gives rise to the somatic cells of the gonad. Array losses within the MS lineage cause sterility within a gonad arm (Figure 1B). The fertility of a specific gonad arm (anterior or posterior) depends on the kin-1 genotype of that gonad arm. For the animal to be completely sterile, independent losses were needed affecting both gonad arms (Figure 1C). kin-1(+; somatic) somatic-loss mosaics exhibit sterility because oocytes fail to undergo meiotic maturation, ovulation, and fertilization, phenocopying acy-4(lf) mutants (Govindan et al. 2009). Complex losses found within the sheath-spermathecal lineages suggest that kin-1 is needed in the gonadal sheath cells, not in the spermatheca, for meiotic maturation (Figure S2).

Genetic and molecular identification of suppressor of acy-4(lf) sterility mutations

To identify new regulators of oocyte meiotic maturation that might function downstream of somatic Goalpha–ACY-4–PKA signaling, we conducted a forward genetic screen for mutations that suppress the sterility of acy-4(ok1806) mutants (Figure 2A; Table 1). We identified 63 suppressors from ~20,000 mutagenized haploid genomes and an additional three suppressors from a related screen (see Materials and Methods). We refer to these suppressors as Sacy mutants (suppressor of acy-4(lf) mutant sterility). All isolated Sacy mutations are recessive. The brood sizes of the Sacy mutants in the acy-4(lf) background are variable and smaller than those of the wild type (~10–90 progeny vs. 339 ± 31 (n = 37); Figure 2B). Linkage analysis and complementation testing indicate that the 66 suppressors represent at least 17 genes (Table 1).

Molecular identification of Sacy genes, an overview

We used a combination of positional cloning and whole-genome sequencing to identify 8 of 17 Sacy loci identified in our screen (Figures 3 and 4; Table 1). Based on their molecular identities, strong loss-of-function phenotypes, and likely modes of action, there appear to be several pathways that function cumulatively to affect the regulation of meiotic maturation downstream of PKA. Among these pathways, sacy-1 acts as a strong negative regulator of meiotic maturation and provides a mechanistic connection to the fundamental germline processes of sex determination and gamete maintenance. We will first describe sacy-1, followed by the nonessential Sacy loci.

SACY-1 DEAD-box helicase functions in the germ line downstream of KIN-1 and is a negative regulator of meiotic maturation in the absence of sperm

Whole-genome sequencing identified three noncomplementing alleles (tn1385, tn1391, and tn1440) mapping to the center of LGI as missense mutations in H27M09.1, which encodes a DEAD-box helicase related to Drosophila Abstrakt and human DDX41 (Figure 4). We confirmed the missense mutations in H27M09.1 using Sanger sequencing (Figure 4) and named H27M09.1 sacy-1. Whole-genome sequencing also identified independent tightly linked missense mutations

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acy-4(lf) sterility, we conducted RNAi experiments in the somatic gonad RNAi-deficient rrf-1(pk1417) genetic background. sacy-1(RNAi) using rrf-1(pk1417); acy-4(ok1806); tnEx37 suppresses acy-4(lf) sterility in the F1 generation (Figure 5A). Thus, reduction of sacy-1 function in the germ line suppresses acy-4(lf) sterility. We used recombineering and biolistic transformation to generate an N-terminal GFP::SACY-1 fusion within the fosmid context (Figure 4C) and assessed its rescuing activity (Figure 5, C and D). The GFP::SACY-1 fusion rescues acy-4(lf) suppression (Figure 5C). We observed that GFP::SACY-1 is expressed in most or all germ-line and somatic cells and localizes to the nucleus and cytoplasm (Figure 4C).

Structural and bioinformatic studies show that DEAD-box RNA helicases contain a highly conserved core helicase domain containing ATP and RNA binding sites (Linder and Jankowsky 2011). Each of the three missense sacy-1 alleles changes a highly conserved glycine residue to an arginine residue in either the DEAD-box or helicase domains (Figure 4B). tn1385 results in a G533R substitution in motif VI, which contributes to ATP binding and hydrolysis (Linder 2006). The R534 residue adjacent to the tn1385 mutation site interacts with the γ-phosphate of ATP (Schutz et al. 2010). tn1440 results in G331R in motif Ib, which contributes to RNA binding (Schutz et al. 2010). tn1391 results in G473R in a region between motifs IV and V, which is surface exposed in the crystal structure of the human ortholog DDX41 (Schutz et al. 2010).

When separated from the acy-4(Ok1806) mutation, each sacy-1 missense allele was comparably viable and fertile as the wild type. For example, sacy-1(tn1385) has a brood size of 349 ± 77 (n = 35), compared to a wild-type brood size of 339 ± 31 (n = 37, P value = 0.49). When placed in trans to the deficiency qDf16, which deletes sacy-1, each missense allele was viable and fertile. Interestingly, when the sacy-1 missense alleles were treated with sacy-1(RNAi) by feeding, we observed increased levels of embryonic lethality compared to the wild type (Figure 5B). Since the wild type is relatively impervious to sacy-1(RNAi) (Figure 5B; ~10% lethality with the full-length dsRNA trigger), an off-target RNAi effect seems an insufficient explanation for the experimental observation. Further, sacy-1 dsRNA triggers that target different portions of the cDNA also cause high levels of embryonic lethality specifically in the sacy-1 missense alleles (Figure 5B). BLAST searches indicate that these dsRNA triggers are highly specific to sacy-1. It seems unlikely that the sacy-1 missense alleles have an enhanced sensitivity to RNAi because even rrf-3(pk1426) mutants, which exhibit enhanced RNAi responses (Sijen et al. 2001), only exhibit a moderate increase in embryonic lethality after sacy-1(RNAi) (Figure 5B). While we are unable to completely exclude the possibility that off-target effects might contribute to the embryonic lethality observed following sacy-1(RNAi), an analysis of the strong loss-of-function sacy-1(tm5503) allele (Figure 4A) shows it to be an essential gene with a zygotic sterile phenotype (see below). Thus, we conclude...
that the sacy-1 mutant alleles isolated as acy-4(If) suppressors are reduction-of-function alleles.

To determine whether sacy-1 is a negative regulator of oocyte meiotic maturation in the absence of sperm, we feminized the strongest of the missense alleles, tn1385, by making double mutants with mutations affecting germline sex determination [e.g., fog-1(e2121), fog-2(oz40), fog-3(q470), and fem-3(e1996)]. In all cases we observed increased numbers of oocytes in the uterus (Figure S3B), indicating that sacy-1 is a negative regulator of meiotic maturation. To test whether sacy-1 functions downstream of Gαs-ACY-4–PKA signaling, we removed kin-1(+) function from the somatic gonad in genetic mosaics generated in a sacy-1(tn1385) genetic background. We observed that all genetic mosaics that lost kin-1(+) function in somatic gonadal lineages were fertile (Table 2). Thus, sacy-1 functions in the germ line downstream of Gαs-ACY-4–PKA signaling. Additional sacy-1 mutant phenotypes are now described.

sacy-1 functions in the hermaphrodite sperm-to-oocyte switch

We serendipitously found that sacy-1(tn1385) strongly suppresses the self-sterility caused by fog-2(oz40) (Table 3), a strong loss-of-function mutation (Y29stop) in fog-2, which
Figure 4  sacy-1 mutations suppress acy-4(lf) sterility. (A) sacy-1 alleles isolated as acy-4(lf) suppressor mutations are shown. The sacy-1(tm5503) deletion is underlined. (B) C. elegans SACY-1 is highly conserved. ClustalW alignment of SACY-1, Drosophila Abstrakt (Iriion and Leptin 1999; Schmucker et al. 2000), and human DDX41. SACY-1 and Abstrakt share 54% (323/603) identity and 70% (424/603) similarity; SACY-1 and DDX41 share 60% (318/533) identity and 75% (401/533) similarity. sacy-1 mutant alleles (triangles) and the DEAD box (boxed in red) are indicated. Ce_SACY-1 (NP_491962.1), Dm_Abstrakt (NP_524220.1), and Hs_DDX41 (NP_057306.2) were used for the analysis. Conserved domains [DEAD-box domain (DEADc), helicase domain (HELICc), and zinc finger domain (ZnF)] and motifs (Q, I, Ia, Ib, II, III, IV, V, and VI) are indicated (Henn et al. 2012). (C) Rescuing GFP::SACY-1 fusion (tnEx159) is broadly expressed in the nuclei and cytoplasm of most or all cells. Embryos (e), spermatheca (sp), oocytes (1, 2, and 3). Bar, 50 μm.
encodes an F-box protein required for spermatogenesis in \textit{C. elegans} hermaphrodites but not males (Schedl and Kimble 1988; Clifford et al. 2000; Nayak et al. 2005). Suppression of \textit{fog-2(oz40)} self-sterility required that the \textit{sacy-1(tn1385)} reduction-of-function mutation be present both maternally and zygotically (Table 3; \textit{mrfzrf}). \textit{sacy-1(tn1385)} also strongly suppressed the self-sterility of \textit{fog-2(q71)} (Table 3), another strong loss-of-function \textit{fog-2} mutation (W148stop; Clifford et al. 2000). The two weaker \textit{sacy-1} missense alleles, \textit{tn1391} and \textit{tn1440}, also suppressed the self-sterility of \textit{fog-2(oz40)} and \textit{fog-2(q71)} to varying degrees (Table 3). The suppression of \textit{fog-2} sterility appears to involve a reinstatement of the hermaphrodite sperm-to-oocyte switch in the absence of \textit{fog-2} function because the suppressed animals appear indistinguishable from wild-type hermaphrodites, and they exclusively produce oocytes in the adult stage. The suppression of \textit{fog-2(lf)} self-sterility by the \textit{sacy-1} missense alleles appears to reflect a loss of \textit{sacy-1} function because \textit{sacy-1(RNAi)} also suppresses \textit{fog-2(oz40)} sterility (Table 3). \textit{sacy-1(RNAi)} in the \textit{rrf-1(pk1417); fog-2(oz40)} background, in which the RNAi response is compromised in the somatic gonad but not the germ line, results in efficient suppression of \textit{fog-2} self-sterility (Table 3), suggesting that \textit{sacy-1} functions in the germ line as a component of the sperm-to-oocyte switch.

Genetic analysis has identified many of the key genes that control sex determination in \textit{C. elegans} (Figure 6; reviewed by Ellis and Schedl 2007; Kimble and Crittenden 2007). Therefore we assessed whether \textit{sacy-1(tm1385)} suppresses the self-sterility of strong loss-of-function mutations in \textit{fem-3, fog-1, \textit{fog-2}}.
and fog-3. In these experiments we ensured that the sacy-1 (tm1385) reduction-of-function mutation was both maternally and zygotically homozygous, but in no case did we find evidence for suppression (Table 3). Biochemical studies established that FOG-2 can form a ternary complex with the KH-domain protein GLD-1 and the 3'-UTR of tra-2 (Clifford et al. 2000), consistent with the proposal that GLD-1 binds the 3'-UTR to mediate translational repression as a key element of the tra-2::gfp extrachromosomal array. The genotype refers to the somatic cells of a gonad arm in the genetic mosaics.

Table 2 sacy-1, spr-5, and twk-1 are epistatic to kin-1

| Genotype | Fertile/total gonad arms | Number of animals screened |
|----------|--------------------------|----------------------------|
| kin-1(ok338) | 0/13 | 1822 |
| sacy-1(tn1385) | 10/10 | 1105 |
| sacy-5(by134) | 12/12 | 1600 |
| twk-1(tm1397) | 11/11 | 1297 |

* Epistasis tests were conducted in the respective double mutant backgrounds by analyzing genetic mosaics with losses of the kin-1(+)-rescuing array in the somatic gonad lineage. Genetic mosaics were sought in animals bearing the tnEx109(kin-1(+) sur-5::gfp) extrachromosomal array. The genotype refers to the somatic cells of a gonad arm in the genetic mosaics.

sterility of tra-2(e2020) mutants (Table 3). Taken together, these results suggest that sacy-1 functions in the hermaphrodite sperm-to-oocyte switch upstream of tra-2.

sacy-1 prevents necrotic cell death of gametes

The analysis thus far relied on weak reduction-of-function sacy-1 alleles recovered as sacy-4(If) suppressors. To address whether sacy-1 plays essential roles during oogenesis, we analyzed the sacy-1(tm5503) deletion allele generated by S. Mitani. sacy-1(tm5503) results from a 619-bp deletion that removes the entire second and third exons and a portion of the fourth (Figure 4A). Potential unspliced or alternatively spliced messages are either predicted to be out of frame or to lack conserved regions of the DEAD-box domain. sacy-1(tm5503) homozygous hermaphrodites produced from heterozygous parents develop to adulthood but are sterile. The gfp::sacy-1 transgene fully rescues the sterility (Figure 5D). sacy-1(tm5503) adult hermaphrodites do not produce fertilized embryos (Table 4); instead they contain oocytes and sperm that become vacuolated and appear to degenerate (Figure 7). We conducted a time-course analysis to examine the onset and progression of gamete degeneration. By DIC microscopy, we observed a mixture of small and large vacuoles in sperm and oocytes on day 1 of adulthood. With time these vacuoles appeared to grow in size or fuse (Figure 8B and Figure S4). Ultimately, we observed the gonad arms to contain gamete remnants in which Brownian

Table 3 sacy-1 functions in the hermaphrodite sperm-to-oocyte switch

| Genotype | Maternal | Zygotic | Fertility (%) | Number scored |
|----------|----------|---------|---------------|---------------|
| sacy-1 RNAi | RNAi | RNAi | 49 | 792 |
| sacy-1 RNAi | RNAi | RNAi | 71 | 753 |
| sacy-1 RNAi | RNAi | RNAi | 0 | 74 |
| sacy-1 RNAi | RNAi | RNAi | 0 | 290 |
| sacy-1 RNAi | RNAi | RNAi | 0 | 342 |
| sacy-1 RNAi | RNAi | RNAi | 0 | 348 |

* Fertility was scored on a per-animal basis, except for the RNAi experiments, in which case fertility was scored on a per-gonad arm basis because sacy-1(RNAi) in fog-2(oz40) or rrf-1(pk1417); fog-2(oz40) backgrounds caused a gamete degeneration phenotype in 3–4% of gonad arms. This phenotype was the same as that caused by sacy-1(tm5503).
* Number scored refers to gonad arms for RNAi experiments and animals for the remainder.
* Brood size of fertile animals was 194 ± 88 (n = 40).
* Brood size of fertile animals was 172 ± 94 (n = 42).
* Brood size of fertile animals was 148 ± 71 (n = 40).
motion was observed to occur in residual cytoplasm. We also observed a similar sperm degeneration phenotype in sacy-1(tm5503) adult males (Figure 7A), which were never observed to sire cross-progeny. The somatic gonad appeared to develop normally in sacy-1(tm5503) hermaphrodites and males. We sought genetic mosaics using the sacy-1(tm5503); unc-119(ed3); tnEx159[gfp::sacy-1 unc-119(+)] strain in which the rescuing GFP::SACY-1 fusion is expressed in most or all germline and somatic cells and serves as a cell-autonomous marker for mosaic analysis. The unc-119(ed3) genetic background was utilized as a marker for the AB lineage so as to identify rare non-Unc mosaics showing the sacy-1(tm5503) gamete degeneration phenotype. We found four genetic mosaics with P1 losses, a single mosaic with a P3 loss (this animal had an independent loss within the C lineage), and two mosaics with P4 losses (Figure 7B; n > 40,000). This result indicates that sacy-1(+) function is required in the germ line to prevent gamete degeneration.

To address whether gamete degeneration in sacy-1(tm5503) is dependent on the apoptotic pathway, we examined double mutants between sacy-1(tm5503) and ced-3(n717) or ced-4(n1162). Both ced-3 and ced-4 are required for apoptosis (Ellis and Horvitz 1986); ced-3 encodes a caspase (Yuan et al. 1993) and ced-4 encodes an Apaf-1–like protein (Yuan and Horvitz 1992). Using DIC microscopy, we observed gamete degeneration in 99% of sacy-1(tm5503); ced-3(n717) adult hermaphrodite gonad arms (n = 150) and in all sacy-1(tm5503); ced-4(n1162) gonad arms (n = 108). Further, we used acridine orange staining to examine early degenerating gametes. We observed that acridine orange stains early degenerating gametes in the proximal gonad of sacy-1(tm5503); ced-3(n717) double mutants, as well as sacy-1(tm5503) single mutant hermaphrodites (Figure 9). Apparently, the sheath cells might engulf and acidify some of these degenerating gametes. These results indicate that gamete degeneration in sacy-1(tm5503) is independent of the chief apoptotic effectors.

As a test of whether gamete degeneration in sacy-1(tm5503) mutant hermaphrodites involves necrotic cell death, we examined a sacy-1(tm5503); unc-68(e540) double mutant. unc-68 encodes the ryanodine receptor (Maryon et al. 1996) and an unc-68 mutation was found to reduce the penetrance of necrotic cell death (Xu et al. 2001). We found that the unc-68(e540) mutation decreased the penetrance of gamete degeneration; whereas all sacy-1(tm5503) gonad arms failed to produce fertilized embryos, 20% of sacy-1(tm5503); unc-68(e540) gonad arms produced fertilized embryos (Table 4). The fertilized embryos in sacy-1(tm5503); unc-68(e540) animals failed to hatch. As controls, we tested mutations in unc-24, unc-32, and unc-33, but found that none were as effective as unc-68(e540) in ameliorating sacy-1(tm5503) gamete degeneration (Table 4). We did observe that 8% of gonad arms were fertile in sacy-1(tm5503); unc-33(tm407) animals (Table 4). unc-33 encodes a microtubule-binding CRMP protein that is exclusively expressed in neurons and is required for normal axon guidance and elongation (Maniar et al. 2012). The slight reduction of gamete necrosis in sacy-1(tm5503); unc-33(tm407) animals might be a secondary physiological consequence of their slow growth.

**Germline feminization delays the onset of gamete degeneration and reveals sacy-1 as a strong negative regulator of meiotic maturation**

We next investigated the genetic requirements for gamete degeneration in sacy-1(tm5503) hermaphrodites. Feminization with strong loss-of-function mutations in the sex determination pathway, fog-2(oz40), fem-3(e1996), fog-1(e2121), or fog-3(q470), delayed the time of onset and the severity of oocyte degeneration in sacy-1(tm5503) females (Figure 8 and Figure S3; S. Kim and D. Greenstein, unpublished results). By contrast, masculinization of the germ line using a gain-of-function mutation in fem-3 (Barton et al. 1987) did not suppress gamete degeneration; we observed vacuolated and morphologically abnormal sperm in sacy-1(tm5503); fem-3(q20gf) animals (Figure S5). Upon mating, sacy-1(tm5503) females produce embryos that arrest without properly undergoing morphogenesis and fail to hatch. We did not explore the basis for this embryonic lethality further. We did not observe mating to wild-type males to overtly increase the penetrance or severity of oocyte degeneration. Possibly, the presence of mutant sperm in the gonad arm might potentiate oocyte degeneration. While the physiological basis for the
delayed onset of oocyte degeneration upon germline feminization is unclear, this phenomenon proved useful in that it enabled us to examine oocytes and embryos produced by $sacy-1(tm5503)$ females. In all female backgrounds tested, we observed oocytes in $sacy-1(tm5503)$ females to undergo meiotic maturation and ovulation at apparently high rates; the uterus filled with unfertilized oocytes (Figure 8A and Figure S3C). We also observed apparently defective ovulation in $sacy-1(tm5503)$ females such that the gonad arms often contained endomitotic oocytes. We confirmed that MSP was undetectable in $sacy-1(tm5503)$ females as expected (Figure S6). Thus, $sacy-1$ is a strong negative regulator of oocyte meiotic maturation in the absence of sperm.

Since only reduction-of-function $sacy-1$ alleles were recovered as $acy-4(lf)$ suppressors, we wished to determine the genetic behavior of a loss-of-function $sacy-1$ allele. Thus, we conducted genetic epistasis analysis between $acy-4$ and $sacy-1$ using strong loss-of-function alleles of both genes. In a hermaphrodite background, we observed gamete degeneration in all $sacy-1(tm5503)$; $acy-4(ok1806)$ animals

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**Figure 7** $sacy-1$ is required for gamete maintenance. (A) $sacy-1(tm5503)$ adult hermaphrodites and males produce gametes that degenerate. Embryos (e), spermatheca (sp), oocytes (−1, −2, and −3), vulva (vu), sperm (s). (B) $sacy-1$ functions in the germ line to prevent gamete necrosis. GFP::SACY-1 fusion rescues $sacy-1(tm5503)$ sterility (top). A genetic mosaic that lost GFP::SACY-1 in the primordial germ cell P4 exhibits gamete necrosis and is sterile (bottom). (C) $sacy-1(tm5503)$ hermaphrodites produce male and female gametes that ultimately degenerate. The yolk receptor RME-2 and MSP were used for markers of oocyte and sperm fates, respectively. Proximal is to the left. Bars, 50 μm.
examined \((n = 94)\). Therefore gamete degeneration is independent of \textit{acy-4} signaling. We therefore employed germline-feminizing mutations to overcome the gamete degeneration phenotype in conducting epistasis experiments between \textit{sacy-1} and \textit{acy-4}. In a \textit{sacy-1(tm5503) fog-3(q470); acy-4(ok1806)} background, we observed oocyte meiotic maturation to occur constitutively; however, ovulation typically failed and endomitotic oocytes accumulated in the gonad arm (Figure S7). All examined \textit{sacy-1(tm5503) fog-3(q470); acy-4(ok1806)} gonad arms contained endomitotic oocytes \((n = 42)\), in contrast
to fog-3(q470); acy-4(ok1806) gonad arms, which exclusively contained oocytes arrested in diakinesis \( (n = 43) \). Thus, sacy-1(\( \text{lfl} \)) is epistatic to acy-4(\( \text{lfl} \)) for meiotic maturation, as was also observed for the reduction-of-function alleles.

Since sacy-1 is a strong negative regulator of oocyte meiotic maturation, we investigated epistasis with oma-1 and oma-2, which encode TIS-11 zinc-finger proteins that are redundantly required for oocyte meiotic maturation (Detwiler et al. 2001). In a hermaphrodite background, we observed gamete degeneration in sacy-1(tm5503); oma-1(zu405te33); oma-2(te51) animals. By contrast, in a sacy-1(tm5503) fog-3(q470); oma-1(zu405te33); oma-2(te51) females, we observed that oocyte degeneration was markedly delayed. We observed that diakinesis-stage oocytes failed to undergo meiotic maturation and ovulation and accumulated in the gonad arms of all quadruple mutant female animals examined \( (n = 30) \). Thus, oma-1 and oma-2 appear to function downstream or in parallel to sacy-1 in the regulation of meiotic maturation.

**Multiple nonessential Sacy genes mediate the somatic control of oocyte meiotic maturation**

In contrast to sacy-1, which is an essential gene, we now describe seven nonessential Sacy loci (pde-6, spr-1–5, and twk-1) that affect the regulation of meiotic maturation by somatic \( \alpha_{s_3} \)-adenylate cyclase–PKA signaling.

**PDE-6 phosphodiesterase is a negative regulator of meiotic progression:** We isolated pde-6 mutations in a related screen for mutations that suppress sterility following gsa-1 (RNAi) yet exhibit normal RNAi responses and are viable and fertile. From this screen, only mutations from a single complementation group, represented by \( \text{tn}1237, \text{tn}1242, \) and \( \text{tn}1336 \), suppress acy-4(\( \text{lfl} \)) sterility. One of these mutations, \( \text{tn}1237 \), was tested and also found to suppress the sterility caused by mosaic loss of gsa-1 activity in the somatic gonad, as expected (Table S5A). A combination of whole-genome and Sanger sequencing identified independent nonsense mutations in each pde-6 allele (Figure 3). We also found that the \( \text{pde}-6(\text{ok}3410) \) deletion allele suppresses acy-4(ok1806) sterility and fails to complement \( \text{tn}1237 \) for suppression of acy-4(\( \text{lfl} \)) sterility. pde-6 encodes a phosphodiesterase and the mutant alleles introduce stop codons prior to or within the coding sequence for the PDE domain (Figure 3), suggesting they represent strong loss-of-function alleles. The likely human ortholog of PDE-6 is PDE8 (Figure S8), the high-affinity cAMP-specific phosphodiesterase, which specifically lowers cAMP levels via phosphodiester bond hydrolysis (Fisher et al. 1998; Soderling et al. 1998). The chromatin localization of the AIR-2 Aurora B kinase in proximal oocytes is a marker for graded MSP responses (Schumacher et al. 1998; Govindan et al. 2009). In an acy-4(\( + \)) background, the pde-6(\( \text{tn}1237 \)) mutation extends AIR-2::GFP chromatin localization distally (Table S5B), suggesting that an enhancement in \( \alpha_{s_3} \)-ACY-4–PKA signaling in gonadal sheath cells results in a heightened MSP response in oocytes.

**Mutations of multiple CoREST components suppress acy-4 (lfl) sterility:** Three noncomplementing alleles, \( \text{tn}1378, \text{tn}1379, \) and \( \text{tn}1394 \), map to the right end of LGI and define
the gene spr-5 (Figure 3). Using tn1394, we genetically mapped the acy-4(If) suppression to the interval between SNPs haw14129 and haw14136, which contains two loci, spr-5 and Y48G10A.6. Previously isolated spr-5 mutations, the strong loss-of-function allele, by134(Y284stop) (Eimer et al. 2002; Nottke et al. 2011), and ar197(A665T) (Jarriault and Greenwald 2002), also suppress acy-4(ok1806) sterility and fail to complement tn1394 for suppression. Consistent with this gene identification, injection of spr-5 dsRNA into acy-4(ok1806); tnEx37[acy-4(+) sur-5::gfp]/spr-5[RNAi] experiments conducted in the somatic gonad RNAi-deficient rrf-1(pk1417); acy-4(ok1806) background indicate that spr-5 depletion in the germ line results in meiotic maturation in the absence of acy-4 (Figure S9). spr-5 by itself does not appear to function as a negative regulator of meiotic maturation in the absence of sperm because oocytes stock in spr-5(by134); fog-2(oz40) females (Table 1).

spr-5 and other spr genes were originally identified as suppressors of the sel-12 presenilin mutant egg-laying defect (Wen et al. 2000; Eimer et al. 2002; Jarriault and Greenwald 2002; Lakowski et al. 2003; reviewed by Lakowski et al. 2006). The spr genes encode multiple chromatin-modifying components that might constitute a C. elegans CoREST-like complex, similar to mammalian CoREST (corepressor for element-1—silencing transcription factor; reviewed by Lakowski et al. 2006). spr-5 encodes an H3K4me2 demethylase that is thought to contribute to transcriptional repression by remodeling chromatin structure (Eimer et al. 2002; Jarriault and Greenwald 2002; Katz et al. 2009). spr-5 mutations have been shown to confer a mortal germline phenotype after more than ~20 generations (Katz et al. 2009) and to exhibit modest defects in meiotic DNA double-strand break repair (Nottke et al. 2011). Suppression of acy-4(If) sterility is observed in the first generation in which spr-5 mutations become homozygous and is efficient in subsequent generations. We have not examined germline mortality in these strains in detail, but did observe declines in fecundity after many generations, consistent with prior findings. spr-5 mutations bypass the sel-12 presenilin requirement for egg laying by derepressing hop-1 presenilin expression (Eimer et al. 2002). Therefore, we considered the possibility that spr-5 mutations might bypass the requirement of acy-4 for meiotic maturation via derepression of other adenylate cyclase(s) in the gonadal sheath cells. We generated C-terminal GFP fusions for ACY-1, ACY-2, and ACY-3 using fosmid recombineering and compared their expression in the wild type and spr-5 mutants. We observed apparently identical expression patterns in the wild type and spr-5 mutants and in no case did we observe expression of an adenylyl cyclase other than acy-4 in the gonadal sheath cells (S. Kim and D. Greenstein, unpublished results). As a more direct test, we conducted genetic mosaic analysis of kin-1 in an spr-5 mutant background. We observed that loss of kin-1(+) activity in the somatic gonad in the spr-5(by134) genetic background resulted in fertility, in contrast to the wild-type background (Table 2). Thus, spr-5 functions downstream or in parallel to kin-1, consistent with a function in the germ line. Prior work identified >100 genes upregulated in spr-5 mutant gonads (Nottke et al. 2011). We tested whether RNAi of any of these candidate genes might restore acy-4(If) sterility to spr-5(by134); acy-4(ok1806) mutants, but in no case was such a result obtained. Possibly spr-5 might regulate genes not identified by the microarray analysis or multiple pathways might contribute to the suppression of acy-4(If) sterility.

During this analysis, we observed that unlike the wild type, acy-4(ok1806) worms exhibit a growth defect on standard nematode growth medium with the bacterial strain HT115(DE3) as a food source, as opposed to OP50-1. Under these conditions, acy-4(ok1806) animals exhibit larval lethality or arrest, or they grow slowly and appear grossly unhealthy (Figure S10). This phenotype is rescued by an acy-4(+) extrachromosomal array (tnEx57), indicating that an absence of acy-4 activity prevents normal growth on the HT115 (DE3) food source. This is an unexpected result because HT115(DE3) is a high-quality food source for C. elegans (Brooks et al. 2009; Y. You, personal communication). We found that spr-5(by134) also suppresses the acy-4(ok1806) HT115 growth defect: double mutant animals are viable and fertile with HT115 as a food source, as they are on standard OP50 bacterial strains. While the basis for this unusual phenotype remains to be determined, we nonetheless screened all Sacy mutants and found two additional loci that suppress the HT115 growth defect. Genetic mapping and DNA sequencing established that these two Sacy genes are spr-2 and spr-4 (Figure 3; Table 1). We therefore tested independently isolated alleles of spr loci and found that spr-2 (ar211) and spr-2(tm4802) suppress acy-4(ok1806) sterility and the HT115 growth defect. We did, however, observe that spr-2(ar199) failed to suppress acy-4(If) sterility. Interestingly, spr-2(ar199) specifically eliminates the expression of SPR-2 in somatic cells after the 50-cell stage (A. Gontijo and B. Lakowski, personal communication), consistent with a role for CoREST function in the germ line downstream of kin-1. Similarly, we observed that spr-4(by105) suppresses acy-4(ok1806) sterility and the HT115 growth defect. We did not isolate spr-3 mutant alleles in our screen, yet both spr-3 (ok2525) and spr-3(by108) suppress acy-4(ok1806) sterility. Neither of these spr-3 alleles suppresses the HT115 growth defect. We constructed acy-4(ok1806) spr-1(g734) double mutants and observed that they were fertile and suppressed for the HT115 growth defect (Figure S10). These results suggest that a CoREST-like complex has a function in the germ line that participates in the regulation of oocyte meiotic maturation by the somatic gonad and sperm signaling. Further, the observation that mutations in spr-1, spr-2, spr-4, and spr-5 suppress the acy-4(If) HT115 growth defect suggests that CoREST-like complex genes and acy-4 might coordinately function in additional processes besides oocyte meiotic maturation.
A two-pore domain potassium (TWIK) channel functions in sheath cells downstream of KIN-1 to regulate meiotic maturation: Whole-genome sequencing identified three noncomplementing alleles (tn1397, tn1398, and tn1403) mapping to the center of LGI as nonsense mutations in twk-1, which encodes a TWIK channel (Figure 3). tn1398 and tn1403 introduce stop codons after the fourth transmembrane domain (W330stop and E272stop, respectively) and tn1397 introduces a stop codon after the second transmembrane domain (Figure 3; W177stop). The crystal structures of TWIK channels suggest that all four transmembrane helices likely participate in the formation of a functional channel complex (Brohawn et al. 2012; Miller and Long 2012). tn1397 is therefore a strong loss-of-function allele. tn1397, but not tn1398 or tn1403, exhibits an adult-onset paralysed uncoordinated (Unc) phenotype in a wild-type genetic background. Thus, tn1398 and tn1403 must reduce but not eliminate twk-1 function. Interestingly, twk-1(tn1397); acy-4(ok1806) animals do not exhibit an adult-onset Unc phenotype. Thus, twk-1 mutant alleles are recessive suppressors of acy-4(lf) sterility, and acy-4(ok1806) is a recessive suppressor of the twk-1(tn1397) movement defect. To test whether twk-1 mutations are causal for the suppression of acy-4(lf) sterility and the adult-onset Unc phenotype, we conducted rescue tests by introducing a twk-1 fomisd clone, WRM0616aE06, into twk-1(tn1397); acy-4(ok1806)/+ and twk-1(tn1397); acy-4(ok1806) genetic backgrounds, respectively. twk-1(tn1397); acy-4(ok1806) animals bearing the twk-1(+) extrachromosomal array were sterile and exhibited the acy-4(lf) defect in meiotic maturation (Table S6A). Further, we also observed rescue of the twk-1(tn1397) Unc phenotype (Table S6B). The suppression of the twk-1(tn1397) Unc phenotype by acy-4(lf) is partially affected by spr-5(+) function because 23% (n = 95) of twk-1(tn1397) spr-5(by134); acy-4(ok1806) animals exhibit the adult-onset Unc phenotype as compared to 0% of twk-1(tn1397); acy-4(ok1806) adults (n = 93). Thus, acy-4, spr-5, and twk-1 genetically interact in a separate biological context.

In C. elegans, high-copy extrachromosomal arrays generated by microinjection, as done for twk-1, are typically silenced in the germ line (Kelly et al. 1997). To test more definitively whether acy-4(ok1806) sterility requires twk-1 (+) function in the somatic gonad, we conducted genetic mosaic analysis of acy-4(lf) suppression. We sought fertile genetic mosaics among twk-1(tn1397); acy-4(ok1806); tnEx180[twk-1(+) sur-5::gfp] animals, produced by acy-4(ok1806)/+ heterozygous parents using nTL1[qls51] as a dominantly marked balancer chromosome for acy-4(ok1806). Two genetic mosaics with losses in the EMS founder cell, which is a precursor to the somatic gonad (Figure 1C), were fertile in both gonad arms. These genetic mosaics produced GFP-containing progeny, indicating that their germ lines were twk-1(+). A third genetic mosaic animal resulted from complex losses within the Z1 lineage (Figure S11). This animal was fertile in the anterior gonad arm, but was sterile in the posterior gonad arm. All gonadal sheath cells in the anterior gonad arm of this mosaic were twk-1(tn1397) mutant, but the germline and several anterior spermathecal cells were twk-1(+). A fourth genetic mosaic, resulting from a loss in the Z4 somatic gonadal precursor cell (and some cells within the C lineage; see Figure 1C), was fertile in the posterior gonad arm, but not the anterior gonad arm. The germ line of this animal was also twk-1(+). We conclude that twk-1 functions downstream of acy-4 in the somatic gonad to regulate meiotic maturation.

To examine TWK-1 expression, we used recombinering to fuse GFP to the C terminus of TWK-1 within the fomisd context and generated transgenic lines in wild-type and twk-1 mutant backgrounds. The twk-1::gfp extrachromosomal arrays rescued both acy-4(lf) suppression and twk-1 Unc phenotypes (Table S6A), indicating that the TWK-1::GFP fusion protein is functional in vivo and might represent the endogenous expression pattern. TWK-1::GFP is expressed in the gonadal sheath cells, the distal tip cell, and a few unidentified neurons, but is not expressed in spermathecal cells (Figure 10). TWK-1 is not itself a strong negative regulator of meiotic maturation as oocytes in unmated twk-1(tn1397); fog-2(oz40) females stack in the gonad arm and do not exhibit elevated rates of meiotic maturation (Table 1). To test whether twk-1 functions downstream or in parallel to somatic PKA signaling needed for oocyte meiotic maturation, we conducted genetic mosaic analysis in twk-1(tn1397) kin-1(ok338); tnEx109[kin-1(+)] sur-5::gfp] animals, similar to what was done for sacy-1 and spr-5. All genetic mosaics that lost kin-1(+) function in somatic gonadal lineages were fertile (Table 2). Thus, twk-1 is epistatic to kin-1 for oocyte meiotic maturation. Taken together, these data suggest that TWK-1 has a function in gonadal sheath cells that contributes to the regulation of meiotic maturation by Gαs–ACY-4–PKA signaling.

TWK channels conduct potassium ions across the plasma membrane to control the negative resting potential of excitable cells. The finding that TWK-1 functions downstream or in parallel to PKA signaling provides additional evidence that the sheath cells play a critical role in regulating C. elegans oocyte meiotic maturation. TWK-1 has several human homologs, including TREK-1 and TREK-2 (Figure S12A). Application of intracellular cAMP or stimulation of a Gαs-coupled receptor block TREK-1 and TREK-2 channel activity (Patel et al. 1998; Lesage et al. 2000). Interestingly, electrophysiological studies found that C-terminal truncation of TREK-1 shortly after the fourth transmembrane domain renders the channel insensitive to cAMP inhibition and thus constitutively open (Patel et al. 1998). Multiple PKA phosphorylation sites in the C terminus were shown to mediate cAMP regulation of the channel (Patel et al. 1998; Murbartian et al. 2005; Kang et al. 2007). We therefore considered the possibility that TWK-1 C terminus might play an essential regulatory function. However, twk-1(tn1403) and twk-1(tn1398) reduce but do not eliminate twk-1 activity and are predicted to truncate the protein at positions 272

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Thus, the TWK-1 channel must retain some function in the absence of the C terminus. As a further test of whether the C terminus of TWK-1 is essential for function, we asked whether a TWK-1 C-terminal truncation (ΔC284) could rescue the strongest loss-of-function allele, twk-1(tn1397). We chose the position of the C-terminal TWK-1 deletion to correspond to the TREK-1 deletion that renders the channel constitutively open. We observed that TWK-1ΔC284::GFP could mediate function for both oocyte maturation and motility (Figure S12B). Another possibility is that the regulation of the channel via the C terminus might be important for closing the channel to facilitate meiotic maturation. If so, then a constitutively open channel, resulting from a C-terminal truncation, might confer a dominant disruptive effect on the meiotic maturation and ovulation process. Such an effect is predicted to be most evident with increased dosage achieved through expression from high-copy arrays. Since TWK-1ΔC284::GFP is functional in rescue assays, it seems unlikely that regulation of TWK-1 by PKA, if it exists in this system, plays an essential role. Alternatively, the C-terminal deletion might produce offsetting effects by concomitantly reducing function and removing inhibitory regulation. Further studies will be needed to assess whether PKA plays a direct role in regulating TWK-1 in the meiotic maturation process.

Sacy genes might act cumulatively downstream of somatic cAMP signaling

Sacy mutations enable partial fertility in the absence of somatic adenylate cyclase signaling, but in no case is fertility restored to wild-type levels. Possibly, Sacy genes might define cumulatively acting pathways that enable somatic control of meiotic maturation to ensure optimal rates of progeny production and sperm utilization. To begin to address this issue, we asked whether mutations in twk-1 or sacy-1 might increase acy-4(lf) brood sizes in combination with a strong-loss-of-function mutation in spr-5. This analysis is complicated by the fact that the strongest loss-of-function mutations in both twk-1 and sacy-1 confer pleiotropic phenotypes that can negatively impact fertility. Nonetheless, we observed that the weak mutations, sacy-1(tn1440) and twk-1(tn1403), further increased brood sizes in the spr-5(by134); acy-4(ok1806) genetic background (Table 5). While not definitive, these results are consistent with the idea that brood size is a complex function of multiple pathways downstream of acy-4. In the case of spr-5 and twk-1, genetic and molecular analyses are consistent with the idea the two pathways might act in combination, but that neither is essential for reproduction. By contrast, genetic analysis of sacy-1 suggests that it functions as a major regulator of oogenesis and a strong negative regulator of meiotic maturation, possibly representing a downstream integrator of the upstream signaling pathways.

Discussion

Suppressor genetics and the somatic control of oocyte meiotic maturation

Oocyte meiotic maturation is a conserved biological process required for sexual reproduction of animals. For the most
part, our understanding in this area largely derives from biochemical and cell biological studies. For example, classical biochemical studies in amphibian oocytes led to the discovery of maturation promoting factor (Masui and Markert 1971). By contrast, comparably fewer forward genetic approaches have been undertaken. Historically, research studies on animal oocytes have benefited greatly from a diversity of experimental systems, including organisms less amenable to forward genetic analyses. Here we have taken the approach of screening for mutations that impact the regulation of the meiotic maturation process in C. elegans.

A tried-and-true approach in developmental genetics is to screen for mutations in which the developmental process of interest is disrupted—here oocyte meiotic maturation. Thus far, comparably few single gene mutations have been described that result in a block in oocyte meiotic maturation. Any mutation that completely feminizes the adult hermaphrodite gonad significantly blocks the process because of the absence of the MSP signal for meiotic maturation (McCarter et al. 1999; Miller et al. 2009). By contrast, strong loss-of-function mutations in acy-4 block meiotic maturation despite the presence of sperm (Govindan et al. 2009). The relatively few single gene mutations found to date that completely block meiotic maturation likely stems from a combination of factors. Meiotic maturation occurs in the adult stage and depends on signaling and cell cycle factors, such as cdk-1, gsa-1, or kin-1, which play earlier developmental roles (Boxem et al. 1999; Govindan et al. 2006, 2009). Further, genetic redundancy, as observed for oma-1 and oma-2 (Detwiler et al. 2001), might contribute to the robustness of the genetic network. Here we have taken advantage of episomal loss of acy-4(af) sterility suppressor mutations. Two technological advances enabled this approach. The first is the wide availability of deletion alleles isolated by the C. elegans Knockout Consortium (Moerman and Barstead 2008; S. Mitani, unpublished results). Many deletion alleles remove functional or catalytic domains in proteins, as does the acy-4(ok1806) deletion we used for screening. This consideration makes it less likely that intragenic or informational suppressors might be isolated. Further, the availability of deletion alleles of many genes facilitates gene identification and the analysis of strong loss-of-function alleles. A second enabling technology is whole-genome sequencing for mutant identification (Hillier et al. 2008; Sarin et al. 2008). In the current instance, this technology made it possible to determine the molecular identities of Sacy genes defined by multiple alleles, in the face of an inability to fine map many of the mutations by conventional means.

A salient feature of oocyte meiotic maturation pathways in many organisms is that the somatic gonad exerts a controlling influence on the germ line. For example, it has long been known that removal of fully grown mammalian oocytes from antral follicles triggers meiotic resumption (Pincus and Enzmann 1935; Edwards 1965). Further, luteinizing hormone promotes meiotic resumption through activation of Gαs-adenylate cyclase–PKA signaling in mural granulosa cells, thereby regulating a cascade of paracrine and gap-junctional signaling pathways, which ultimately results in lowering intra-oocyte cAMP levels to trigger MPF activation (reviewed by Sun et al. 2009; Downs 2010). In mammals, somatic gonadal control of meiotic maturation, in part, provides a means to link reproduction to pituitary hormone control. By contrast, in C. elegans, control by the somatic gonad provides a means to link oocyte meiotic maturation to sperm availability. In C. elegans, somatic Gαs–adenylate cyclase–PKA signaling is required for all described germline responses to the MSP hormone (Govindan et al. 2009; this work; Figure 11). Further, Gαs–adenylate cyclase–PKA pathway activation can drive meiotic maturation at high rates in the absence of sperm (Govindan et al. 2006, 2009), approaching ~50% of the maximal hermaphrodite rate, depending on the specific method of pathway activation.

In this work, we used suppressor genetics to ask two questions: (1) What are the molecular pathways that impose and mediate somatic control of meiotic maturation? (2) What happens to reproduction when signaling is perturbed? We identified mutations in at least 17 Sacy genes that enable partial fertility in the absence of somatic adenylate cyclase signaling. Cumulatively, we molecularly characterized mutations in 10 Sacy genes (sacy-1, pde-6, spr-1–5, twk-1, tom-1, and uev-1). In no case was fertility restored to wild-type levels, however. Given the scale of the suppressor screen, it seems doubtful that any viable single gene mutation can fully restore the fertility of acy-4(af) hermaphrodites to wild-type levels. We identified two classes of mutations. The first class of suppressor is exemplified by sacy-1, which encodes a highly conserved DEAD-box helicase. While we recovered reduction-of-function sacy-1 alleles as acy-4(af) suppressor mutations, our screens could not recover strong loss-of-function sacy-1 alleles because of its essential functions.

| Genotype                              | Brood size (± SD) | Number of animals scored |
|---------------------------------------|-------------------|--------------------------|
| acy-4(ok1806)                         | 1 (± 2)           | 30                       |
| spr-5(by134); acy-4(ok1806)           | 81 (± 35)         | 40                       |
| twk-1(tn1403); acy-4(ok1806)          | 32 (± 25)         | 36                       |
| sacy-1(tn1440); acy-4(ok1806)         | 20 (± 19)         | 39                       |
| twk-1(tn1403) spr-5(by134); acy-4(ok1806) | 128* (± 46)     | 40                       |
| sacy-1(tn1440) spr-5(by134); acy-4(ok1806) | 108* (± 52)     | 40                       |

* P < 0.01 compared to spr-5(by134); acy-4(ok1806) using Student’s t-test.
needed for the dependence of meiotic maturation on the G
and OMA-2. CoREST-like complex has a function in the germ line that is
germ line upstream of, or in parallel to, the positive regulators OMA-1
a strong negative regulator of meiotic maturation that functions in the
data) and INX-14 and INX-22 in oocytes (Govindan
in the gonadal sheath cells (T. Starich and D. Greenstein, unpublished
via gap-junctional communication involving the innexins INX-8 and INX-9
maturation. The gonadal sheath cells inhibit meiotic maturation in part
function in the gonadal sheath cells as negative regulators of meiotic
ACY-4
MSP binding activity are displayed in a localized fashion, though this is
PKA sheath cell pathway. For illustrative purposes, TWK-1 and sheath cell
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lishes or maintains the dependence of meiotic maturation on
proteins that might interfere with the circuitry that estab-
lished a CoREST-like chromatin regulators
a PKA activity in the gonadal sheath cells. PDE-6 and TWK-1 may
in the gonadal sheath cells as negative regulators of meiotic
maturation. The gonadal sheath cells inhibit meiotic maturation in part
via gap-junctional communication involving the innexins INX-8 and INX-9
in the gonadal sheath cells (T. Starich and D. Greenstein, unpublished
data) and INX-14 and INX-22 in oocytes (Govindan et al. 2009). SACY-1 is
a strong negative regulator of meiotic maturation that functions in the
germ line upstream of, or in parallel to, the positive regulators OMA-1
and OMA-2. CoREST-like complex has a function in the germ line that is
needed for the dependence of meiotic maturation on the Gαs–ACY-4–
PKA sheath cell pathway. For illustrative purposes, TWK-1 and sheath cell
MSP binding activity are displayed in a localized fashion, though this is
unlikely to be the case (Govidan et al. 2009; this work).

needed for gamete maintenance and embryogenesis. SACY-1
is a strong negative regulator of oocyte meiotic maturation in
the absence of sperm, and it likely functions upstream of OMA-1
and OMA-2 (Figure 11), which are redundantly required for
meiotic maturation (Dettwiler et al. 2001). In contrast to
SACY-1, the second suppressor class, exemplified by mutations
affecting a CoREST-like complex and the TWK-1 two-
pore potassium channel, is not absolutely required for mei-
otic maturation or its negative regulation in the absence of
sperm (Figure 11). These genes and perhaps other Sacy loci
appear to function cumulatively to enable somatic control of
meiotic maturation.

Control of transcription and translation in the germ line
and the regulation of meiotic maturation

Our findings suggest that CoREST-like chromatin regulators
function in the germ line to ensure the somatic control of
oocyte meiotic maturation. One potential model is that a
CoREST-like complex prevents the germline expression of
proteins that might interfere with the circuitry that estab-
lishes or maintains the dependence of meiotic maturation on
somatic Gαs–adenylate cyclase–PKA and MSP signaling. In
this model, the role of CoREST in oocyte meiotic maturation
would be reminiscent of the synthetic multivulval (SynMuv)
genes in vulval development (Fay and Yochem 2007). The
SynMuv genes establish a necessary precondition for vulval
induction by repressing ectopic transcription of lin-3, which
encodes the anchor cell signal (Cui et al. 2006; Saffer et al.
2011). SynMuvB genes define a chromatin regulatory path-
way involving the Rb retinoblastoma ortholog LIN-35 (Lu
and Horvitz 1998). Interestingly, lin-35 and spr-1 display
a synthetic genetic interaction affecting gonadogenesis
(Bender et al. 2007), suggesting that CoREST may mediate
multiple functions needed for optimal germline develop-
ment and reproduction. These functions might involve the
regulation of transcription in the germ line.

By contrast, SACY-1 might function in post-transcriptional
gene regulation important for the control of oocyte meiotic
maturation, the hermaphrodite sperm-to-oocyte switch, and
gamete maintenance. Genetic epistasis analysis suggests that
sacy-1 functions upstream of oma-1 and oma-2. Since oocytes
are transcriptionally quiescent (Starck 1977; Gibert et al.
1984; Schisa et al. 2001; Walker et al. 2007), and OMA-1
and OMA-2 are cytoplasmically localized (Dettwiler et al.
2001), an attractive hypothesis is that these two TIS11
zinc-finger proteins regulate meiotic maturation at a post-
transcriptional level. Indeed, OMA-1 and OMA-2 have been
shown to repress the translation of several mRNAs in
oocytes and embryos (Jadhav et al. 2008; Li et al. 2009;
Guven-Ozkan et al. 2010). Our finding that SACY-1 func-
tions as a component of the hermaphrodite sperm-to-oocyte
switch likely upstream of tra-2 is consistent with its potential
involvement in post-transcriptional gene regulation (reviewed
by Thomas et al. 2012). Both SACY-1 and its Drosophila
ortholog Abstract are found in the nucleus and cytoplasm
(Irion and Leptin 1999), and thus these factors might func-
tion at a variety of levels to impact gene expression. We
suggest that SACY-1 is a positive factor for TRA-2 expres-
sion, perhaps playing a similar role as Abstract, which pro-
motes expression of the Insucutable protein (Irion et al.
2004). If SACY-1 functions in a similar manner to regulate
meiotic maturation, then it might function in part by pro-
moting the translation of an inhibitory factor that restrains
cell cycle progression. Interestingly, DDX41, the human
ortholog of SACY-1, was recently found to be one of five
genes recurrently mutated in patients with relapsing acute
myeloid leukemia (Ding et al. 2012). DDX41 also functions
in a signaling pathway that detects invading viral double-
stranded DNA in the cytoplasm and initiates an antiviral
response (Zhang et al. 2011), but other components of this
innate immunity pathway appear not to be conserved in
nematodes. Intriguingly, sacy-1 is required to prevent the
necrotic cell death of gametes. Therefore, sacy-1 establishes
a mechanistic link among three developmental processes
critical for sexual reproduction: germline sex determination,
somatic control of meiotic maturation, and preservation of
gamete quality. The molecular genetic tools described in this

Figure 11 Model for the control of oocyte meiotic maturation in C.
Figure 11 Model for the control of oocyte meiotic maturation in C.
elegans. MSP signaling for oocyte meiotic maturation requires Gαs–
ACY-4–PKA activity in the gonadal sheath cells. PDE-6 and TWK-1 may
function in the gonadal sheath cells as negative regulators of meiotic
maturation.
work will facilitate the dissection of these key reproductive processes.

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SACY-1 DEAD-Box Helicase Links the Somatic Control of Oocyte Meiotic Maturation to the Sperm-to-Oocyte Switch and Gamete Maintenance in Caenorhabditis elegans

Seongseop Kim, J. Amaranath Govindan, Zheng Jin Tu, and David Greenstein
Figure S1  Location of Sacy alleles of tom-1. Each tom-1 gene isoform is depicted. The start codon (+1) of the tom-1a gene isoform is indicated. Newly identified tom-1 tn alleles and the corresponding amino acid changes are shown. Deletion alleles are underlined. Apparently, tom-1 is a complex locus and only ok2437, tn1463, and tn1454 suppress acy-4(If) sterility—the tom-1 isoform(s) relevant to this observation are unclear.
**Figure S2**  *kin-1* genetic mosaics with complex losses within the somatic gonadal cell lineages. Genetic-mosaic animals with complex losses of the *kin-1(+) array in the somatic gonad (see Figure 1 in the main text) are described in more detail. Green characters represent the presence of the *kin-1(+) array and red characters represent the absence of the *kin-1(+) array. Uterine cells were not scored. One sterile genetic mosaic animal (star) resulted from multiple independent losses within both the Z1 and Z4 lineages. This animal is consistent with a requirement for *kin-1(+) function in the gonadal sheath cells for meiotic maturation.
**Figure S3**  
*sacy-1* is a negative regulator of oocyte meiotic maturation in the absence of sperm. (A) Control female mutants. Oocytes are stacked within the gonad arm and the uterus is empty. Spermatheca (sp). (B) *sacy-1(tn1385)* inhibits oocyte meiotic maturation in the absence of sperm. Oocytes undergo maturation and ovulation at an increased frequency and are observed in the uterus despite the absence of sperm. A similar phenotype was observed in *fog-1(e2121) sacy-1(tn1385)* females (S. Kim and D. Greenstein, unpublished results). (C) *sacy-1(tm5503)* is a strong negative regulator of oocyte meiotic maturation in the absence of sperm. Many unfertilized oocytes are observed in the uterus of *sacy-1(tm5503)* females, suggesting that *sacy-1* inhibits oocyte meiotic maturation in the absence of sperm. The frequent occurrence of defective ovulation in *sacy-1(tm5503)* females prevented us from directly measuring meiotic maturation rates. *fog-1(e2121) sacy-1(tm5503)* females exhibit a similar phenotype (S. Kim and D. Greenstein, unpublished results). All images were obtained from day-1 adults (24 hr post-L4) examined by DIC microscopy. *fog-3(q470)* and *sacy-1(tm5503) fog-3(q470)* images were duplicated from Figure 8 for comparison. Scale bar, 50 µm.
Figure S4  Representative DIC images of day-1 adults illustrating the qualitative scoring criteria used to evaluate the severity of the gamete degeneration phenotype of sacy-1(tm5503) hermaphrodites and females. Scale bar, 50 μm.
**Figure S5**  Masculinization of the hermaphrodite germ line does not suppress *sacy-1(tm5503)* gamete degeneration. A *sacy-1(tm5503); fem-3(q20gf)* hermaphrodite (bottom) exhibits the gamete degeneration phenotype. A *sacy-1(tm5503)/+; fem-3(q20gf)* hermaphrodite (top) produces excessive amounts of sperm. Scale bar, 50 μm.
Figure S6  sacy-1(tm5503) females do not produce MSP. Western blot of MSP from wild-type (WT), fog-1(e2121) sacy-1(tm5503), and sacy-1(tm5503) fog-3(q470) adults. Because oocyte meiotic maturation occurs frequently in sacy-1(tm5503) females despite the absence of MSP, sacy-1 is a strong negative regulator of meiotic maturation. Ten animals of each genotype were lysed and immunoblotting was conducted using a rabbit C-terminal-specific anti-MSP antibody (C3196; 1:20,000) (Kosinski et al., 2005). Mouse anti-actin C4 antibody (Millipore; 1:20,000) served as a loading control.
Figure S7  sacy-1(tm5503) is epistatic to acy-4(ok1806) for oocyte meiotic maturation. Oocyte meiotic maturation occurs frequently in sacy-1(tm5503) fog-3(q470); acy-4(ok1806) females, though ovulation often fails and endomitotic oocytes are observed in the gonad arm. In contrast, oocytes arrest in diakinesis in fog-3(q470); acy-4(ok1806) females. Day-1 adults were examined by DIC microscopy of living animals (A) and DNA was detected by DAPI staining of dissected and fixed gonads (B). Proximal is to the left side. Scale bar, 50 µm.
Figure S8  Phylogenetic comparison C. elegans and human phosphodiesterases. The likely human ortholog of C. elegans PDE-6 is PDE8, the high-affinity cAMP-specific phosphodiesterase. Red characters represent six C. elegans phosphodiesterases, and blue characters represent 11 human phosphodiesterases. The following amino acid sequences were used for the analysis; Ce_PDE-1 (NP_493343.1), Ce_PDE-2 (NP_001022705), Ce_PDE-3 (NP_871943.2), Ce_PDE-4 (NP_001040798.1), Ce_PDE-5 (NP_491544.2), Ce_PDE-6 (NP_490787.1), Hs_PDE1 (NP_000915.1), Hs_PDE2 (NP_002590.1), Hs_PDE3 (NP_000913.2), Hs_PDE4 (NP_001098101.1), Hs_PDE5 (NP_001074.2), Hs_PDE6 (NP_000274.2), Hs_PDE7 (NP_001229247.1), Hs_PDE8 (NP_001025024.1), Hs_PDE9 (NP_002597.1), Hs_PDE10 (NP_001124162.1), Hs_PDE11 (NP_058649.3).
Figure S9  spr-5 RNAi suppresses acy-4(lf) sterility. spr-5 dsRNAs, or a buffer control, were injected into the gonad arms of acy-4(ok1806); tnEx37[acy-4(+)] sur-5::gfp or rrf-1(pk1417); acy-4(ok1806); tnEx37[acy-4(+)] sur-5::gfp adult hermaphrodites, and non-array-bearing F1 animals were scored by DIC microscopy for suppression of acy-4(ok1806) sterility. Fertile animals were defined as having more than two embryos in the spermathecae and/or uterus. The numbers of animals scored are indicated.
Figure S10 Growth defect of acy-4(ok1806) on HT115 bacteria. (A) acy-4(ok1806) animals exhibit larval lethality, larval arrest, or slow growth on standard NGM plates with E. coli HT115 as the food source. In contrast, wild-type, acy-4(ok1806); tnEx37[acy(+):sur-5::gfp], and acy-4(ok1806) spr-1(gk734) animals grow well with HT115 as the food source. Likewise, spr-2, spr-4, and spr-5 mutations suppress the acy-4(ok1806) growth defect (not shown). Embryos isolated by bleach treatment were cultured on HT115-seeded plates for approximately 72 hrs. acy-4(ok1806) homozygotes were the GFP-negative progeny of array-bearing parents. Scale bar, 1 mm. (B) DIC images of wild type (WT) and acy-4(ok1806) animals grown on HT115-seeded medium for 72 hrs. Scale bar, 50 µm.
Figure S11  

*twk-1* functions in the somatic gonad downstream of *acy-4*. A genetic mosaic animal resulting from multiple independent losses of the *twk-1(+)*-bearing extrachromosomal array in the anterior somatic gonadal lineage (Z1). This animal was fertile in the anterior arm, indicating suppression of *acy-4(lf)* sterility, but sterile in the posterior arm. Green characters represent the presence of the *twk-1(+) array*, and red characters represent the absence of the *twk-1(+) array*. Uterine cells were not scored.
Figure S12  The TWK-1 C-terminus is not essential for function. (A) ClustalW alignment of C. elegans TWK-1 and human TREK-2 (NP_612190). Using RT-PCR, we found that the TWK-1 prediction (NP_492054.2) was incorrect in that a small predicted intron was found to be coding, resulting in an additional 14 amino acids. The RT-PCR data is in agreement with the gene prediction based on the FN890018 cDNA. We thank Bernard Lakowski for pointing out the discrepancy in the tkw-1 gene prediction. The four transmembrane domains (M1-M4) and two pore domains (P1 and P2) are underlined. The three tkw-1 nonsense mutations and the position of the ΔC284 deletion are indicated. TWK-1 exhibits less similarity to TREK-1 (not shown) in the C-terminal region because of the latter's smaller size. (B) The TWK-1DC284::GFP fusion (tnEx188) rescues tkw-1(tn1397). Error bars represent one standard deviation. Student’s t-test was used for statistical analysis.
### Table S1  Molecular identity of Sacy mutations in tn alleles

| Gene | Allele | Nucleotide change | Amino acid change$^a$ |
|------|--------|-------------------|----------------------|
| **pde-6** | tn1237 | CAG → TAG | Q668* |
| | tn1242 | TGG → TGA | W444* |
| | tn1336 | TGG → TGA | W664* |
| **tom-1** | tn1454 | Tc1 insertion | |
| | tn1463 | GGT → AGT | G498S |
| **sacy-1** | tn1385 | GGA → AGA | G533R |
| | tn1391 | GGA → AGA | G473R |
| | tn1440 | GGA → AGA | G331R |
| **twk-1** | tn1397 | TGG → TAG | W177* |
| | tn1398 | TGG → TAG | W330* |
| | tn1403 | GAA → TAA | E272* |
| **spr-4** | tn1383 | CGA → TGA | R1230* |
| | tn1402 | CGA → TGA | R1230* |
| | tn1404 | CAT → TAT | H845Y |
| | tn1438 | CGA → TGA | R486* |
| | tn1444 | CAG → TAG | Q1128* |
| | tn1467 | CAG → TAG | Q45* |
| **spr-5** | tn1378 | TGG → TAG | W666* |
| | tn1379 | GAG → AGA | E164K |
| | tn1394 | GGT → GAT | G619D |
| **uev-1** | tn1381 | GGA → GAA | G17E |
| | tn1382 | GGT → GAT | G47D |
| **spr-2** | tn1380 | GAT → GCT | D42A |
| | tn1436 | Tc1 insertion | |

$^a$Premature stop codons are indicated by asterisks. The numbering of amino acids refers to the isoforms PDE-6A, TOM-1A, SPR-4A, and SPR-2B.
| Strain   | Genotype                                                                 |
|----------|---------------------------------------------------------------------------|
| N2       | Wild type, Bristol isolate                                                |
| CB4856   | Hawaiian isolate                                                         |
| DG2730   | tnl1[N2=>CB4856, acy-4(OK1806)] V; tnEx37[acy-4(+); sur-5::gfp]            |
| DG3051   | tnEx131[acy-1::gfp rol-6(su1006d)]                                       |
| DG3064   | tnEx133[acy-2::gfp rol-6(su1006d)]                                       |
| DG3066   | tnEx134[acy-3::gfp rol-6(su1006d)]                                       |
| DG3395   | tnEx175[twk-1::gfp rol-6(su1006d)]                                       |
| DG3441   | tnEx181[twk-1::gfp str-1::gfp]                                            |
| DG3521   | fog-1[e2121] sacy-1(tm5503) l/hT2[blI-4(e937) let-7[q782] qls48] (I;III) |
| DG3524   | fog-1[e2121] sacy-1[tn1385] l/hT2[blI-4(e937) let-7[q782] qls48] (I;III) |
| DG3585   | fog-3[q470] l/hT2[blI-4(e937) let-7[q782] qls48] (I;III); acy-4(OK1806) V; tnEx37 |
| DG3586   | gso-1(pk75) l/hT2[blI-4(e937) let-7[q782] qls48] (I;III); fog-2(oz40) V    |
| DG3393   | kin-1(ok338) l; tnEx109[kin-1(+); sur-5::gfp]                             |
| DG3360   | pbrm-1(gk1195) I; acy-4(OK1806) V; tnEx37                                 |
| VC10166  | pbrm-1(gk1195) I; octr-1(gk1196) X                                        |
| DG2292   | pde-6[tn1237] I                                                         |
| DG2397   | pde-6[tn1237] I; unc-46(e177) acy-4(OK1806) V                            |
| DG2096   | pde-6[tn1242] I                                                         |
| DG2454   | pde-6[tn1242] I; unc-46(e177) acy-4(OK1806) V                            |
| DG2111   | pde-6[tn1336] I                                                         |
| DG2834   | rrf-1(pk1417) I; acy-4(OK1806) V; tnEx37                                 |
| DG3606   | rrf-1(pk1417) I; fog-2(oz40) V                                           |
| DG3544   | sacy-1(tm5503) [fog-3(q470) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III) |
| DG3589   | sacy-1(tm5503) [fog-3(q470) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); acy-4(OK1806) V; tnEx37 |
| DG3557   | sacy-1(tm5503) [fog-3(q470)]; unc-13(e1091) lin-11(n566) I; oma-1[2u405te33] IV/nT1[qls51] (IV;V); oma-2[te51] V/nT1[qls51] (IV;V) |
| DG3492   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III)             |
| DG3587   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); acy-4(OK1806) V; tnEx37 |
| DG3514   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); ced-3(n717) IV |
| DG3566   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); ced-4(n1162) III/lhT2[blI-4(e937) let-7?q782] qls48] (I;III) |
| DG3528   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); fem-3(e1996)/unc-24(e138) dpy-20(e1282) V |
| DG3570   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); fem-3(q20gf) V |
| DG3505   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); fog-2(oz40) V |
| DG3512   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); fog-2(q71) V |
| DG3486   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); him-8(tm611) IV |
| DG3561   | sacy-1(tm5503) l/hT2[blI-4(e937) let-7?q782] qls48] (I;III); unc-24(e138) IV |
sacy-1(tm5503) I/hT2[blt-4(e937) let-?(q782) qls48] (I;III); unc-32(e189) III/hT2[blt-4(e937) let-?(q782) qls48] (I;III)

sacy-1(tm5503) I/hT2[blt-4(e937) let-?(q782) qls48] (I;III); unc-33(mn407) IV

sacy-1(tm5503) I/hT2[blt-4(e937) let-?(q782) qls48] (I;III); unc-68(e540) V

sacy-1(tm5503) I; tnEx159[gfp::sacy-1 unc-119(+)]

sacy-1(tm5503) I; unc-119(ed3) III; tnEx159[gfp::sacy-1 unc-119(+)]

sacy-1(tm5503)/unc-13(e1091) lin-11(n566) I; fem-3(e1996) V/hT1[qls51] (IV;V)

sacy-1(tm5503)/unc-13(e1091) lin-11(n566) I; oma-1(zu405te33) IV/hT1[qls51] (IV;V); oma-2(te51) V/hT1[qls51] (IV;V)

sacy-1(tm1385) fag-3(q470) I/hT2[blt-4(e937) let-?(q782) qls48] (I;III)

sacy-1(tm1385) I

sacy-1(tm1385) I; acy-4(ok1806) V

sacy-1(tm1385) I; fem-3(e1996) IV/hT1[qls51] (IV;V)

sacy-1(tm1385) I; fog-2(az40) V

sacy-1(tm1385) I; fog-2(q71) V

sacy-1(tm1385) kin-1(ok338) I; tnEx109

sacy-1(tm1385) pbrm-1(tm1475) I; acy-4(ok1806) V

sacy-1(tm1385) pbrm-1(tm1475) I; acy-4(ok1806) V; tnEx37

sacy-1(tm1391) I

sacy-1(tm1391) I; acy-4(ok1806) V

sacy-1(tm1391) I; fog-2(oz40) V

sacy-1(tm1391) I; fog-2(q71) V

sacy-1(tm1391) I; unc-119(ed3) III; acy-4(ok1806) V; tnEx37; tnEx159

sacy-1(tm1391) I

sacy-1(tm1440) I; acy-4(ok1806) V

sacy-1(tm1440) I; acy-4(ok1806) V

sacy-1(tm1440) I; fog-2(az40) V

sacy-1(tm1440) I; fog-2(q71) V

sacy-1(tm1440) pbrm-1(tm1476) I; acy-4(ok1806) V

sacy-1(tm1440) pbrm-1(tm1476) I; acy-4(ok1806) V; tnEx37

sacy-1(tm1440) spr-5(by134) I; acy-4(ok1806) V

spr-4(by105) I; acy-4(ok1806) V

spr-4(tm1383) I; acy-4(ok1806) V

spr-4(tm1402) I; acy-4(ok1806) V

spr-4(tm1404) I; acy-4(ok1806) V

spr-4(tm1438) I; acy-4(ok1806) V

spr-4(tm1444) I; acy-4(ok1806) V

spr-4(tm1467) I; acy-4(ok1806) V

spr-5(ar197) I; acy-4(ok1806) V

spr-5(by134) I; acy-4(ok1806) V
DG3185  spr-5(by134); fag-2(oz40) V
DG3122  spr-5(by134) kin-1(ok338) I; tnEx109
DG2553  spr-5(tn1378) I; acy-4(ok1806) V
DG2564  spr-5(tn1379) I; acy-4(ok1806) V
DG2522  spr-5(tn1394) I; acy-4(ok1806) V
NM1815  tom-1(ok188)
DG3192  tom-1(ok188) I; acy-4(ok1806) V; tnEx37
RB1887  tom-1(ok2437) I
DG3079  tom-1(ok2437) I; acy-4(ok1806) V
VC223   tom-1(ok285) I
DG3217  tom-1(ok285) I; acy-4(ok1806) V; tnEx37
FX04724  tom-1(tm4724) I
DG3245  tom-1(tm4724) I; acy-4(ok1806) V; tnEx37
DG3118  tom-1(tn1454) I; acy-4(ok1806) V
DG3097  tom-1(tn1463) I; acy-4(ok1806) V
DG3408  twk-1(tn1397) I
DG3095  twk-1(tn1397) I; acy-4(ok1806) V
DG3447  twk-1(tn1397) I; acy-4(ok1806) V/nT1[qls51] (IV;V); tnEx180[twk-1(+)] sur-5::gfp
DG3490  twk-1(tn1397) I; acy-4(ok1806) V/nT1[qls51] (IV;V); tnEx188[twk-1(ΔC284)::gfp str-1::gfp]
DG3451  twk-1(tn1397) kin-1(ok338) I; tnEx109
DG3389  twk-1(tn1398) I
DG3083  twk-1(tn1398) I; acy-4(ok1806) V
DG3390  twk-1(tn1403) I
DG3113  twk-1(tn1403) I; acy-4(ok1806) V
DG3459  twk-1(tn1403) spr-5(by134) I; acy-4(ok1806) V
RB1976  uev-1(ok2610) I
DG3409  uev-1(ok2610) I; acy-4(ok1806) V
DG3076  uev-1(tn1381) I; acy-4(ok1806) V
DG3080  uev-1(tn1382) I; acy-4(ok1806) V
CB3778  tra-2(e2020) II
CB3843  fem-3(e1996)/unc-24(e138) dpy-20(e1282) IV
CA257   him-8(tm611) IV
DG2836  spr-2(ar199) dpy-20(e1282) IV; acy-4(ok1806) V; tnEx37
DG2943  spr-2(ar211) dpy-20(e1282) IV; acy-4(ok1806) V
DG3136  spr-2(tm4802) IV; acy-4(ok1806) V
DG2558  spr-2(tn1380) IV; acy-4(ok1806) V
DG2592  spr-2(tn1436) IV; acy-4(ok1806) V
DG3612  acy-4(ok1806) spr-1(gk734) V
DG3604  acy-4(ok1806) unc-23(e25) V; tnEx37
|     |                        |                        |
|-----|------------------------|------------------------|
| DG3145 | acy-4(ok1806) V; spr-3(by108) X | DG3129 | acy-4(ok1806) V; spr-3(ok2525) X |
| DG3391 | acy-4(ok1806) V; tnEx37 | BSS53 | fog-2(oz40) V |
| CB4108 | fog-2(q71) V           |
| Name          | Sequence                                                                 | Use                                      |
|---------------|---------------------------------------------------------------------------|------------------------------------------|
| ok1806F1      | CGGGTTGATGAATCTTGATCATTCTC                                              | acy-4(ok1806) genotyping                |
| ok1806R1      | CATGATACAAAGCTGTGACGAGAATC                                             | acy-4(ok1806) genotyping                |
| ok1806F2      | TCAGTCTCGTATTGTTGTCTGTC                                                 | acy-4(ok1806) genotyping                |
| gk1195_F1     | TTCATCTGAATGCATTCAATCCTTGGATC                                           | pbrm-1(gk1195) genotyping               |
| gk1195_R1     | TTTCAGGGAAGCGGAAGTGGAAATGGAAG                                           | pbrm-1(gk1195) genotyping               |
| H27M09.1_F5   | ACTGCTGGAAATATATCATCTTATCGTC                                            | sacy-1(tm5503) genotyping               |
| tm5503_F1     | GTTATGGACGAATACAGAGCGCAATTAATG                                         | sacy-1(tm5503) genotyping               |
| tm5503_R1     | CTTTGATCTGAATAGCTGTCGCGTTLCAC                                           | sacy-1(tm5503) genotyping               |
| H27M09.1_RNAi_F | TTTTAATACGACTCACTATAGGGGAATCTGATGCGATG                                  | sacy-1 RNAi                              |
| H27M09.1_RNAi_R | TTTTAATACGACTCACTATAGGGGAATCTGATGCGATG                                  | sacy-1 RNAi                              |
| H27M09.1_cDNA_F1 | TTTCCCGGGAATATGAGAAGGGCGACGCGCAGAAATTCC                              | sacy-1 cDNA 5’ half RNAi                |
| H27M09.1_cDNA_R1 | TTTTCGAGCTCCAATAGCTTTAGTGTCGGCGAATTG                                   | sacy-1 cDNA 5’ half RNAi                |
| H27M09.1_cDNA_F2 | TTTCCCGGCAATTTGGCGAACAAGCTAAAGACGGTACAG                               | sacy-1 cDNA 3’ half RNAi                |
| H27M09.1_cDNA_R2 | TTTTCGAGCAATGCTGGCTGTTTTTCTGCGATT                                       | sacy-1 cDNA 3’ half RNAi                |
| acy-1_Exon13_F | AAGGTGATGGAATACCTCATAATAGGGGAATACTGATGCAAGAAGAAACAGGTTTCAC              | Recombining for acy-1::gfp              |
| acy-1_Exon13_R | TGTGAAAGGAATATAGGAGGTTTAAAGGCAAATGTAATG                                 | Recombining for acy-1::gfp              |
| acy-2_Exon17_F | CCGCGGAGAACAACCAAACATAGTAAACAAATAATTGTCACA                              | Recombining for acy-2::gfp              |
| acy-2_Exon17_R | GAGAGGAAAGTGGAATGCGAATGGAGGTAAAATACAGAAC                                 | Recombining for acy-2::gfp              |
| acy-3_Exon14_F | ACAATTTCCGAGACCTCACAATTCACAATTCGATGATAATG                               | Recombining for acy-3::gfp              |
| acy-3_Exon14_R | AGACATGAGAATAAGGAACATATATTTGCAAAAAATG                                   | Recombining for acy-3::gfp              |
| twk-1_GalK_F2 | AGGTTTCCAGATTCAGAAGAAGCCTGAAATTGTTTGGAGGCC                               | Recombining for galk                    |
| twk-1_GalK_R2 | TCCGGCTGGATCCAAATCCTGGCTCCAGCAGAATGTGACCAGAT                            | Recombining for galk                    |
| twk-1_C-ter_trunc | AGGTTTCCAGATTCAAGAAGAAGCCTGAAATTGTTTGGAGGCC                           | Recombining for C-terminal deletion      |

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| Sampling Name | Sequence | Description |
|---------------|----------|-------------|
| KIN-1R 50bp   | TCCCAATTCTTTGTA AATCAACAGTGATGCAACATTCACACAAAAAGTTACGAAA | Recombineering for extending kin-1 promoter in the WRM069aA02 fosmid |
|               | CTCTGTGG |             |
| KIN-1OF 50bp  | TAAAGTTGGGTAACGCCAGGGTTTCCCCAGTCACGACGTTGTA AAACGACGAGAGACACTGCTCCTGGAGAGTCAC | Recombineering for extending kin-1 promoter in the WRM069aA02 fosmid |
|               |           |             |
| kin-1 galk F  | TAAGTTGGGTAACGCCAGGGTTTCCCCAGTCACGACGTTGTA AAACGACGCTGTGACAATTAATCATCGGCA | Recombineering for galk insertion into the WRM069aA02 fosmid |
|               |           |             |
| kin-1 galk R  | AATCAACAGTGATGCAACATTCACACAAAAAGTTACGAAA | Recombineering for galk insertion into the WRM069aA02 fosmid |
|               | CTCTGTGGTACAGCAGCTGTCTGGCTTCTCTT |             |
| H27M09.1_gfpF1| TTTTAAGTATCTTAAAAAGTAGAAACAGAAAAATTCGTTTAGTA TTCGAAATGAGTAAAGGATAAGAACTTTTCATGC | Recombineering for gfp::sacy-1 |
|               |           |             |
| H27M09.1_gfpR1| CGCCGGCGGTCTTCATATTTTGTCGCCGCTTCACACATCTG TGCTCATTCTTGTATAATGGATCCATGCAATTCG | Recombineering for gfp::sacy-1 |
|               |           |             |
| twk-1_GFP_F1 | AAGCTCAGGCATACAGTGATGTCAGACTTTTTACTGCAACA AGATGTGATGAAAGGAGAACTTTTCATG | Recombineering for twk-1::gfp |
|               |           |             |
| twk-1_GFP_R1 | ATATCCGTTTCTTTTTTTTGTCCGAAATACATAAATTTGCAAA TATCTATTCTGTATAGTTCCTGCATGCAATTCG | Recombineering for twk-1::gfp |
|               |           |             |
| twk-1_F1     | GACAAATAATGAAAGCAGCATCCTGTGGATAG | twk-1 RT-PCR |
| twk-1_R1     | GTGACCAAATCCTTGACAGATCATCATTGT | twk-1 RT-PCR |
Table S4  *pbrm-1* is not a Sacy mutation

| Genotype                                      | Brood size (± SD) | Number of animals scored |
|-----------------------------------------------|-------------------|--------------------------|
| *acy*-4(ocr1806)                             | 1 (± 2)           | 30                       |
| *pbrm*-1(ocr1195)*                          | 102 (± 86)        | 30                       |
| *pbrm*-1(ocr1195); *acy*-4(ocr1806)          | 0 (± 2)           | 35                       |
| *sacy*-1(ocr1385); *acy*-4(ocr1806)          | 14 (± 15)         | 39                       |
| *sacy*-1(ocr1385) *pbrm*-1(ocr1475)*; *acy*-4(ocr1806) | 23 (± 18)       | 30                       |
| *sacy*-1(ocr1440); *acy*-4(ocr1806)          | 20 (± 19)         | 39                       |
| *sacy*-1(ocr1440) *pbrm*-1(ocr1476)*; *acy*-4(ocr1806) | 12 (± 11)       | 30                       |

*a*VC10166 [*pbrm*-1(ocr1195) I; *octr*-1(ocr1196) X] strain was used. *pbrm*-1(ocr1195) deletes 126 nucleotides, including an intron and part of exon 6 of the *pbrm*-1a gene isoform.

*b* *pbrm*-1(ocr1475) induces a missense mutation (A1182T) in the *pbrm*-1a gene isoform.

*c* P=0.025 compared to *sacy*-1(ocr1385); *acy*-4(ocr1806).

*d* *pbrm*-1(ocr1476) induces a missense mutation (A157T) in the *pbrm*-1a gene isoform.

*e* P=0.048 compared to *sacy*-1(ocr1440); *acy*-4(ocr1806).

Student’s t-test was used for statistical analysis.
Table S5  *pde-6* negatively regulates oocyte meiotic progression

A. *pde-6*(tn1237) is epistatic to *gsa-1*(pk75) for oocyte meiotic maturation

| Genotype                      | Fertile gonad arms/Total |
|-------------------------------|--------------------------|
| *gsa-1*(pk75)                 | 0/9                      |
| *gsa-1*(pk75); *pde-6*(tn1237) | 5/6                      |

Genetic mosaics in which the sheath/spermathecal lineages were mutant for *gsa-1* were sought using *tnEx31[gsa-1(+)] sur-5::gfp* for mosaic analysis (Govindan et al., 2009).

B. *pde-6*(tn1237) extends AIR-2::GFP to oocyte chromatin in a hermaphrodite background

| Genotype                      | Chromatin localization of AIR-2::GFP | Oocyte position in the gonad arm |
|-------------------------------|------------------------------------|---------------------------------|
|                               | –1       | –2     | –3     | –4     | –5     |
| Wild type (n=72)              | 95.8%    | 32%    | 1.4%   | 0%     | 0%     |
| *pde-6*(tn1237) (n=35)        | 100%     | 97.1%  | 70.6%  | 10.3%  | 0%     |

Strains were homozygous for *its14*, which expresses AIR-2::GFP in the germ line. MSP and Gαcy-ACY-4 signaling promotes the localization of AIR-2::GFP to oocyte chromatin (Govindan et al., 2009). The control data were published (Govindan et al., 2009), and the *pde-6* data were from a contemporaneous experiment.
### Table S6 Genetic analysis of twk-1(ifs) for meiotic maturation and Unc phenotypes

#### A. twk-1(ifs) suppresses acy-4(ifs) sterility

| Genotype                        | Brood size (± SD) | Number of animals scored |
|---------------------------------|-------------------|--------------------------|
| acy-4(ok1806)                   | 1 (± 2)           | 30                       |
| twk-1(tn1397); acy-4(ok1806)    | 36 (± 25)         | 36                       |
| twk-1(tn1397); acy-4(ok1806); tnEx180\(^a\) | 2\(^c\) (± 7)     | 46                       |
| twk-1(tn1397); acy-4(ok1806); tnEx181\(^b\) | 1\(^c\) (± 1)     | 40                       |

\(^a\)tnEx180[twk-1(+) sur-5::gfp]. tnEx180 itself does not reduce brood size because twk-1(tn1397); acy-4(ok1806)/nT1[qIs51]; tnEx180 hermaphrodites have 267 ± 36 (n=25) progeny.

\(^b\)tnEx181[twk-1::gfp str-1::gfp]. twk-1(tn1397); acy-4(ok1806)/+ animals bearing tnEx180 or tnEx181 are not Unc, indicating rescue of the twk-1 adult-onset Unc phenotype.

\(^c\)P<0.00001 compared to twk-1(tn1397); acy-4(ok1806) using Student’s t-test.

#### B. The adult-onset Unc phenotype of twk-1(tn1397) requires acy-4(+)

| Genotype                        | Percentage of Unc animals\(^a\) | Number of animals scored |
|---------------------------------|---------------------------------|--------------------------|
| Wild type                       | 0                               | 152                      |
| twk-1(tn1397)                   | 76                              | 106                      |
| twk-1(tn1397); tnEx180\(^b\)    | 0                               | 126                      |
| twk-1(tn1397); acy-4(ok1806)    | 0                               | 93                       |
| twk-1(tn1397); acy-4(ok1806); tnEx37\(^c\) | 93\(^c\)                       | 94                       |

\(^a\)L4 hermaphrodites were transferred to new NGM plates and observed approximately 24 hrs later for the twk-1(tn1397) Unc phenotype. All L4s tested did not show the Unc phenotype at the time of picking.

\(^b\)tnEx180[twk-1(+) sur-5::gfp].

\(^c\)tnEx37[acy-4(+)] sur-5::gfp.
**Figure S1** Location of Sacy alleles of *tom-1*. Each *tom-1* gene isoform is depicted. The start codon (+1) of the *tom-1a* gene isoform is indicated. Newly identified *tom-1* *tn* alleles and the corresponding amino acid changes are shown. Deletion alleles are underlined. Apparently, *tom-1* is a complex locus and only *ok2437, tn1463, and tn1454* suppress *acy-4(lf)* sterility—the *tom-1* isoform(s) relevant to this observation are unclear.
Figure S2  *kin-1* genetic mosaics with complex losses within the somatic gonadal cell lineages. Genetic-mosaic animals with complex losses of the *kin-1(+) array in the somatic gonad (see Figure 1 in the main text) are described in more detail. Green characters represent the presence of the *kin-1(+) array and red characters represent the absence of the *kin-1(+) array. Uterine cells were not scored. One sterile genetic mosaic animal (star) resulted from multiple independent losses within both the Z1 and Z4 lineages. This animal is consistent with a requirement for *kin-1(+) function in the gonadal sheath cells for meiotic maturation.
**Figure S3** *sacy-1* is a negative regulator of oocyte meiotic maturation in the absence of sperm. (A) Control female mutants. Oocytes are stacked within the gonad arm and the uterus is empty. Spermatheca (sp). (B) *sacy-1*(tn1385) inhibits oocyte meiotic maturation in the absence of sperm. Oocytes undergo maturation and ovulation at an increased frequency and are observed in the uterus despite the absence of sperm. A similar phenotype was observed in *fog-1*(e2121) *sacy-1*(tn1385) females (S. Kim and D. Greenstein, unpublished results). (C) *sacy-1*(tm5503) is a strong negative regulator of oocyte meiotic maturation in the absence of sperm. Many unfertilized oocytes are observed in the uterus of *sacy-1*(tm5503) females, suggesting that *sacy-1* inhibits oocyte meiotic maturation in the absence of sperm. The frequent occurrence of defective ovulation in *sacy-1*(tm5503) females prevented us from directly measuring meiotic maturation rates. *fog-1*(e2121) *sacy-1*(tm5503) females exhibit a similar phenotype (S. Kim and D. Greenstein, unpublished results). All images were obtained from day-1 adults (24 hr post-L4) examined by DIC microscopy. *fog-3*(q470) and *sacy-1*(tm5503) *fog-3*(q470) images were duplicated from Figure 8 for comparison. Scale bar, 50 µm.
Figure S4  Representative DIC images of day-1 adults illustrating the qualitative scoring criteria used to evaluate the severity of the gamete degeneration phenotype of sacy-1(tm5503) hermaphrodites and females. Scale bar, 50 µm.
Figure S5  Masculinization of the hermaphrodite germ line does not suppress sacy-1(tm5503) gamete degeneration. A sacy-1(tm5503); fem-3(q20gf) hermaphrodite (bottom) exhibits the gamete degeneration phenotype. A sacy-1(tm5503)/+; fem-3(q20gf) hermaphrodite (top) produces excessive amounts of sperm. Scale bar, 50 µm.
Figure S6  *sacy-1(tm5503)* females do not produce MSP. Western blot of MSP from wild-type (WT), *fog-1(e2121) sacy-1(tm5503)*, and *sacy-1(tm5503) fog-3(q470)* adults. Because oocyte meiotic maturation occurs frequently in *sacy-1(tm5503)* females despite the absence of MSP, *sacy-1* is a strong negative regulator of meiotic maturation. Ten animals of each genotype were lysed and immunoblotting was conducted using a rabbit C-terminal-specific anti-MSP antibody (C3196; 1:20,000) (Kosinski et al., 2005). Mouse anti-actin C4 antibody (Millipore; 1:20,000) served as a loading control.
Figure S7  *sacy-1(tm5503)* is epistatic to *acy-4(ok1806)* for oocyte meiotic maturation. Oocyte meiotic maturation occurs frequently in *sacy-1(tm5503) fog-3(q470); acy-4(ok1806)* females, though ovulation often fails and endomitotic oocytes are observed in the gonad arm. In contrast, oocytes arrest in diakinesis in *fog-3(q470); acy-4(ok1806)* females. Day-1 adults were examined by DIC microscopy of living animals (A) and DNA was detected by DAPI staining of dissected and fixed gonads (B). Proximal is to the left side. Scale bar, 50 μm.
Figure S8  Phylogenetic comparison *C. elegans* and human phosphodiesterases. The likely human ortholog of *C. elegans* PDE-6 is PDE8, the high-affinity cAMP-specific phosphodiesterase. Red characters represent six *C. elegans* phosphodiesterases, and blue characters represent 11 human phosphodiesterases. The following amino acid sequences were used for the analysis; *Ce_PDE-1* (NP_493343.1), *Ce_PDE-2* (NP_001022705), *Ce_PDE-3* (NP_871943.2), *Ce_PDE-4* (NP_001040798.1), *Ce_PDE-5* (NP_491544.2), *Ce_PDE-6* (NP_490787.1), *Hs_PDE1* (NP_000915.1), *Hs_PDE2* (NP_002590.1), *Hs_PDE3* (NP_000913.2), *Hs_PDE4* (NP_001098101.1), *Hs_PDE5* (NP_001074.2), *Hs_PDE6* (NP_000274.2), *Hs_PDE7* (NP_001229247.1), *Hs_PDE8* (NP_001025024.1), *Hs_PDE9* (NP_002597.1), *Hs_PDE10* (NP_001124162.1), *Hs_PDE11* (NP_058649.3).
Figure S9  spr-5 RNAi suppresses acy-4(1f) sterility. spr-5 dsRNAs, or a buffer control, were injected into the gonad arms of acy-4(ok1806); tnEx37[acy-4(+)] sur-5::gfp or rrf-1(pk1417); acy-4(ok1806); tnEx37[acy-4(+)] sur-5::gfp adult hermaphrodites, and non-array-bearing F1 animals were scored by DIC microscopy for suppression of acy-4(ok1806) sterility. Fertile animals were defined as having more than two embryos in the spermathecae and/or uterus. The numbers of animals scored are indicated.
Figure S10  Growth defect of acy-4(ok1806) on HT115 bacteria. (A) acy-4(ok1806) animals exhibit larval lethality, larval arrest, or slow growth on standard NGM plates with E. coli HT115 as the food source. In contrast, wild-type, acy-4(ok1806); tnEx37[acy-4(+);sur-5::gfp], and acy-4(ok1806) spr-1(gk734) animals grow well with HT115 as the food source. Likewise, spr-2, spr-4, and spr-5 mutations suppress the acy-4(ok1806) growth defect (not shown). Embryos isolated by bleach treatment were cultured on HT115-seeded plates for approximately 72 hrs. acy-4(ok1806) homozygotes were the GFP-negative progeny of array-bearing parents. Scale bar, 1 mm. (B) DIC images of wild type (WT) and acy-4(ok1806) animals grown on HT115-seeded medium for 72 hrs. Scale bar, 50 µm.
**Figure S11**  *twk-1* functions in the somatic gonad downstream of *acy-4*. A genetic mosaic animal resulting from multiple independent losses of the *twk-1(+)*-bearing extrachromosomal array in the anterior somatic gonadal lineage (Z1). This animal was fertile in the anterior arm, indicating suppression of *acy-4(/f)* sterility, but sterile in the posterior arm. Green characters represent the presence of the *twk-1(+) array, and red characters represent the absence of the *twk-1(+) array. Uterine cells were not scored.*
Figure S12  The TWK-1 C-terminus is not essential for function. (A) ClustalW alignment of *C. elegans* TWK-1 and human TREK-2 (NP_612190). Using RT-PCR, we found that the TWK-1 prediction (NP_492054.2) was incorrect in that a small predicted intron was found to be coding, resulting in an additional 14 amino acids. The RT-PCR data is in agreement with the gene prediction based on the FN890018 cDNA. We thank Bernard Lakowski for pointing out the discrepancy in the *twk-1* gene prediction. The four transmembrane domains (M1-M4) and two pore domains (P1 and P2) are underlined. The three *twk-1* nonsense mutations and the position of the ΔC284 deletion are indicated. TWK-1 exhibits less similarity to TREK-1 (not shown) in the C-terminal region because of the latter’s smaller size. (B) The TWK-1:DC284::GFP fusion (*tnEx188*) rescues *twk-1(tn1397)*. Error bars represent one standard deviation. Student’s t-test was used for statistical analysis.
| Gene | Allele | Nucleotide change | Amino acid change$^a$ |
|------|--------|------------------|---------------------|
| *pde-6* | tn1237 | CAG → TAG | Q668* |
|       | tn1242 | TGG → TGA | W444* |
|       | tn1336 | TGG → TGA | W664* |
| *tom-1* | tn1454 | Tc1 insertion | |
|       | tn1463 | GGT → AGT | G498S |
| *sacy-1* | tn1385 | GGA → AGA | G533R |
|       | tn1391 | GGA → AGA | G473R |
|       | tn1440 | GGA → AGA | G331R |
| *twk-1* | tn1397 | TGG → TAG | W177* |
|       | tn1398 | TGG → TAG | W330* |
|       | tn1403 | GAA → TAA | E272* |
| *spr-4* | tn1383 | CGA → TGA | R1230* |
|       | tn1402 | CGA → TGA | R1230* |
|       | tn1404 | CAT → TAT | H845Y |
|       | tn1438 | CGA → TGA | R486* |
|       | tn1444 | CAG → TAG | Q1128* |
|       | tn1467 | CAG → TAG | Q45* |
| *spr-5* | tn1378 | TGG → TAG | W666* |
|       | tn1379 | GAG → AGA | E164K |
|       | tn1394 | GGT → GAT | G619D |
| *uev-1* | tn1381 | GGA → GAA | G17E |
|       | tn1382 | GGT → GAT | G47D |
| *spr-2* | tn1380 | GAT → GCT | D42A |
|       | tn1436 | Tc1 insertion | |

$^a$Premature stop codons are indicated by asterisks. The numbering of amino acids refers to the isoforms PDE-6A, TOM-1A, SPR-4A, and SPR-2B.
| Strain | Genotype |
|--------|----------|
| N2     | Wild type, Bristol isolate |
| CB4856 | Hawaiian isolate |
| DG2730 | tnl1[N2->CB4856, acy-4(ok1806)] V; tnEx37[acy-4(+); sur-5::gfp] |
| DG3051 | tnl1[acy-1::gfp rol-6[su1006d]] |
| DG3064 | tnl1[acy-2::gfp rol-6[su1006d]] |
| DG3066 | tnl1[acy-3::gfp rol-6[su1006d]] |
| DG3395 | tnl1[acy-4::gfp rol-6[su1006d]] |
| DG3441 | tnl1[acy-5::gfp str-1::gfp] |
| DG3521 | fog-1[e2121] sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III) |
| DG3524 | sacy-1(1385) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III) |
| DG3585 | sacy-1(1385) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); acy-4(ok1806) V; tnEx37 |
| DG3393 | kin-1(ok338) I; tnEx109[kin-1(+); sur-5::gfp] |
| DG3395 | pbrm-1[gk1195] I; acy-4(ok1806) V; tnEx37 |
| VC10166| pbrm-1[gk1195] I; octr-1[gk1196] X |
| DG2292 | pde-6[t1237] I |
| DG2397 | pde-6[t1237] I; unc-46(e177) acy-4(ok1806) V |
| DG2096 | pde-6[t1242] I |
| DG2454 | pde-6[t1242] I; unc-46(e177) acy-4(ok1806) V |
| DG2111 | pde-6[t1336] I |
| DG2834 | rrf-1[pk1417] I; acy-4(ok1806) V; tnEx37 |
| DG3606 | rrf-1[pk1417] I; fog-2[oz40] V |
| DG3544 | sacy-1(tm5503) fog-3[q470] l/hT2[bl1::e937] let-?:{q782} qls48] (I;III) |
| DG3589 | sacy-1(tm5503) fog-3[q470] l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); acy-4(ok1806) V; tnEx37 |
| DG3557 | sacy-1(tm5503) fog-3[q470]; unc-13(e1091) lin-11(n566) I; oma-1[2u405te33] IV/nT1[qsl51] (IV;V); oma-2[te51] V/nT1[qsl51] (IV;V) |
| DG3492 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III) |
| DG3587 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); acy-4(ok1806) V; tnEx37 |
| DG3514 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); ced-3(n717) IV |
| DG3566 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); ced-4(n1162) III/hT2[bl1::e937] let-?:{q782} qls48] (I;III) |
| DG3528 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); fem-3[e1996]/unc-24[e138] dpy-20(e1282) V |
| DG3570 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); fem-3[q20g] V |
| DG3505 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); fem-3[82g] V |
| DG3512 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); fem-2[171] V |
| DG3486 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); him-8(tm611) IV |
| DG3561 | sacy-1(tm5503) l/hT2[bl1::e937] let-?:{q782} qls48] (I;III); unc-24[e138] IV |
DG3560  sacy-1(tm5503) I/hT2[bli-4(e937) let-?(q782) qls48] (I;III); unc-32(e189) III/hT2[bli-4(e937) let-?(q782) qls48] (I;III)

DG3571  sacy-1(tm5503) I/hT2[bli-4(e937) let-?(q782) qls48] (I;III); unc-33(mn407) IV

DG3547  sacy-1(tm5503) I/hT2[bli-4(e937) let-?(q782) qls48] (I;III); unc-68(e540) V

DG3485  sacy-1(tm5503) I; tnEx159[gfp::sacy-1 unc-119(+)]

DG3611  sacy-1(tm5503) I; unc-119(ed3) III; tnEx159[gfp::sacy-1 unc-119(+)]

DG3520  sacy-1(tm5503)/unc-13(e1091) lin-11(n566) I; fem-3(e1996) V/hT1[qls51] (IV;V)

DG3539  sacy-1(tm5503)/unc-13(e1091) lin-11(n566) I; oma-1[zu405te33] IV/hT1[qls51] (IV;V); oma-2[te51]

DG3543  V/nT1[qls51] (IV;V)

DG3430  sacy-1(tn1385) I

DG3373  sacy-1(tn1385) I; acy-4(ok1806) V

DG3542  sacy-1(tn1385) I; fem-3(e1996) IV/nT1[qls51] (IV;V)

DG3449  sacy-1(tn1385) I; fog-2(oz40) V

DG3460  sacy-1(tn1385) I; fog-2(q71) V

DG3453  sacy-1(tn1385) kin-1(ok338) I; tnEx109

DG3070  sacy-1(tn1385) pbrm-1(tn1475) I; acy-4(ok1806) V

DG3254  sacy-1(tn1385) pbrm-1(tn1475) I; acy-4(ok1806) V; tnEx37

DG3249  sacy-1(tn1391) I

DG3060  sacy-1(tn1391) I; acy-4(ok1806) V

DG3414  sacy-1(tn1391) I; fog-2(oz40) V

DG3461  sacy-1(tn1391) I; fog-2(q71) V

DG3314  sacy-1(tn1391) I; unc-119(ed3) III; acy-4(ok1806) V; tnEx37; tnEx159

DG3431  sacy-1(tn1440) I

DG3362  sacy-1(tn1440) I; acy-4(ok1806) V

DG3478  sacy-1(tn1440) I; fog-2(oz40) V

DG3450  sacy-1(tn1440) I; fog-2(q71) V

DG3115  sacy-1(tn1440) pbrm-1(tn1476) I; acy-4(ok1806) V

DG3256  sacy-1(tn1440) pbrm-1(tn1476) I; acy-4(ok1806) V; tnEx37

DG3458  sacy-1(tn1440) spr-5(by134) I; acy-4(ok1806) V

DG2964  spr-4(by105) I; acy-4(ok1806) V

DG3073  spr-4(tn1383) I; acy-4(ok1806) V

DG3074  spr-4(tn1402) I; acy-4(ok1806) V

DG3107  spr-4(tn1404) I; acy-4(ok1806) V

DG3108  spr-4(tn1438) I; acy-4(ok1806) V

DG3109  spr-4(tn1444) I; acy-4(ok1806) V

DG3110  spr-4(tn1467) I; acy-4(ok1806) V

DG2942  spr-5(ar197) I; acy-4(ok1806) V

DG2941  spr-5(by134) I; acy-4(ok1806) V
DG3185  spr-5(by134) I; fog-2(az40) V
DG3122  spr-5(by134) kin-1(ok338) I; tnEx109
DG2553  spr-5(tn1378) I; acy-4(ok1806) V
DG2564  spr-5(tn1379) I; acy-4(ok1806) V
DG2522  spr-5(tn1394) I; acy-4(ok1806) V
NM1815  tom-1(ok188)
DG3192  tom-1(ok188) I; acy-4(ok1806) V; tnEx37
RB1887  tom-1(ok2437) I
DG3079  tom-1(ok2437) I; acy-4(ok1806) V
VC223  tom-1(ok285) I
DG3217  tom-1(ok285) I; acy-4(ok1806) V; tnEx37
FX04724  tom-1(tm4724) I
DG3245  tom-1(tm4724) I; acy-4(ok1806) V; tnEx37
DG3118  tom-1(tn1454) I; acy-4(ok1806) V
DG3097  tom-1(tn1463) I; acy-4(ok1806) V
DG3408  twk-1(tn1397) I
DG3095  twk-1(tn1397) I; acy-4(ok1806) V
DG3447  twk-1(tn1397) I; acy-4(ok1806) V; tnEx37
DG3490  twk-1(tn1397) I; acy-4(ok1806) V; tnEx180
DG3451  twk-1(tn1397) kin-1(ok338) I; tnEx109
DG3389  twk-1(tn1398) I
DG3083  twk-1(tn1398) I; acy-4(ok1806) V
DG3390  twk-1(tn1403) I
DG3113  twk-1(tn1403) I; acy-4(ok1806) V
DG3459  twk-1(tn1403) spr-5(by134) I; acy-4(ok1806) V
RB1976  uev-1(ok2610) I
DG3409  uev-1(ok2610) I; acy-4(ok1806) V
DG3076  uev-1(tn1381) I; acy-4(ok1806) V
DG3080  uev-1(tn1382) I; acy-4(ok1806) V
CB3778  tra-2(e2020) II
CB3843  fem-3(e1996)/unc-24(e138) dpy-20(e1282) IV
CA257  him-8(tm611) IV
DG2836  spr-2(ar199) dpy-20(e1282) IV; acy-4(ok1806) V; tnEx37
DG2943  spr-2(ar211) dpy-20(e1282) IV; acy-4(ok1806) V
DG3136  spr-2(tm4802) IV; acy-4(ok1806) V
DG2558  spr-2(tn1380) IV; acy-4(ok1806) V
DG2592  spr-2(tn1436) IV; acy-4(ok1806) V
DG3612  acy-4(ok1806) spr-1(gk734) V
DG3604  acy-4(ok1806) unc-23(e25) V; tnEx37
|    | Genotype                        |
|----|--------------------------------|
| DG3145 | acy-4(ak1806); spr-3(by108) X |
| DG3129 | acy-4(ak1806); spr-3(ak2525) X |
| DG3391 | acy-4(ak1806); tnEx37          |
| BS553  | fog-2(az40) V                  |
| CB4108 | fog-2(q71) V                   |
| Name                  | Sequence                                                                 | Use                                  |
|-----------------------|--------------------------------------------------------------------------|--------------------------------------|
| ok1806F1              | CGGGTTGATGAATCTTGATCATTTCTC                                               | acy-4(ok1806) genotyping             |
| ok1806R1              | CATGATACAAACATTGTCAGGAGACT                                               | acy-4(ok1806) genotyping             |
| ok1806F2              | TCAGTCTCGTATTGTTGGTCTGTC                                                 | acy-4(ok1806) genotyping             |
| gk1195_F1             | TTCATCTGAATTGCCATTCACTCTTGATC                                             | pbrm-1(gk1195) genotyping            |
| gk1195_F2             | TTTCAAGGAAAGCGGAACTGCAATTTTGGAAG                                       | pbrm-1(gk1195) genotyping            |
| gk1195_R1             | CTCTTGTATACCTCAATCTCGGTCTC                                                | pbrm-1(gk1195) genotyping            |
| H27M09.1_F5           | ACTGCTGGAAAGATATACCTCTTAATAGTC                                          | sacy-1(tm5503) genotyping            |
| tm5503_F1             | GTTATTTGACGAATCCAGCGCAGAAATTTTGGAAG                                      | sacy-1(tm5503) genotyping            |
| tm5503_R1             | CTTGATCTGAAATGCCTGCGCTGTC                                                | sacy-1(tm5503) genotyping            |
| H27M09.1_RNAi_F       | TTTTAATACGACTCATACTAGGGAAGCTGATGAGGAG                                 | sacy-1 RNAi                          |
| H27M09.1_RNAi_R       | TTTTAATACGACTCATACTAGGGAAGCTGATGAGGAG                                 | sacy-1 RNAi                          |
| H27M09.1_cDNA_F1      | TTTCCGGGGAATATGAGGACCAGCAGGGAAATTTTGGAAG                                 | sacy-1 cDNA 5' half RNAi             |
| H27M09.1_cDNA_R1      | TTTCTCGACGTCTAACTGCTTTGAGCGTCCGCAATTTG                                  | sacy-1 cDNA 5' half RNAi             |
| H27M09.1_cDNA_F2      | TTTCCGGGGAATATGAGGACCAGCAGGGAAATTTTGGAAG                                 | sacy-1 cDNA 3' half RNAi             |
| H27M09.1_cDNA_R2      | TTTCTCGACGTCTAATGCGCTGCCTGATTGCAATTTG                                  | sacy-1 cDNA 3' half RNAi             |
| acy-1_13_F            | AAGGTTGATGAATCTCATACTACAGGGAAGCTGATGAGGAG                               | Recombining for acy-1::gfp            |
| acy-1_13_R            | TGTGAAGGAAATTGATAGGATTTTAAAGCAAATGATG                                   | Recombining for acy-1::gfp            |
| acy-2_17_F            | CCGGCAGGACCAAAAACCATGAGAAACAAATATATATGACATG                             | Recombining for acy-2::gfp            |
| acy-2_17_R            | GAGAGAAGGATGATGCGATGAGGAGGAAAATCAAGAGC                                  | Recombining for acy-2::gfp            |
| acy-3_14_F            | ACAATTTCGGAGACCTCTCACTCACTCTCACTTGATGATG                                | Recombining for acy-3::gfp            |
| acy-3_14_R            | AGACCTCAAGAAGTAAAGAAACATATAATATTGAAAGAAAAATG                             | Recombining for acy-3::gfp            |
| acy-2_13_R            | GTACACCTCATGAAAGGAGAAAACATATTTCCACATG                                   | Recombining for acy-2::gfp            |
| twk-1_GalK_F2         | AGGTTTCCAGATCCAGAAGAGCTGAATTTTGTTGAGGAGG                                 | Recombining for galk                  |
| twk-1_GalK_R2         | TCCGGCGTTGATCCAAATTTTCAGCCACACAACTGACAAATTTTGTTGAGGAGG                   | Recombining for galk                  |
| twk-1_C-ter_trunc     | AGGTTTCCAGATCCAGAAGAGCTGAATTTTGTTGAGGAGG                                 | Recombining for C-terminal deletion   |
| Sequence | Description |
|----------|-------------|
| TCCCAATTCTTGGTA | Recombineering for extending *kin-1* promoter in the WRM069aA02 fosmid |
| KIN-1R 50bp | |
| AATCAACAGTGATGCAACACATTCACAAAAAGTTACCAGAA | |
| CTCTGTGG | |
| KIN-1OF 50bp | Recombineering for extending *kin-1* promoter in the WRM069aA02 fosmid |
| TAAAGTTGGGATAACGCCAGGATTTCACGTCAGTGTGTA | |
| AAACGACGAGAGACACCTGCTCTCGGAGAGTCA | |
| kin-1 galk F | Recombineering for *galk* insertion into the WRM069aA02 fosmid |
| TAAGTTGGTAAACCGCAGGTTTTTCCAGTCAGCAGTTGTA | |
| AAACGACGCTTGTGACAATTAATCATCGGCA | |
| kin-1 galk R | |
| AATCAACACGTAGTACAGATTCACAAAAAGTTACCAGAAA | |
| CTCTGTGGTCACGACTGCTCCCTT | |
| H27M09.1_gfpF1 | Recombineering for *gfp::sacy* |
| TTTTAAGTATCTTTAAAGTAGAAACAGAAAAATTCGTTTAAGTAGA | |
| TTCGGAATGAGTAAGGAGAAACACTTTTCCACGTA | |
| H27M09.1_gfpR1 | Recombineering for *gfp::sacy* |
| CGCGCGGCGTCTCCTATTTCGTCGCGCGTTCTCCACATCTG | |
| TGCTCATTTTTGATAGTATCCATCCATGCTCGA | |
| twk-1_GFP_F1 | Recombineering for *twk-1::gfp* |
| AAGCTCATCGGATCACAGTGTAGTCACCTTTTACTCAAAACACCA | |
| AGATGTGATGATGAGGTGACACATTTTACTCA | |
| twk-1_GFP_R1 | Recombineering for *twk-1::gfp* |
| ATATCCGTTTTTCTTTTTTTTCGAAAAATCTAAATTGCAAAATTCGTTTAAGTAGA | |
| TATACTTTTGCATTAGTCCTCATTGATCATG | |
| twk-1_F1 | |
| GACAAATAGAAGATCCTTTCTCCCTTGATAGGAT | |
| twk-1_R1 | |
| GTGACCAATCCCTGACAGTCATCATTG | |
| twk-1 RT-PCR | |

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### Table S4  *pbrm-1* is not a *Sacy* mutation

| Genotype                                      | Brood size (± SD) | Number of animals scored |
|-----------------------------------------------|-------------------|--------------------------|
| acy-4(ok1806)                                 | 1 (± 2)           | 30                       |
| pbrm-1(gk1195)*                              | 102 (± 86)        | 30                       |
| pbrm-1(gk1195); acy-4(ok1806)                | 0 (± 2)           | 35                       |
| sacy-1(tn1385); acy-4(ok1806)                | 14 (± 15)         | 39                       |
| sacy-1(tn1385) pbrm-1(tn1475)*; acy-4(ok1806) | 23 (± 18)         | 30                       |
| sacy-1(tn1440); acy-4(ok1806)                | 20 (± 19)         | 39                       |
| sacy-1(tn1440) pbrm-1(tn1476)*; acy-4(ok1806) | 12 (± 11)         | 30                       |

*VC10166 [pbrm-1(gk1195) I; octr-1(gk1196) X] strain was used. pbrm-1(gk1195) deletes 126 nucleotides, including an intron and part of exon 6 of the pbrm-1a gene isoform.

*pbrm-1(tn1475) induces a missense mutation (A1182T) in the pbrm-1a gene isoform.*

>P=0.025 compared to sacy-1(tn1385); acy-4(ok1806).

*pbrm-1(tn1476) induces a missense mutation (A157T) in the pbrm-1a gene isoform.*

>P=0.048 compared to sacy-1(tn1440); acy-4(ok1806).

Student’s *t*-test was used for statistical analysis.
Table S5  *pde-6* negatively regulates oocyte meiotic progression

A. *pde-6*(tn1237) is epistatic to *gsa-1(pk75)* for oocyte meiotic maturation

| Genotype               | Fertile gonad arms/Total |
|------------------------|--------------------------|
| gsa-1(pk75)            | 0/9                      |
| gsa-1(pk75); pde-6(tn1237) | 5/6                      |

Genetic mosaics in which the sheath/spermathecal lineages were mutant for *gsa-1* were sought using *tnEx31[gsa-1(+)] sur-5::gfp* for mosaic analysis (Govindan et al., 2009).

B. *pde-6*(tn1237) extends AIR-2::GFP to oocyte chromatin in a hermaphrodite background

| Genotype               | Chromatin localization of AIR-2::GFP | Oocyte position in the gonad arm |
|------------------------|-------------------------------------|---------------------------------|
|                        | −1        | −2       | −3       | −4       | −5       |
| Wild type (n=72)       | 95.8%     | 32%      | 1.4%     | 0%       | 0%       |
| *pde-6*(tn1237) (n=35) | 100%      | 97.1%    | 70.6%    | 10.3%    | 0%       |

Strains were homozygous for *itIs14*, which expresses AIR-2::GFP in the germ line. MSP and Gαs-ACY-4 signaling promotes the localization of AIR-2::GFP to oocyte chromatin (Govindan et al., 2009). The control data were published (Govindan et al., 2009), and the *pde-6* data were from a contemporaneous experiment.
Table S6 Genetic analysis of twk-1(lf) for meiotic maturation and Unc phenotypes

A. twk-1(lf) suppresses acy-4(lf) sterility

| Genotype | Brood size (± SD) | Number of animals scored |
|----------|-------------------|--------------------------|
| acy-4(ok1806) | 1 (± 2) | 30 |
| twk-1(tn1397); acy-4(ok1806) | 36 (± 25) | 36 |
| twk-1(tn1397); acy-4(ok1806); tnEx180 | 2 (± 7) | 46 |
| twk-1(tn1397); acy-4(ok1806); tnEx181 | 1 (± 1) | 40 |

*tnEx180[twk-1(+); sur-5::gfp]. tnEx180 itself does not reduce brood size because twk-1(tn1397); acy-4(ok1806)/nT1[qIs51]; tnEx180 hermaphrodites have 267 ± 36 (n=25) progeny.

*btnEx181[twk-1::gfp str-1::gfp]. twk-1(tn1397); acy-4(ok1806)/+ animals bearing tnEx180 or tnEx181 are not Unc, indicating rescue of the twk-1 adult-onset Unc phenotype.

*P<0.00001 compared to twk-1(tn1397); acy-4(ok1806) using Student’s t-test.

B. The adult-onset Unc phenotype of twk-1(tn1397) requires acy-4(+)

| Genotype | Percentage of Unc animals | Number of animals scored |
|----------|---------------------------|--------------------------|
| Wild type | 0 | 152 |
| twk-1(tn1397) | 76 | 106 |
| twk-1(tn1397); tnEx180 | 0 | 126 |
| twk-1(tn1397); acy-4(ok1806) | 0 | 93 |
| twk-1(tn1397); acy-4(ok1806); tnEx37 | 93 | 94 |

*L4 hermaphrodites were transferred to new NGM plates and observed approximately 24 hrs later for the twk-1(tn1397) Unc phenotype. All L4s tested did not show the Unc phenotype at the time of picking.

*tnEx180[twk-1(+); sur-5::gfp].

*tnEx37[acy-4(+); sur-5::gfp].