Developmental Biology **239,** 177–189 (2001)
doi:10.1006/dbio.2001.0394, available online at http://www.idealibrary.com on IDEAL®

REVIEW

RNAi As a Tool for Understanding Germline Development in *Caenorhabditis elegans*: Uses and Cautions

Eleanor M. Maine[1]

Department of Biology, Syracuse University, 108 College Place, Syracuse, New York 13244

RNA-mediated genetic interference (RNAi) has become a very useful tool for analyzing gene function in development and other processes. RNAi can be used as a complement to traditional genetic studies or as a primary means of determining biological function. However, the efficacy of RNAi depends on a variety of factors that the researcher must take into consideration. This review focuses on germline development in the nematode, *Caenorhabditis elegans*, and discusses the uses and limitations of RNAi in providing new information about gene function as well as the possible endogenous role RNAi plays in germline physiology.

***Key Words: Caenorhabditis elegans*; *C. elegans*; RNA interference; RNAi; germ line; germ cells; development; germline development.**

INTRODUCTION

Within the last few years, RNA-mediated genetic interference (RNAi) has become an increasingly important tool for the study of gene function. In this era of vast genomic sequence information, RNAi can be a very quick method for determining the loss-of-function phenotype of a gene for which no genetic mutants are available. RNAi can also provide complementary information when coupled with more traditional approaches. This review discusses the utility of RNAi within a specific context: the study of germline development in the soil nematode, *Caenorhabditis elegans*. Different ways in which RNAi can be used are introduced in general terms first, and then specific examples are provided.

PRACTICAL CONSIDERATIONS WHEN USING RNAi

RNAi regulates gene expression at the posttranscriptional level, by depleting mRNA, which in turn depletes protein. Guo and Kemphues (1995) found that injection of *in vitro* transcribed antisense or sense strand RNA could produce a severe loss-of-function phenotype. Subsequent studies showed that injection of *in vitro* synthesized double-stranded (ds) RNA causes mRNA that is highly related in sequence to the dsRNA trigger to be degraded (Fire *et al*., 1998; Montgomery *et al*., 1998). Corresponding protein products are depleted over a period of time that depends on the stability of the protein in question, causing a "phenocopy" of the affected gene. Typically, the RNAi phenocopy resembles a strong loss-of-function phenotype, but cannot be counted on to produce a null phenotype. Therefore, this review refers to RNAi "depletion" of gene product rather than elimination and the resulting phenocopy is referred to as *gene-x(RNAi)*. In contrast, a genetic mutation is referred to as *gene-x(−)*. Another term used in some literature is RNAi "knockdown" of gene product, in contrast to a genetic "knockout" mutation.

The details of RNAi are not understood, but work in a variety of systems is providing us with a basic mechanism. The dsRNA trigger is cleaved to ~22 nucleotide (nt) fragments by a RNase III-type nuclease, Dicer (Bernstein *et al*., 2001). Once formed, dsRNA fragments complex with another nuclease to form an RNA-induced silencing complex (RISC) (Hammond *et al*., 2000). With the dsRNA component as a guide, RISC then binds to specific (complementary) mRNA molecules and cleaves them, thus preventing their translation (Hammond *et al*., 2000; Zamore *et al*.,

[1] Fax: (315) 443-2156. E-mail: emmaine@syr.edu.

0012-1606/01 $35.00

2000; Elbashir *et al*., 2001). Although Dicer and RISC have been studied in *Drosophila* tissue culture, the homologous *C. elegans* enzymes will no doubt be analyzed soon.

In *C. elegans*, RNAi experiments typically involve treating an adult hermaphrodite with dsRNA and examining her progeny for a phenotype. This procedure often depletes maternal gene product that is put into the egg as well as zygotically encoded gene product that is produced during embryonic or larval development. In the case of germline-expressed genes (and some somatic genes), a phenotype can often be seen in the treated mother herself after some period of time. Many factors influence the RNAi phenotype that one obtains, and it is important to take them into consideration when planning experiments and analyzing phenotypes. Critical factors include: (1) dsRNA delivery method, (2) developmental stage(s) at which dsRNA is applied, (3) choice of dsRNA sequence, (4) stability of the target protein(s), (5) temperature, and (6) nature of the gene being tested.

Timing and method of dsRNA treatment. Traditionally, dsRNA is injected into the body cavity (pseudocoelom), gonad, or intestine. However, animals can also be soaked in concentrated dsRNA solutions (Tabara *et al*., 1998) or fed a bacterial strain that synthesizes dsRNA (Timmons and Fire, 1998; Timmons *et al*., 2001). Soaking and feeding are advantageous because they are quicker than injection, and they allow L1–L3 larvae, which are too small for injection, to be treated. When studying a gene that is expressed at multiple times in development, it may be important to deplete the gene product at different developmental stages. For example, treatment of young larvae can in some cases bypass a maternal effect phenotype and allow a zygotic phenotype to be examined. As an alternative method, dsRNA can be encoded by a transgene (Tavernakis *et al*., 2000; see below).

Choice of trigger. DsRNA can deplete mRNAs that are less than 100% identical in sequence. In fact, silencing has been reported with only 80% trigger–target identity (e.g., Tabara *et al*., 1998). Thus, if the intended target gene is a member of a multigene family, then a dsRNA trigger synthesized from that gene may also silence the other family member. Given our current understanding of the mechanism of RNAi, perhaps short (~22 nucleotide) regions of high identity within a context of lower identity will trigger RNAi. However, Parrish *et al*. (2000) have shown that, for at least some genes, this does not occur, while the overall percent sequence identity between trigger and target sequences is critical.

Characteristics of the gene/protein. RNAi efficiency seems to depend on characteristics of the target gene. For example, when examining a maternal effect of the P0 generation, the time between dsRNA treatment and phenotypic response is highly variable. To some extent, the response time depends on stability of the target protein. Any protein existing at the time of treatment must turn over before the treated animals can take on a phenocopy. As another example, two or three adjacent *C. elegans* genes are sometimes transcribed together as one long primary transcript, which is then processed into discrete mRNAs. If RNAi were able to target pre-mRNA, then dsRNA corresponding to one gene in an "operon" might silence the other, cotranscribed genes, as well. Such cross-silencing has been reported in one case (Bosher *et al*., 1999), suggesting that RNAi can target nuclear pre-mRNA as well as cytoplasmic mRNA. However, in other cases, RNAi specifically silences an individual member of an operon (e.g., Korf *et al*., 1998).

Temperature. For reasons that are unclear, the response of some genes to RNAi is temperature dependent. Oftentimes, a gene will respond best at high temperature (e.g., 25°C); in rare cases, it responds best at low temperature (e.g., 15°C). Therefore, if no RNAi phenotype is seen at one temperature, it is worthwhile checking others. In addition, a steady temperature will provide the most reliable comparison from one experiment to another (e.g., see Kuznicki *et al*., 2000).

SPECIFIC USES OF RNAi

In *C. elegans*, RNAi is often used to investigate the homologs of genes of known function in other organisms. However, it can be useful as a complement to genetic studies and as a method for investigating the function of genes identified by biochemical approaches. The various uses of RNAi are outlined in this section. Specific examples are provided within the context of germline development, below. Tables 1–3 contain additional examples.

RNAi as a complement to genetic studies. RNAi can provide additional information that may not be gleaned from a traditional genetic approach. (1) RNAi can reveal a maternal effect phenotype that is not apparent from analysis of genetic mutations. Mutations that disrupt germline development often cause sterility. Therefore, unless a conditional allele is available, it is not possible to examine the progeny of mutant mothers to assess whether maternally contributed gene product functions in the next generation. RNAi often depletes stores of maternal message that would normally go into the oocyte and can reveal a maternal effect. However, maternally contributed *protein* that is already present at the time of RNAi treatment will not be depleted. (2) RNAi can be used to look at phenotypic interactions. For technical reasons, it may be difficult to look at the genetic interactions among genes with similar phenotypes. Instead, RNAi can be used to deplete some or all of the gene products. As RNAi does not usually produce a null phenotype, the most rigorous way to test for interactions is to confirm that different combinations of genetic mutations and RNAi [e.g., *gene-1(−) gene-2(RNAi)* and *gene-1(RNAi); gene-2(−)*] produce the same phenotype. (3) RNAi can provide a relatively strong loss-of-function phenotype compared to certain missense mutations.

Analysis of multigene families. Traditional genetic screens rarely recover mutations in functionally redundant genes. RNAi provides a means to detect functional redun-

TABLE 1
RNAi As a Complement to Genetic Studies

Gene(s)	Gene product	Germline function	Reference
cye-1	cyclin E	cell cycle progression	Fay and Han, 2000
sel-8 (lag-3)	effector in GLP-1 mediated signaling	proliferation	Doyle *et al.*, 2000; see also Petcherski and Kimble, 2000
mlc-4	myosin light chain	cytokinesis	Shelton *et al.*, 1999
lin-5	Novel	mitotic/meiotic spindle formation	Lorson *et al.*, 2000
mei-1, mei-2	katinin microtubule-severing complex	meiotic spindle assembly	Clark-Maguire and Mains, 1994; Srayko *et al.*, 2000
cyk-1	FH gene	cytokinesis	Swan *et al.*, 1998

dancy among structurally related gene products. This is especially helpful in a species like *C. elegans* where multicopy gene families are prevalent. Silencing an individual member of a highly conserved gene family may require careful selection of a portion of the transcript that is least conserved, and in some cases may not be possible. Also, when more than one gene is targeted at a time, the RNAi response of individual genes may be weakened (e.g., Gonczy *et al.*, 2000), perhaps reflecting saturation of the RNAi machinery (see Parrish *et al.*, 2000). However, combinatorial RNAi has clearly been effective in a number of cases (e.g., Kuznicki *et al.*, 2000; Schubert *et al.*, 2000).

Analysis of C. elegans homologs of genes of known function. RNAi can be used to investigate the function of regulatory genes that have been identified in other species. Two basic approaches are seen in the literature: in-depth analysis of a *C. elegans* gene or gene family; and in-depth analysis of gene function in another species, with an evaluation (often cursory) of the RNAi phenotype associated with a *C. elegans* homolog. One note of caution here: *C. elegans* phenotypes can be difficult to evaluate for the uninitiated, so it is best to seek expert advice when doing so. There are published examples of incorrectly scored phenotypes (data not shown)!

Analysis of genes identified by biochemical means. RNAi can detect the biological importance of a gene identified by two- or three-hybrid screens or microarray analysis. In these studies, antibody staining is often used to monitor how efficient RNAi is at depleting the protein of interest.

Regulation of development in other nematode species. Researchers are beginning to compare developmental regulation in related nematode species. RNAi provides a quick way to compare gene function in different species because it circumvents the need for doing extensive background genetics. Unfortunately, some nematode species appear to be resistant to RNAi (F. Piano, personal communication).

TABLE 2
Examples of Multigene Families/Functional Redundancy Examined by RNAi

Gene(s)	Gene products	Germline function	Reference
nos	Nanos-related	viability	Subramaniam and Seydoux, 1999; Kraemer *et al.*, 1999
puf and *fbf*	Pumilio-related	viability	Subramaniam and Seydoux, 1999; Zhang *et al.*, 1997
glh	Vasa/RNA helicase family	P granule components; proliferation/gametogenesis	Gruidl *et al.*, 1996; Kuznicki *et al.*, 2000
ncc-1 and 5 relatives	cdc2-related kinases	cell cycle progression	Boxem *et al.*, 1999
cdk-4	CDK4/6	cell cycle progression	Park and Krause, 1999
cdc-25.1–cdc-25.4	cdc25-related phosphatases	cell cycle progression	Ashcroft *et al.*, 1999
ima	importin α subunits	*ima-3:* meiotic progression	Geles and Adam, 2001
cpb	cytoplasmic polyadenylation element binding protein family	spermatogenesis; sex determination[a]; others?	Luitjens *et al.*, 2000
histone H1	histone H1 isoforms	viability	Jedrusik and Schulz, 2001

[a] *fog-1,* a CPEB family member studied by traditional genetic means (Barton and Kimble, 1990).

TABLE 3
Examples of *C. elegans* Homologs Examined by RNAi

Gene	Product	Germline Function	Reference
pkc-3	protein kinase C isoform	establishment	Tabuse *et al.*, 1998
nmy-2	nonmuscle myosin	establishment	Guo and Kemphues, 1996
T07D3	GERp95/Piwi-related	proliferation	Cikaluk *et al.*, 1999
prg-1, prg-2	Piwi-related	proliferation	Cox *et al.*, 1998
cks-1	cks/suc1-related	meiotic progression	Polinko and Strome, 2000
cyd-1	cyclin D	cell cycle progression	Park and Krause, 1999
lmn-1	nuclear lamin	cell cycle progression	Liu *et al.*, 2000
mdf-2 and *mdf-1*	MAD-2- and MAD-1-related	checkpoint control	Kitgawa and Rose, 1999; Gartner *et al.*, 2000
glc-1	protein phosphatase 1	chromosome condensation	Hsu *et al.*, 2000
air-2	Aurora-like kinase	cytokinesis; chromosome condensation	Schumacher *et al.*, 1998b; Severson *et al.*, 2000; Hsu *et al.*, 2000
bir-1	BIR-related	cytokinesis	Speliotes *et al.*, 2000
plk-1	Polo-like kinase	NEBD	Chase *et al.*, 2000
air-1	Aurora-like kinase	mitotic spindle formation	Schumacher *et al.*, 1998a
czw-1	ZW10-related	kinetochore function	Starr *et al.*, 1997
hcp	holocentric protein-related	kinetochore function	Bushwitz *et al.*, 1999; Moore *et al.*, 1999
dhc-1	dynein heavy chain	oocyte meiotic maturation (various events)	Gonczy *et al.*, 1999
dnc-1	$p150^{Glued}$-related	similar to *dhc-1*	Gonczy *et al.*, 1999; Skop and White, 1998
dnc-2	p50/dynamitin-related		
cds-1, cds-2	Cds1/Chk2 protein kinase family	meiotic recombination	Oishi *et al.*, 2001; Higashitani *et al.*, 2000
ce-rdh-1	recA-like	meiotic recombination	Takanami *et al.*, 1998
mag-1	Mago nashi-related	sex determination	Li *et al.*, 2000
ptc-1	Patched-related	cytokinesis/membrane formation	Kuwabara *et al.*, 2000
syn-4	syntaxin (t-SNARE)	membrane fusion	Jantsch-Plunger and Glotzer, 1999
various	protein trafficking machinery	endocytosis of yolk	Grant and Hirsch, 1999; Greener *et al.*, 2001
spk-1	SR protein kinase	proliferation and/or differentiation	Kuroyanagi *et al.*, 2000
srp genes, *CeSF2*, and others	RNA processing machinery: SR and SR-related proteins	proliferation and/or differentiation	Kawano *et al.*, 2000; Longman *et al.*, 2000; Kuroyanagi *et al.*, 2000

Note. BIR, baculoviral inhibitor-of-apoptosis repeat; NEBD, nuclear envelope break down; CBEP, cytoplasmic polyadenylation element binding.

Molecular studies. Traditionally, when cloning a gene, one confirms the gene identification by DNA-mediated transformation rescue of a mutant phenotype. However, the traditional *C. elegans* transformation method is generally ineffective in the germ line (see Kelly *et al.*, 1997; see below). RNAi is routinely used as an alternative means of verifying gene identity (Guo and Kemphues, 1995). RNAi can also be used to confirm that the phenotype associated with a deletion allele results from the loss of a single gene function (e.g., Karashima *et al.*, 2000).

Large-scale screens for biological function. High throughput RNAi is being used to investigate the loss-of-function phenotypes associated with large sets of genes or cDNAs. These studies can identify developmental functions for previously unstudied genes.

GERMLINE DEVELOPMENT IN *C. elegans*

C. elegans is an excellent model organism for developmental studies because its cell lineage is largely invariant, allowing the precursor cells for each tissue and structure to be identified with confidence. Also, its body is transparent, allowing examination of living cells and tissues and active morphogenesis. *C. elegans* germline development is outlined in brief here. For details, see Hubbard and Greenstein

(2000) and Seydoux and Schedl (2001). The morphology of the developing *C. elegans* germ line was revealed by lineage analysis and detailed microscopy (Hirsh *et al.*, 1976; Klass *et al.*, 1976; Kimble and Hirsh, 1979; Crittenden *et al.*, 1994; Hall *et al.*, 1999). Laser ablation and genetic studies have since identified cell-signaling events that regulate germline proliferation, meiotic progression, ovulation, and oocyte maturation (Kimble and White, 1981; McCarter *et al.*, 1997, 1999).

The germ line is set aside during the initial embryonic cleavage divisions but does not proliferate or differentiate until larval development. The earliest marker for germ line precursor cells are P granules, cytoplasmic structures composed of protein and RNA (see Seydoux and Schedl, 2001; Seydoux and Strome, 1999; see also Schisa *et al.*, 2001). In the adult germ line, P granules are associated with nuclear pores (Pitt *et al.*, 2000). They are deposited into the oocyte cytoplasm and segregate to the germ lineage of the embryo (Fig. 1A; Strome and Wood, 1982). By the fourth cleavage division, a dedicated germ cell precursor, P4, has formed (Fig. 1A). P4 remains quiescent until it divides again later in embryogenesis to form Z2 and Z3. Z2 and Z3 begin to proliferate after the embryo hatches as a L1 larva, and germline proliferation continues throughout the life of the animal. The dividing germ cells are closely associated with the developing somatic gonad. In fact, after the first two to three rounds of mitosis, subsequent germ cell proliferation depends on a signal from two somatic cells at the tips of the gonad, called the distal tip cells (Kimble and White, 1981; Austin and Kimble, 1987). Germ cell divisions, like somatic divisions, also depend on the availability of food (see Hong *et al.*, 1998; Subramaniam and Seydoux, 1999).

As larval development proceeds, the gonad elongates to form a tube and eventually reflexes to form one or two U-shaped arms in males and hermaphrodites, respectively (Fig. 1B). Germ cells at the distal end of the tube remain in mitosis while proximal germ cells enter meiosis. Meiotic chromosomes are first visible during the L3 larval stage, and sperm differentiation is first visible during the L4 stage. In hermaphrodites, the germ line switches from spermatogenesis to oogenesis at about the time of the adult molt. Both males and hermaphrodites continue to make gametes throughout adulthood. In older larvae and adults, the germ line is a syncytium where each nucleus is associated with its own local region of cytoplasm and is partially surrounded by a plasma membrane (Hirsh *et al.*, 1976; Hall *et al.*, 1999). For simplicity, each nuclear-cytoplasmic unit is often referred to as a germ cell. In hermaphrodites, a central cytoplasmic core or rachis is present for much of the length of the germ line. Many oocyte components are synthesized by meiotic nuclei, secreted into the core, and later incorporated in oocytes (Gibert *et al.*, 1984).

Traditional genetic and molecular studies have identified regulators of germline specification, proliferation, and differentiation. RNAi has greatly accelerated the pace of research progress by providing a quick way to identify gene function and examine gene interactions. In the next section, examples of the various uses of RNAi are provided within the context of germline development.

MULTIGENE INTERACTIONS: SPECIFICATION OF THE GERM LINE

Maternally expressed gene products are critical for specifying the germ line in the early embryo. RNAi has been an important tool for testing interactions among many of these genes. In *C. elegans*, the anterior–posterior (A-P) axis of the embryo is determined by the position of sperm entry (Goldstein and Hird, 1996; see review by Goldstein, 2000). This initial cue is interpreted by a set of polarity proteins that include several PAR (embryonic **par**titioning) proteins (reviewed by Kemphues, 2000). The posterior cortical cytoplasm of the early embryo becomes enriched in certain polarity proteins (e.g., PAR-1, PAR-2) while the anterior cortical cytoplasm becomes enriched in others (e.g., PAR-3, PAR-6, PKC-3). Embryonic A-P polarity is important for establishing regional patterns of gene expression in the early embryo and for P granule localization (Fig. 1C).

Analysis of the MEX-5 and MEX-6 (**m**esoderm in **ex**cess) proteins suggests that they translate the A-P positional information into regional patterns of gene expression (see Figs. 1A and 1C; Schubert *et al.*, 2000; see Kemphues, 2000). *mex-5* was identified in genetic screens for genes that regulate blastomere fate, and *mex-6* was identified by its close sequence similarity to *mex-5*. RNAi was used to examine the biological role of MEX-5 and MEX-6 and their interactions with other regulatory proteins in the early embryo. Despite the sequence similarity between *mex-5* and *mex-6*, Schubert *et al.* were able to design gene-specific triggers. *mex-5* mutants produce embryos with defects in cell fate but the germ line is unaffected. *mex-6(RNAi)* animals produce viable, fertile embryos. However, *mex-5(−);mex-6(RNAi)* animals produce embryos with additional defects, including the lack of a germ line. Schubert *et al.* isolated a *mex-6* deletion allele by reverse genetics and showed that *mex-5(−);mex-6(RNAi)*, *mex-5(RNAi);mex-6(RNAi)* and *mex-5(−);mex-6(−)* animals all produce embryos with the same set of defects. Therefore, the RNAi and mutant phenotypes of *mex-5* and *mex-6* are indistinguishable from each other. Schubert *et al.* went on to use RNAi to examine interactions between *mex-5*, *mex-6*, and other regulatory genes such as *pie-1* (**p**harynx and **i**ntestine in **e**xcess), *par-1*, and *par-3*. They also used *mex-5(RNAi);mex-6(RNAi)* animals to show that the distribution of all known lineage-specific proteins depends on MEX-5/MEX-6 activity.

ANALYSIS OF MULTIGENE FAMILIES CONTAINING KNOWN GERMLINE REGULATORS IN OTHER SPECIES: *glh* AND *nos/puf* GENES

All known P granule protein components are RNA binding proteins, consistent with a role for P granules in RNA metabolism. Four members of the RNA helicase family, the

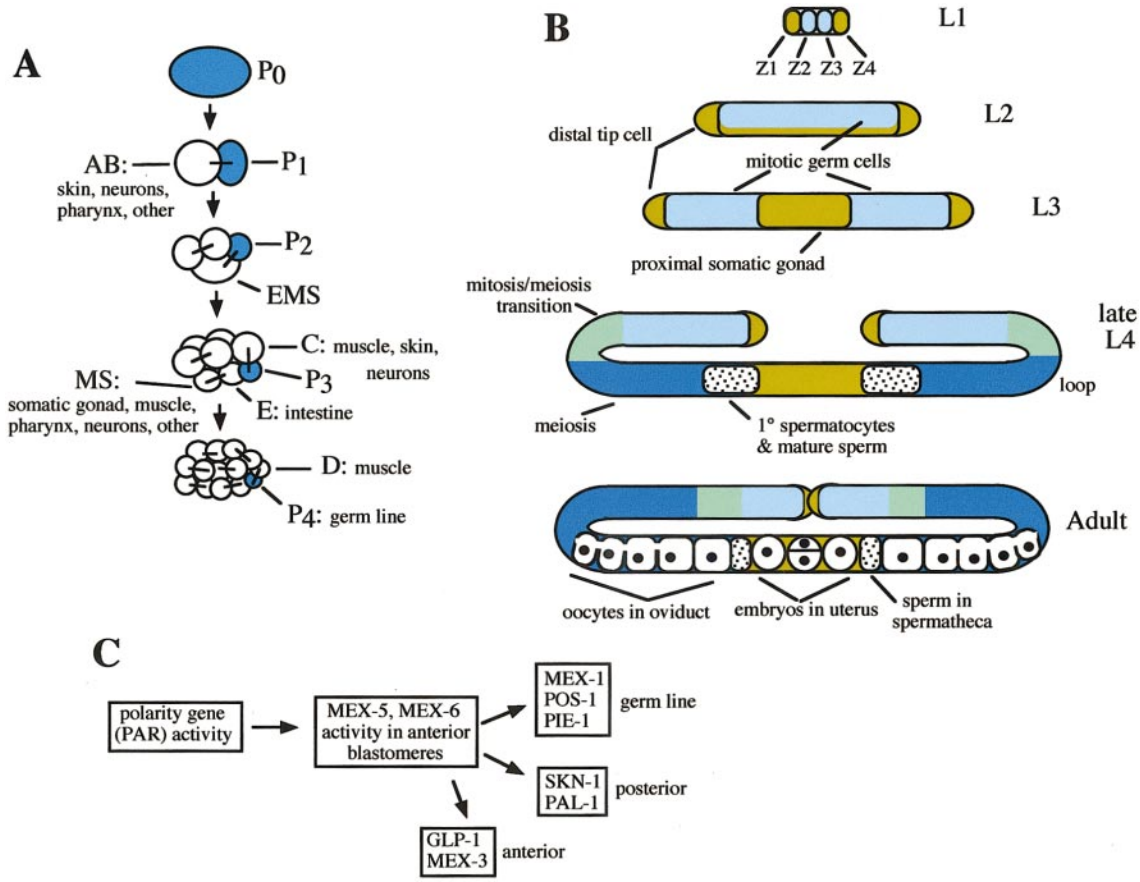

FIG. 1. Overview of germline development. (A) Formation of blastomeres with specified fates, the so-called "founder" cells, AB, C, D, E, MS, and P4. Sister cells are indicated by a line. P granules become localized to the P lineage (shaded) at each round of cell division. Germline-specific proteins are expressed in this lineage as well. (B) Germline development in hermaphrodite larvae and adults. L1 larva hatches with two germline precursors, Z2 and Z3, which divide to form a germline syncytium. Distal tip cells and proximal somatic gonad are shaded gold. Myoepthelial cells associated with the meiotic germ line form the oviduct; they are not expressly indicated. Late-stage oocytes become separated from the syncytium shortly before ovulation, as indicated. Light blue shading, mitotic portion of the germ line; blue green, mitotic-to-meiosis transition region; deep blue, pachytene and later meiotic regions. (C) Hierarchy of gene expression in early blastomeres. PAR and other polarity proteins set up anterior-posterior polarity in the embryo, which then leads to MEX-5/MEX-6 activity in the anterior blastomeres. Consequently, anterior-, posterior- and germline-specific patterns of gene expression arise. In the absence of MEX-5/MEX-6 activity, posterior and germline proteins are also made in the anterior blastomeres, and anterior proteins are absent from the embryo altogether (Schubert *et al.*, 2000).

GLHs (Gruidl *et al.*, 1996; Kuznicki *et al.*, 2000), and a protein called PGL-1 (**P g**ranu**l**e component; Kawasaki *et al.*, 1998) are associated with P granules throughout development. In addition, at least five other RNA binding proteins are associated with P granules in the early embryo (see Strome and Seydoux, 1999; Seydoux and Schedl, 2001). The GLHs (**g**erm**l**ine **h**elicases) are related to *Drosophila* Vasa, which is a component of "polar" granules, the *Drosophila* equivalent of P granules. Vasa has RNA helicase activity (Liang *et al.*, 1994) and functions in germ cell specification (reviewed by Liang *et al.*, 1994). Bennett and co-workers used RNAi to show that the four GLHs are critical for germline proliferation and gametogenesis (Gruidl *et al.*, 1996; Kuznicki *et al.*, 2000). They also used combinatorial RNAi to show that *C. elegans* GLH-1 and GLH-4 are functionally redundant (Kuznicki *et al.*, 2000). However, it is unclear whether these factors function in germline specification in the embryo because injection of dsRNA into adults does not deplete maternally contributed GLH proteins (as shown by labeling with anti-GLH antibodies). Protein stability is a general consideration in RNAi studies because it influences the rate at which animals respond to dsRNA. If protein is very slow to turn over, then RNAi may not be able to produce a phenocopy. With the *glh* genes, perhaps treatment of L3 or early L4 larvae (by feeding or soaking) will deplete maternal protein in the adult so that the contribution of maternal GLH to embryonic development can be addressed. Kuznicki *et al.* found that RNAi

was more effective at higher temperatures (25°C vs 20°C); therefore, temperature can be used to modulate the *glh(RNAi)* phenotype. One point to bear in mind here is that all existing mutations in the P granule component, PGL-1, are temperature-sensitive (Kawasaki *et al.*, 1998). Therefore, P granule assembly or function may be a temperature-sensitive process.

In *C. elegans*, germline viability is maintained during larval development through the action of four *mes* (**m**aternal **e**ffect **s**terile; Capowski *et al.*, 1991) genes, three *nos* (**N**an**os**-like; Subramaniam and Seydoux, 1999; Kraemer *et al.*, 1999) genes, and eight *puf/fbf* (**Pu**milio **f**amily; *fbf*, *f*em-3 **b**inding **f**actor; Zhang *et al.*, 1997) genes. See Seydoux and Strome (1999) and Seydoux and Schedl (2001) for reviews. The *mes* genes were identified in genetic screens (Capowski *et al.*, 1991). In contrast, *nos* and *puf* genes were studied because they encode proteins related to Nanos and Pumilio, two regulators of primordial germ cell formation and development in *Drosophila* (Subramaniam and Seydoux, 1999). Two additional Pumilio-like proteins, FBF-1 and FBF-2, were identified in biochemical assays for regulators of germline sex determination (Zhang *et al.*, 1997; see below). Based on RNAi studies, the activity of most *nos* and *puf/fbf* genes promotes germline viability, and members of each family are functionally redundant. In the most penetrant Nos phenotype seen by Subramaniam and Seydoux (1999), 97% of *nos-1(RNAi); nos-2(RNAi)* and *nos-1(−);nos-2(RNAi)* animals had slowly dividing germ lines that died during L3 stage by a combination of apoptotic and nonapoptotic mechanisms. Addition of *nos-3(RNAi)* had no effect on the phenotype. In contrast, Kraemer *et al.* (1999) obtained quantitatively different results; in their hands, germline death occured in only 41% of *nos-1(RNAi);nos-2(RNAi)* animals; addition of *nos-3(RNAi)* increased this sterility to 79%. The different results may reflect differences in the trigger sequences used (full-length vs. partial cDNA) and/or treatment conditions such as temperature, amount of dsRNA delivered, and time period after treatment during which embryos were collected for analysis. In addition, the N2 wild-type strains used by each laboratory may have subtle differences in their response to RNAi.

Using RNAi, Subramaniam and Seydoux also found that members of the Pumilio family promote similar aspects of germ cell fate as do the NOS proteins. Five-way combinatorial RNAi with *puf-6*, *puf-7*, *puf-8*, *fbf-1*, and *fbf-2* produced 99.2% F_1 progeny with a phenotype similar to *nos-1(RNAi);nos-2(RNAi)* animals. RNAi with individual or pairs of *puf/fbf* genes produced weak, if any, phenotypes. However, neither *puf-6* and *puf-7* nor *fbf-1* and *fbf-2* were assayed individually because the two sets of genes are closely related in sequence. In principle, when working with a multigene family, one might be able to design and synthesize a relatively short trigger that is highly related to one gene and not to others in the family. In some cases, the only suitable sequences might be in the 3′ untranslated region.

RNAi AS A COMPLEMENT TO GENETIC STUDIES: THE ROLE OF *him-3* IN EARLY MEIOSIS

Researchers are beginning to look at the regulation of early meiotic events such as entry into and progress through early meiosis, chromosome pairing and recombination (see Albertson *et al.*, 1997; Seydoux and Schedl, 2001). Analysis of the role of *him-3* in meiotic recombination provides an example of how RNAi can be used to complement genetic studies. See Table 3 for additional examples. If chromosomes pair incorrectly or fail to undergo recombination in early meiotic prophase, then they often segregate improperly during the meiotic divisions (Dernburg *et al.*, 1998). Consequently, mutants with disrupted pairing and recombination often have aneuploid, inviable offspring and a **h**igh **i**ncidence of **m**ale progeny (a Him phenotype). The Him phenotype reflects a defect in chromosome segregation because *C. elegans* males are XO and form as a result of X chromosome non-disjunction. Zetka *et al.* (1999) used RNAi to complement their genetic and molecular studies of *him-3* function and show that HIM-3 is required for chromosome pairing during early meiotic prophase. When they did their study, the available *him-3* mutations caused a reduction in meiotic recombination, but did not decrease the level of HIM-3 protein. In contrast, the HIM-3 level dropped substantially in *him-3(RNAi)* animals. Characterization of the *him-3(RNAi)* phenotype revealed germ lines with severe defects in meiotic chromosome pairing and recombination. Comparison of the RNAi and mutant phenotypes suggested further that the existing mutations specifically influence cross-over frequency rather than synapsis.

DETECTING A REQUIREMENT FOR MATERNAL GENE PRODUCT IN THE EMBRYO: *zen-4*

Many genes act at more than one point in development. In particular, early embryonic development relies on many maternal gene products that are supplied to the oocyte. Often, zygotic expression of the same genes is critical later in development. It can be very difficult to detect a maternal effect using standard genetic mutations without doing genetic mosaic analysis, which is quite laborious. An example is outlined below. Additional examples are included in Tables 1–3; most of them are involved in cell cycle progression or regulation.

Mutations in *zen-4* (**z**ygotic **en**closure) were isolated in screens for morphogenesis-defective mutants that failed to undergo ventral enclosure (Raich *et al.*, 1998). In ventral enclosure, hypodermal epithelium migrates from the dorsal side of the embryo to enclose the ventral and lateral regions. Because the mutant phenotype is lethal, it was not possible to recover *zen-4* mutant mothers and examine their progeny. Instead, RNAi was used to deplete maternal *zen-4*

mRNA. When wildtype animals were treated with *zen-4* dsRNA, their progeny failed to undergo cytokinesis during the oocyte meiotic divisions (which occur after fertilization in *C. elegans*) and subsequent embryonic mitotic divisions. The end result is a multi-nucleate single-celled embryo.

FUNCTIONAL ANALYSIS OF GENES IDENTIFIED VIA BIOCHEMICAL MEANS: THE ROLE OF *fbf-1*, *fbf-2*, AND *fog-2* IN SEX DETERMINATION

Somatic and germline sex determination in *C. elegans* has been studied extensively using traditional genetics as reviewed by Schedl (1997), Meyer (1997), and Kuwabara (1999). Recently, RNAi has provided information about aspects of sex determination that are particular to the hermaphrodite germ line: the process of making a male germ line in a female soma during larval stages and the subsequent switch to a female germ line in the adult. Two very nice cases where a biochemical result was validated using RNAi are in the analysis of the *fbf* (*f*em-3 **b**inding **f**actor) genes (Zhang *et al*., 1997) and *fog-2* (Clifford *et al*., 2000).

To understand better the sperm-oocyte switch in hermaphrodites, Zhang *et al*. (1997) used the yeast three-hybrid assay to identify proteins that bind to the *fem-3* 3′ UTR. Previously, it had been shown that these sequences are required for negative regulation of *fem-3* and for the sperm-to-oocyte switch (Ahringer and Kimble, 1991). Zhang and colleagues used RNAi to examine the biological function of *fem-3* 3′ UTR binding proteins (FBFs). FBFs are encoded by *fbf-1* and *fbf-2*, two genes that are 93% identical at the nucleotide level. Because of the sequence similarity, both genes are silenced by *fbf-1* dsRNA. Zhang *et al*. observed that *fbf(RNAi)* produced a masculinized germ line with excess sperm, as expected for a negative regulator of *fem-3*. Other *fbf(RNAi)* defects included reduced germ cell numbers and abnormal oogenesis.

Genetic analysis of *fog-2* (**f**eminization **o**f the **g**erm line) had identified it as a critical regulator of hermaphrodite germline sex determination (Schedl and Kimble, 1988). *fog-2* activity in larval hermphrodites masculinizes the germ line and ensures that sperm are produced. Clifford *et al*. carried out a yeast two-hybrid screen for proteins that interact with GLD-1 and identified an interactor that maps close to the presumed location of *fog-2* on the physical map (Clifford *et al*., 2000). They tested the RNAi phenotype of this interactor and found that it is Fog. Molecular characterization of *fog-2* mutations confirmed that the interactor gene was indeed *fog-2*. Based on previous genetic and molecular analysis of *gld-1*, it is quite conceivable that GLD-1 might interact with FOG-2 to promote the male sexual fate (Francis, *et al*., 1995; Jones *et al*., 1996).

GERMLINE DEVELOPMENT IN OTHER NEMATODE SPECIES

RNAi is a potentially valuable tool for comparing gene function across species, and researchers are beginning to use RNAi to study germline events in other nematode species. So far, the work has centered on sex determination and germline proliferation. The feminizing gene, *tra-2*, and the masculinizing gene, *her-1*, were silenced in related male/female speies, *C. briggsae* and *C. remanei*, and found to have similar functions to what had been described in *C. elegans* (Kuwabara, 1996; Haag and Kimble, 2000; Streit *et al*., 1999). Rudel and Kimble (2001) investigated the role of *glp-1* in the *C. briggsae* and *C. remanei* germ lines. *glp-1* encodes a Notch-like receptor that is expressed in the *C. elegans* germ line and mediates a proliferative signal from the somatic gonad (Austin and Kimble, 1987, 1989; Yochem *et al*., 1989). GLP-1 and a related receptor, LIN-12, mediate inductive signaling in somatic tissues during embryonic and larval development, as well (see Seydoux and Schedl, 2001). The *Cb-glp-1(RNAi)* and *Cr-glp-1(RNAi)* phenotypes resembled *Ce-glp-1* in the germ line, however intriguing differences were seen in the *Cb-glp-1* somatic phenotype. It appears that *glp-1* function has generally been conserved, but has been expanded in the *C. briggsae* soma.

LARGE SCALE SCREENS FOR BIOLOGICAL FUNCTION

High throughput RNAi has been done to examine the developmental function of most of the predicted genes on individual *C. elegans* chromosomes (Fraser *et al*., 2000; Gonzcy *et al*., 2000) and to examine the function of large collections of tissue-specific or random cDNAs (Shelton *et al*., 1999; Piano *et al*., 2000; Maeda *et al*., 2001). In general, large-scale studies may be most useful for identifying genes with essential embryonic functions rather than post-embryonic functions. For example, the chromosome surveys of Gonczy *et al*. (2000) and Fraser *et al*. (2000) were quite successful at detecting known embryonic phenotypes but much less successful at detecting known post-embryonic phenotypes. However, Maeda *et al*. detected known embryonic and post-embryonic phenotypes quite readily. The results from any given study almost certainly depend in part on differences in method (e.g., temperature, use of genomic vs. cDNA templates, dsRNA delivery method, and parameters used for accepting a RNAi phenotype as valid). In fact, Maeda *et al*. developed a variation on the soaking method of Tabara *et al*. to greatly increase the efficacy of RNAi, at least for some genes.

F. Piano, V. Reinke, and colleagues did an explicit comparison of results from different large-scale studies (personal communication). They are using RNAi to survey the phenotypes associated with a large collection of germline-enriched cDNAs (Reinke *et al*., 2000; personal communication), and many of these genes were previously tested in the chromosome I or III studies of Fraser *et al*. (2000) or

Gonczy *et al.* (2000). By comparing the results from the different large scale studies, they estimate a level of false negatives that ranges from less than 10% to over 30% (F. Piano, personal communication). That is, one study failed to detect a phenotype that the other study did detect. In some cases, one group discounted a valid RNAi phenotype because the penetrance was low; however, most of the differences seem to be methodological. This result emphasizes the important point that a negative result with RNAi provides no information about gene function. It may be that one has not used optimal RNAi conditions for the gene in question.

Maeda *et al.* (2001) detected numerous new genes that play a role in germline development. They identified a large set of cDNAs that cause P0 sterility among treated worms and a smaller set that produce F_1 lethality. Maeda *et al.* focused their phenotypic analysis on the latter as a set of genes that are likely to have a germline-specific function during development. Based on the RNAi data, these genes are likely to function in germline proliferation or maintenance, oogenesis, or gonadogenesis.

DOES RNAi FUNCTION IN GERMLINE DEVELOPMENT?

Screens for RNAi resistant mutants have identified genes that do not seem to be important for development or physiology per se, although they are important in some cases for regulating the transposition of repeated DNA elements (Tabara *et al.*, 1999; Ketting *et al.*, 1999). However, the *ego-1* gene provides a link between development and RNAi. *ego-1* is a germline-expressed gene that is required for germline development and fertility and also promotes RNAi in the germ line (Smardon *et al.*, 2000; Qiao *et al.*, 1995). A large proportion of germline-expressed genes—although not all genes—require *ego-1* activity in order to respond to dsRNA (Smardon *et al.*, 2000; E.M. and V. Vought, unpublished data). Given that some RNAi defective mutants are fertile (Tabara *et al.*, 1999; Ketting *et al.*, 1999), *ego-1* may have entirely separate functions in development and RNAi. Alternatively, *ego-1* may act upstream of the RNAi-specific genes identified by Tabara *et al.* to accomplish a function that is required for both RNAi and development.

Many mutations that prevent RNAi in the germ line also cause genome instability, e.g., high rates of transposition of repeated DNA elements and a Him phenotype (Ketting *et al.*, 1999; Tabara *et al.*, 1999). One hypothesis is that RNAi prevents transposition, and defects in RNAi will allow transposition to occur (Ketting *et al.*, 1999). However, other genes are known to affect only transposition (e.g., *mut-6*) or RNAi (e.g, *rde-1*), suggesting that the relationship between these two events is complex. As suggested by Tabara *et al.* (1999), perhaps there is a transposon-specific RNAi mechanism that is distinct from a more general RNAi mechanism. Genes such as *mut-7* would function in both mechanisms while genes such as *rde-1* would only function in the general mechanism. Another alternative, parallel to the hypothesis described above for *ego-1*, is that genes such as *mut-7* act upstream of RNAi-specific genes to accomplish a function that is required for both RNAi and transposition.

FUTURE DIRECTIONS

RNAi has the potential for wide use in other animal species besides *C. elegans*. As we learn more about the mechanism of RNAi, it should become possible to design more efficient triggers and better assay conditions, and these parameters may be species-specific. For example, injection of *Drosophila* embryos with dsRNA can elicit a strong RNAi phenotype for embryonic genes (Kennerdell and Carthew, 1998) while dsRNA-encoding transgenes work well for eliciting an RNAi response in older animals (Fortier and Belote, 2000; Lam and Thummel, 2000; Martinek and Young, 2000). Indeed, Tavernakis *et al.* (2000) have shown that dsRNA produced *in vivo* from dsRNA-encoding transgenes can produce a strong RNAi response in the nervous system, a tissue that is refractory to exogenously supplied dsRNA. Nonetheless, RNAi may not be useful for studying all genes or all tissues in all organisms. For example, a tissue may remain resistant to RNAi if its physiology requires that it not express certain components of the RNAi machinery.

ACKNOWLEDGMENTS

I thank Linda Ambrosio, John Belote, Jane Hubbard, Fabio Piano, Tim Schedl, Jill Schumacher, and Geraldine Seydoux for insightful comments on the manuscript and Fabio Piano for communicating unpublished data. E.M.M. is supported by the National Science Foundation.

REFERENCES

Ahringer, J., and Kimble, J. (1991). Control of the sperm-oocyte switch in *Caenorhabditis* elegans hermaphrodites by the *fem-3* 3′ untranslated region. *Nature* **349,** 346–348.

Albertson, D. G., Rose, A. M., and Villeneuve, A. M. (1997). Chromosome organization, mitosis, and meiosis. In "*C. elegans* II" (D. L. Riddle, T. Blumenthal, B.J. Meyer, and J. R. Priess, Eds.), pp. 47–78. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ashcroft, N. R., Srayko, M., Kosinski, M. E., Mains, P. E., and Golden, A. (1999). RNA-mediated interference of a cdc25 homolog in *Caenorhabditis elegans* results in defects in the embryonic cortical membrane, meiosis, and mitosis. *Dev. Biol.* **206,** 15–32.

Austin, J., and Kimble, J. (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. *Cell* **51,** 589–599.

Austin, J., and Kimble, J. (1989). Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of *C. elegans*. *Cell* **58,** 565–571.

Barton, M. K., and Kimble, J. (1990). *fog-1*, a regulatory gene required for specification of spermatogenesis in the germ line of *Caenorhabditis elegans*. *Genetics* **125,** 29–39.

Bernstein, E., Caudy, A. A., Hammond, S. M., and Hannon, G. J. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409,** 363–366.

Bosher, J. M., Dufoufcq, P., Sookhareea, S., and Labouesse, M. (1999). RNA interference can target pre-mRNA: Consequences for gene expression in a *Caenorhabditis elegans*. *Genetics* **153,** 1245–1256.

Boxem, M., Srinivasan, D. G., and van den Heuvel, S. (1999). The *Caenorhabditis elegans* gene *ncc-1* encodes a cdc2-related kinase required for M phase in meiotic and mitotic cell divisions, but not for S phase. *Development* **126,** 2227–2239.

Bushwitz, B. J., Ahmad, K., Moore, L. L., Roth, M. B., and Henikoff, S. (1999). A histone-H3-like protein in *C. elegans*. *Nature* **401,** 547–548.

Capowski, E. E., Martin, P., Garvin, C., and Strome, S. (1991). Identification of grandchildless loci whose products are required for normal germ-line development in the nematode *Caenorhabditis elegans*. *Genetics* **129,** 1061–1072.

Chase, D., Serafinas, C., Ashcroft, N., Kosinski, M., Longo, D., Ferris, D. K., and Golden, A. (2000). The polo-like kinase PLK-1 is required for nuclear envelope breakdown and the completion of meiosis in *Caenorhabditis elegans*. *Genesis* **26,** 26–41.

Cikaluk, D. E., Tahbaz, N., Hendricks, L. C., DiMattia, G. E., Hansen, D., Pilgrim, D., and Hobman, T. C. (1999). GERp95, a membrane-associated protein that belongs to a family of proteins involved in stem cell differentiation. *Mol. Biol. Cell* **10,** 3357–3372.

Clark-Maguire, S., and Mains, P. E. (1994). Localization of the mei-1 gene product of *Caenorhabditis elegans*, a meiotic-specific spindle component. *J. Cell Biol.* **126,** 199–209.

Clifford, R., Lee, M.-H., Nayak, S., Ohmachi, M., Giorgini, F., and Schedl, T. (2000). FOG-2, a novel F-box containing protein, associates with the GLD-1 RNA binding protein and directs male sex determination in the *C. elegans* hermaphrodite germline. *Development* **127,** 5265–5276.

Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D., and Lin, H. (1998). A novel class of evolutionarily conserved genes defined by *piwi* are essential for stem cell self-renewal. *Genes Dev.* **12,** 3715–3727.

Crittenden, S. L., Troemel, E. R., Evans, T. C., and Kimble, J. (1994). GLP-1 is localized to the mitotic region of the *C. elegans* germ line. *Development* **120,** 2901–2911.

Dernburg, A. F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., and Villeneuve, A. M. (1998). Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94,** 387–398.

Doyle, T. G., Wen, C., and Greenwald, I. (2000). SEL-8, a nuclear protein required for LIN-12 and GLP-1 signaling in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **97,** 7877–7881.

Elbashir, S. M., Lendeckel, W., and Tuschl, T. (2001). RNA interference is mediated by 21- and 23-nucleotide RNAs. *Genes Dev.* **15,** 188–200.

Fay, D. S., and Han, M. (2000). Mutations in *cye-1*, a *Caenorhabditis elegans* cyclin E homolog, reveal coordination between cell-cycle control and vulval development. *Development* **127,** 4049–4060.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391,** 806–811.

Fortier, E., and Belote, J. (2000). Temperature-dependent gene silencing by an expressed inverted repeat in *Drosophila*. *Genesis* **26,** 240–244.

Francis, R., Maine, E., and Schedl, T. (1995). Analysis of the multiple roles of *gld-1* in germline development: Interactions with the sex determination cascade and the *glp-1* signaling pathway. *Genetics* **139,** 607–630.

Fraser, A. G., Kamath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Ahringer, J. (2000). Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* **408,** 325–330.

Gartner, A., Milstein, S., Ahmed, S., Hodgkin, J., and Hengartner, M. O. (2000). A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C. elegans*. *Mol. Cell* **5,** 435–443.

Geles, K. G., and Adam, S. A. (2001). Germline and developmental roles of the nuclear transport factor importin α3 in *C. elegans*. *Development* **128,** 1817–1830.

Gibert, M.-A., Starck, J., and Beguet, B. (1984). Role of the gonad cytoplasmic core during oogenesis of the nematode *Caenorhabditis elegans*. *Biol. Cell* **50,** 77–86.

Goldstein, B. (2000). Embryonic polarity: A role for microtubules. *Curr. Biol.* **10,** R820–R822.

Goldstein, B., and Hird, S. N. (1996). Specification of the anteroposterior axis in *Caenorhabditis elegans*. *Development* **122,** 1467–1474.

Gonczy, P., Pichler, S., Kirkham, M., and Hyman, A. A. (1999). Cytoplasmic dynein is required for distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage *Caenorhabditis elegans* embryo. *J. Cell Biol.* **147,** 135–150.

Gonczy, P., Echeverri, G., Oegema, K., Coulson, A., Jones, S. J., Copley, R. R., Duperon, J., Oegema, J., Brehm, M., Cassin, E., Hannak, E., Kirkham, M., Pichler, S., Flohrs, K., Goessen, A., Leidel, S., Alleaume, A. M., Martin, C., Ozlu, N., Bork, P., and Hyman, A. A. (2000). Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature* **408,** 331–336.

Grant, B., and Hirsh, D. (1999). Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte. *Mol. Biol. Cell* **10,** 4311–4326.

Greener, T., Grant, B., Shang, Y., Wu, X., Greene, L. E., Hirsh, D., and Eisenberg, E. (2001). *Caenorhabiditis elegans* auxilin: A J-domain protein essential for clathrin-mediated endocytosis *in vivo*. *Nat. Cell Biol.* **3,** 215–219.

Gruidl, M. E. Smith, P. A., Kuznicki, K. A., McCrone, J. S., Kirchner, J., Roussell, D. L., Strome, S., and Bennett, K. L. (1996). Multiple potential germ-line helicases are components of the germ-line-specific P granules of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **93,** 13837–13842.

Guo, S., and Kemphues, K. (1995). *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81,** 611–620.

Haag, E. S., and Kimble, J. (2000). Regulatory elements required for development of *Caenorhabditis elegans* hermaphrodites are conserved in the *tra-2* homologue of *C. remanei*, a male/female sister species. *Genetics* **155,** 105–116.

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

Hammond, S. M., Bernstein, E., Beach, D., and Hannon, G. J. (2000). An RNA-directed nuclease mediate post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404,** 293–296.

Higashitani, A., Aoki, H., Mori, A., Sasagawa, Y., Takanami, T., and Takahashi, H. (2000). *Caenorhabditis elegans* Chk2-like gene is essential for meiosis but dispensable for DNA repair. *FEBS Lett.* **485,** 35–39.

Hirsh, D., Oppenheim, D., and Klass, M. (1976). Development of the reporductive system of *Caenorhabditis elegans*. *Dev. Biol.* **49,** 200–219.

Hong, Y., Roy, R., and Ambros, V. (1998). Developmental regulation of a cyclin-dependent kinase inhibitor controls postembryonic cell cycle progression in *Caenorhabditis elegans*. *Development* **125,** 3585–3597.

Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F., Lin, R., Smith, M. M., and Allis, C. D. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* **102,** 279–291.

Hubbard, E. J. A., and Greenstein, D. (2000). The *Caenorhabditis elegans* gonad: A test tube for cell and developmental biology. *Dev. Dyn.* **218,** 2–22.

Jantsch-Plunger, V., and Glotzer, M. (1999). Depletion of syntaxins in the early *Caenorhabditis elegans* embryo reveals a role for membrane fusion events in cytokinesis. *Curr. Biol.* **9,** 738–745.

Jedruski, M. A., and Schulze, E. (2001). A single histone H1 isoform (H1.1) is essential for chromatin silencing and germline development in *Caenorhabditis elegans*. *Development* **128,** 1069–1080.

Jones, A. R., Francis, R., and Schedl, T. (1996). GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during *Caenorhabditis elegans* germline development. *Dev. Biol.* **180,** 165–183.

Karashima, T., Sugimoto, A., and Yamamoto, M. (2000). *Caenorhabditis elegans* homologue of the human azoospermia factor DAZ is required for oogenesis but not for spermatogenesis. *Development* **127,** 1069–1079.

Kawasaki, I., Shim, Y.-H., Kirchner, J., Kaminker, J., Wood, W. B., and Strome, S. (1998). PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in *C. elegans*. *Cell* **94,** 635–645.

Kawano, T., Fujita, M., and Sakamoto, H. (2000). Unique and redundant functions of SR proteins, a conserved family of splicing factors, in *Caenorhabditis elegans* development. *Mech. Dev.* **95,** 67–76.

Kelly, W. G., Xu, S., Montgomery, M. K., and Fire, A. (1997). Distinct requirements for somatic and germline expression of a generally expressed *C. elegans* gene. *Genetics* **146,** 227–238.

Kemphues, K. (2000). PARsing embryonic polarity. *Cell* **101,** 345–348.

Kennerdell, J. R., and Carthew, R. W. (1998). Use of ds-RNA-mediated genetic interference to demonstrate that *frizzled* and *frissled 2* act in the Wingless pathway. *Cell* **95,** 1017–1026.

Ketting, R. F., Haverkamp, T. H. A., van Leunen, H. G. A. M., and Plasterk, R. H. A. (1999). *mut-7* of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrom helicase and RNaseD. *Cell* **99,** 133–141.

Kimble, J., and Hirsh, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* **70,** 396–417.

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

Kitagawa, R., and Rose, A. M. (1999). Components of the spindle-assembly checkpoint are essential in *Caenorhabditis elegans*. *Nat. Cell Biol.* **1,** 514–521.

Klass, M., Wold, N., and Hirsh, D. (1976). Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. *Dev. Biol.* **52,** 1–18.

Korf, I., Fan, Y., and Strome, S. (1998). The Polycomb group in *Caenorhabditis elegans* and maternal control of germline development. *Development* **125,** 2469–2478.

Kraemer, B., Crittenden, S., Gallegos, M., Moulder, G., Barstead, R., Kimble, J., and Wickens, M. (1999). NANOS-3 and FBF proteins physically interact to control the sperm-oocyte switch in *Caenorhabditis elegans*. *Curr. Biol.* **9,** 1009–1018.

Kuroyanagi, H., Kimura, T., Wada, K., Hisamoto, N., Matsumoto, K., and Hagiwara, M. (2000). SPK-1, a *C. elegans* SR protein kinase homologue, is essential for embryogenesis and required for germline development. *Mech. Dev.* **99,** 51–64.

Kuwabara, P. E. (1996). Interspecies comparison reveals evolution of control regions in the nematode sex-determining gene *tra-2*. *Genetics* **144,** 597–607.

Kuwabara, P. E. (1999). Developmental genetics of *Caenorhabditis elegans* sex determination. *Curr. Top. Dev. Biol.* **41,** 99–132.

Kuwabara, P. E., Lee, M. H., Schedl, T., and Jefferis, G. S. (2000). A *C. elegans* patched gene, *ptc- 1*, functions in germ-line cytokinesis. *Genes Dev.* **14,** 1933–1944.

Kuznicki, K. A., Smith, P. A., Leung-Chiu, W. M., Estevez, A. O., Scott, H. C., and Bennett, K. L. (2000). Combinatorial RNA interference indicates GLH-4 can compensate for GLH-1; these two P granule components are critical for fertility in *C. elegans*. *Development* **127,** 2907–2916.

Lam, G., and Thummel, C. S. (2000). Inducible expression of double-stranded RNA directs specific genetic interference in *Drosophila*. *Curr. Biol.* **10,** 957–963.

Li, W., Boswell, R., and Wood, W. B. (2000). *mag-1*, a homolog of *Drosophila mago nashi*, regulates hermaphrodite germ-line sex determination in *Caenorhabditis elegans*. *Dev. Biol.* **218,** 172–182.

Liang, L., Dielh-Jones, W., and Lasko, P. (1994). Localization of vasa protein to the *Drosophila* pole plasm is independent of its RNA-binding and helicase activities. *Development* **120,** 1201–1211.

Liu, J., Ben-Shahar, T. R., Riemer, D., Treinin, M., Spann, P., Weber, K., Fire, A., and Gruenbaum, Y. (2000). Essential roles for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. *Mol. Biol. Cell* **11,** 3937–3947.

Longman, D., Johnstone, I. L., and Caceres, J. F. (2000). Functional characterization of SR and SR-related genes in *Caenorhabditis elegans*. *EMBO J.* **19,** 1625–1637.

Lorson, M. A., Horvitz, H. R., and van den Heuvel, S. (2000). LIN-5 is a novel component of the spindle apparatus required for chromosome segregation and cleavage plane specification in *Caenorhabditis elegans*. *J. Cell Biol.* **148,** 73–86.

Luitjens, C., Gallegos, M., Kraemer, B., Kimble, J., and Wickens, M. (2000). CPEB proteins control two key steps in spermatogenesis in *C. elegans*. *Genes Dev.* **14,** 2596–2609.

Maeda, I., Kohara, Y., Yamamoto, M., and Sugimoto, A. (2001). Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Curr. Biol.* **11,** 171–176.

Martinek, S., and Young, M. W. (2000). Specific genetic interference with behavioral rhythms in *Drosophila* by expression of inverted repeats. *Genetics* **156,** 1717–1725.

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

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

Meyer, B. J. (1997). Sex determination and X chromosome dosage compensation.. In "*C. elegans* II" (D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, Eds.), pp. 209–240. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Montgomery, M. K., Xu, S., and Fire, A. (1998). RNA as a target of double-stranded RNA-mediated genetic interference in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **95,** 15502–15507.

Moore, L. L., Morrison, M., and Roth, M. B. (1999). HCP-1, a protein involved in chromosome segregation, is localized to the centromere of mitotic chromosomes in *Caenorhabditis elegans*. *J. Cell Biol.* **147,** 471–480.

Oishi, I., Iwai, K., Kagohashi, Y., Fujimoto, H., Kariya, K. I., Kataoka, T., Sawa, H., Okano, H., Otani, H., Yamamura, H., and Minami, Y. (2001). Critical role of *Caenorhabditis elegans* homologs of Cds1 (Chk2)-related kinases in meiotic recombination. *Mol. Cell. Biol.* **21,** 1329–1335.

Park, M., and Krause, M. W. (1999). Regulation of postembryonic G1 cell cycle progression in *Caenorhabditis elegans* by a cyclin D/CDK-like complex. *Development* **126,** 4849–4860.

Parrish, S., Fleenor, J., Xu, S., Mello, C., and Fire, A. (2000). Functional anatomy of a dsRNA trigger: Differential requirement for the two trigger strands in RNA interference. *Mol. Cell* **6,** 1077–1087.

Petcherski, A. G., and Kimble, J. (2000). LAG-3 is a putative transcriptional activator in the *C. elegans* Notch pathway. *Nature* **405,** 364–368.

Piano, F., Schetter, A. J., Mangone, M., Stein, L., and Kemphues, K. (2000). RNAi analysis of genes expressed in the ovary of *Caenorhabditis elegans*. *Curr. Biol.* **10,** 1619–1622.

Pitt, J. N., Schisa, J. A., and Priess, J. R. (2000). P granules in the germ cells of *Caenorhabditis elegans*, adults are associated with clusters of nuclear pores and contain RNA. *J. Cell Biol.* **219,** 315–333.

Polinko, E. S., and Strome, S. (2000). Depletion of a Cks homolog in *C. elegans* embryos uncovers a post-metaphase role in both meiosis and mitosis. *Curr. Biol.* **10,** 1471–1474.

Qiao, L., Lissemore, J. L., Shu, P., Smardon, A., Gelber, M. B., and Maine, E. M. (1995). Enhancers of *glp-1*, a gene required for cell-signaling in *Caenorhabditis elegans*, define a set of genes required for germline development. *Genetics* **141,** 551–569.

Raich, W. B., Moran, A. N., Rothman, J. H., and Hardin, J. (1998). Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4. *Mol. Biol. Cell* **9,** 2037–2049.

Reinke, V., Smith, H. E., Nance, J., Wang, J., Van Doren, C., Begley, R., Jones, S. J., Davis, E. B., Scherer, S., Ward, S., and Kim, S. K. (2000). A global profile of germline gene expression in *C. elegans*. *Mol. Cell* **6,** 605–616.

Rudel, D., and Kimble, J. (2001). Conservation of *glp-1* regulation and function in nematodes. *Genetics* **157,** 639–654.

Schedl, T. (1997). Developmental genetics of the germ line. In "*C. elegans* II" (D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, Eds.), pp. 241–269. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schedl, T., and Kimble, J. (1988). *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. *Genetics* **119,** 43–61.

Schisa, J. A., Pitt, J. N., and Priess, J. R. (2001). Analysis of RNA associated with P granules in germ cells of *C. elegans* adults. *Development* **128,** 1287–1298.

Schubert, C. M., Lin, R., de Vries, C. J., Plasterk, R. H. A., and Priess, J. R. (2000). MEX-5 and MEX-6 function to establish soma/germline asymmetry in early *C. elegans* embryos. *Mol. Cell* **5,** 671–682.

Schumacher, J. M., Ashcroft, N., Donovan, P. J., and Golden, A. (1998). A highly conserved centrosomal kinase, AIR-1, is required for accurate cell cycle progression and segregation of developmental factors in *C. elegans* embryos. *Development* **125,** 4391–4402.

Schumacher, J. M., Golden, A., and Donovan, P. J. (1998). AIR-2: An Auraro/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. *J. Cell Biol.* **143,** 1635–1646.

Severson, A. F., Hamill, D. R., Carter, J. C., Schumacher, J., and Bowerman, B. (2000). The aurora-related kinase AIR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis. Curr. Biol. **10,** 1162–1171.

Seydoux, G., and Strome, S. (1999). Launching the germline in *Caenorhabditis elegans*: Regulation of gene expression in early germ cells. *Development* **126,** 3275–3283.

Seydoux, G., and Schedl, T. (2001). The germline in *C. elegans*: Origins, proliferation, and silencing. *Int. Rev. Cytol.* **203,** 139–186.

Shelton, C. A., Carter, J. C., Ellis, G. C., and Bowerman, B. (1999). The nonmuscle myosin regulatory light chain gene *mlc-4* is required for cytokinesis, anterior-posterior polarity, and body morphology during *Caenorhabditis elegans* embryogenesis. *J. Cell Biol.* **146,** 439–451.

Skop, A. R., and White, J. G. (1998). The dynactin complex is required for cleavage plane specification in early *Caenorhabditis elegans* embryos. *Curr. Biol.* **8,** 1110–1116.

Smardon, A., Spoerke, J. M., Stacey, S. C., Klein, M. E., Mackin, N., and Maine, E. M. (2000). EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*. *Curr. Biol.* **10,** 169–178.

Speliotes, E. K., Uren, A., Vaux, D., Horvitz, H. R. (2000). The survivin-like *C. elegans* BIR-1 protein acts with the Aurora-like kinase AIR-2 to affect chromosomes and the spindle midzone. *Mol. Cell* **6,** 211–223.

Srayko, M., Buster, D. W., Bazirgan, O. A., McNally, F. J., and Mains, P. E. (2000). MEI-1/MEI-2 katanin-like microtubule severing activity is required for *Caenorhabditis elegans* meiosis. *Genes Dev.* **14,** 1072–1084.

Starr, D. A., Williams, B. C., Li, Z., Etemad-Moghadam, B., Dawe, R. K., and Goldberg, M. L. (1997). Conservation of the centromere/kinetochore protein ZW10. *J. Cell Biol.* **138,** 1289–1301.

Streit, A., Li, W., Robertson, B., Schein, J., Kamal, I. H., Marra, M., and Wood, W. B. (1999). Homologs of the *Caenorhabditis elegans* masculinizing gene *her-1* in *C. briggsae* and the filarial parasite *Brugia malay*i. *Genetics* **152,** 1573–1584.

Strome, S., and Wood, W. B. (1982). Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. *Cell* **35,** 15–25.

Subramaniam, K., and Seydoux, G. (1999). *nos-1* and *nos-2*, two genes related to *Drosophila nanos*, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. *Development* **126,** 4861–4871.

Swan, K. A., Severson, A. F., Carter, J. C., Martin, P. R., Schnabel, H., Schnabel, R., and Bowerman, B. (1998). *cyk-1*: A *C. elegans* FH gene required for a late step in embryonic cytokinesis. *J. Cell Sci.* **111,** 2017–2027.

Tabara, H., Grishok, A., and Mello, C. C. (1998). RNAi in *C. elegans*: Soaking in the genome sequence. *Science* **282,** 430–431.

Tabara, H., Sarkissian, M., Kell, W. G., Fleenor, J., Grishok, A., Timmons, L., Fire, A., and Mello, C. C. (1999). The *rde-1* gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* **99,** 123–132.

Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K. J., Miwa, J., and Ohno, S. (1998). Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. *Development* **125,** 3607–3614.

Takanami, T., Sato, S., Ishihara, T., Katsura, I., Takahashi, H., and Higashitani, A. (1998). Characterization of a *Caenorhabditis elegans* recA-like gene *Ce-rdh-1* involved in meiotic recombination. *DNA Res.* **5,** 373–377.

Tavernakis, N., Wang, S. L., Dorovkov, M., Ryazanov, A., and Driscoll, M. (2000). Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. *Nat. Genet.* **24,** 180–183.

Timmons, L., and Fire, A. (1998). Specific interference by ingested dsRNA. *Nature* **395,** 584.

Timmons, L., Court, D. L., and Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263,** 103–112.

Yochem, J., and Greenwald, I. (1989). *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *C. elegans*, encode similar transmembrane proteins. *Cell* **58,** 553–563.

Zamore, P. D., Tuschl, T., Sharp, P. A., and Bartel, D. P. (2000). RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101,** 25–33.

Zetka, M. C., Kawasaki, I., Strome, S., and Muller, F. (1999). Synapsis and chiasma formation in *Caenorhabiditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. *Genes Dev.* **13,** 2258–2270.

Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J., and Wickens, M. P. (1997). A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. *Nature* **390,** 477–484.

Received for publication April 25, 2001
Revised June 18, 2001
Accepted June 18, 2001
Published online September 7, 2001