Dominant mutations in the *Caenorhabditis elegans* Myt1 ortholog *wee-1.3* reveal a novel domain that controls M-phase entry during spermatogenesis

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**SUMMARY**

Regulatory phosphorylation of the Cdc2p kinase by Wee1p-type kinases prevents eukaryotic cells from entering mitosis or meiosis at an inappropriate time. The canonical Wee1p kinase is a soluble protein that functions in the eukaryotic nucleus. All metazoa also have a membrane-associated Wee1p-like kinase named Myt1, and we describe the first genetic characterization of this less well-studied kinase. The *Caenorhabditis elegans* Myt1 ortholog is encoded by the *wee-1.3* gene, and six dominant missense mutants prevent primary spermatocytes from entering M phase but do not affect either oocyte meiosis or any mitotic division. These six dominant *wee-1.3(gf)* mutations are located in a four amino acid region near the C terminus and they cause self-sterility of hermaphrodites. Second-site intragenic suppressor mutations in *wee-1.3(gf)* restore self-fertility to these dominant sterile hermaphrodites, permitting genetic dissection of this kinase. Ten intragenic *wee-1.3* suppressor mutations were recovered and they form an allelic series that includes semi-dominant, hypomorphic and null mutations. These mutants reveal that WEE-1.3 protein is required for embryonic development, germline proliferation and initiation of meiosis during spermatogenesis. This suggests that a novel, sperm-specific pathway negatively regulates WEE-1.3 to allow the G2/M transition of male meiosis I, and that dominant *wee-1.3* mutants prevent this negative regulation.

Key words: Cell cycle, Meiosis, Wee1p, Myt1, Spermatogenesis, *C. elegans*

**INTRODUCTION**

The eukaryotic cell division cycle coordinates cell growth with chromosomal replication so that two daughter cells containing all required components form at the appropriate time (Nurse, 2000). This process is divided into phases of gap 1 (G1 phase), DNA synthesis (S phase), a second gap (G2 phase) and mitosis (M-phase). Transit through the eukaryotic cell cycle is governed by evolutionarily conserved cyclin-dependent kinases (CDKs) that are tightly regulated by numerous kinases, phosphatases and small inhibitory proteins (Pavletich, 1999). Yeast use a single mitotic CDK and multiple cyclins to control cell cycle progression (Stern and Nurse, 1996). However, higher eukaryotes possess multiple mitotic CDKs (Nigg, 1995) and each mitotic CDK associates with a specific cyclin that can vary with the cell cycle stage (Shulman, 1998). Successive activation of multiple CDK/cyclin complexes is thought to promote cell cycle progression in higher eukaryotes.

Mitotic entry is controlled by activation of a protein complex that is composed of the Cdc2p kinase and cyclin B (Dunphy et al., 1988). This protein complex accumulates during late G2 phase, but phosphorylation of threonine-14 and tyrosine-15 on Cdc2p prevents its activation (Coleman and Dunphy, 1994). Dephosphorylation of these residues constitutes the major mitotic entry signal in eukaryotes. In fission yeast, Y15 of Cdc2p is phosphorylated by the Wee1p and Mik1p kinases (Lundgren et al., 1991), while dephosphorylation is carried out by the Cdc25p phosphatase (Russell and Nurse, 1986; Berry and Gould, 1996; Gautier et al., 1991). In metazoans, T14 phosphorylation is catalyzed exclusively by a member of the Wee1p kinase family called Myt1 (Fattaey and Booher, 1997). The Myt1 kinase can also phosphorylate Y15 of Cdc2p, is about 40% identical and 70% similar to the canonical *S. pombe* Wee1p kinases in its kinase domain, contains a predicted transmembrane domain, and has a C-terminal domain of poorly understood function. The membrane-spanning domain mediates Myt1 endoplasmic reticulum and Golgi localization in human cell culture lines when this protein is overexpressed (Liu et al., 1997). Thus, Myt1 is a distinct member of the Wee1p kinase family that appears to function in the cytoplasm to regulate Cdc2p.

We have analyzed rare dominant mutants that affect *C. elegans* spermatogenesis (*spe* mutants) and show that they contain mutations in the *C. elegans* *wee-1.3* gene. The *wee-1.3* gene is the *C. elegans* Myt1 ortholog and these are the first Myt1 kinase mutations recovered in any organism. These
dominant spe mutants cannot perform the G2/M transition during spermatogenesis so hermaphrodites lack mature spermatozoa and exhibit, consequently, self-sterility. Genetic suppression of this self-sterility allowed recovery of loss-of-function mutants in *C. elegans* wee-1.3. These loss-of-function mutations reveal that WEE-1.3 is required for the kinase domain. This suggests that these putative candidate suppressors. They were outcrossed to *dpy-2 unc-4/mnl1* GFP+ males and F1 GFP+ nonUnc progeny were picked. Balanced *wee-1.3* gf* unc-4/mnl1* GFP+ were identified as hermaphrodites that failed to segregate DpyUnc progeny.

For γ-ray mutagenesis, *L4* hermaphrodites of genotype *dpy-2 wee-1.3* ([e1947];*dpy-2 unc-4* were mutagenized with 1500 Rads from a 137Cs source. These hermaphrodites were crossed to *dpy-2 unc-4/mnl1* males and 125 Dpy outcross progeny were each picked to a separate plate. A single suppressor of genotype *wee-1.3* ([e1947];*sup*/dpy-2 unc-4* was identified as a self-fertile hermaphrodite that segregated Dpy and DpyUnc progeny. This candidate was crossed to *dpy-2 unc-4/mnl1* males. Non-Dpy outcross progeny were picked to plates and a balanced line of putative genotype *dpy-2 wee-1.3* ([e1947];*sup*/mnl1) was established from hermaphrodites that failed to segregate DpyUnc progeny. No extragenic suppressors were identified in either the γ-ray or ENU screen.

**Materials and Methods**

**Strains, culture and nomenclature**

Nematode culture and handling were performed as previously described (Brenner, 1974). All strains are derived from the wild-type N2 strain (var. Bristol), and all animals were maintained at 20°C unless otherwise noted. The following genetic markers and balancers were used.

LG II: *dpy-2(e8), dpy-10(e128), unc-4(e120)* (Brenner, 1974); *mab-3(e1240), edf21* (Shen and Hodgkin, 1988); *mln1[dpy-10(e128) mls14]* (Edgley and Riddle, 2001); *mln1[dpy-10(e128) unc-52(e444)], let-241(mn228), mnDf12, mnDf28, mnDf29, mnDf30, mnDf57, mnDf58, mnDf60, mnDf63, mnDf71 and mnDf88* (Sigurdson et al., 1984).

LG III: *dpy-1(e1)* (Brenner, 1974) and *smg-6(r896)* (Hodgkin et al., 1989).

LG IV: *dpy-20(e1282)* (Hosono, 1982).

*mls14* bears a dominant GFP+ phenotype in pharyngeal muscle, gut, and in four- to 60-cell embryos, but only the pharyngeal marker was used during these studies (Edgley and Riddle, 2001).

All dominant spe mutants were recovered following EMS mutagenesis under standard conditions (Brenner, 1974) and each bears a mutation in a gene initially called spe-37, but now named *wee-1.3* for nomenclatural clarity. *wee-1.3* ([e1947]) was isolated as a self-fertile hermaphrodite of genotype *dpy-2 unnatural 1* of 4 or 12, and all animals were maintained at 20°C unless otherwise noted. The following genetic markers and balancers were used.

**Nucleic acid methods**

The chromosome II deficiency *ebDf1* was mapped by polymerase chain reaction (PCR)-based methods described previously (Williams, 1995). Each PCR had a control primer pair that would produce a product from the genomic DNA present in *ebDf1* homozygotes and a second pair of test primers. PCR on wild-type embryos was carried out in parallel, and reactions were visualized on ethidium bromide stained agarose gels. At least five PCRs were attempted with each pair of test primers to determine if they could produce a product from *ebDf1* template DNA. This approach revealed that the left *ebDf1* breakpoint is within a 4 kb interval present in cosmid ZK1320 and the right breakpoint is within a 23 kb interval starting in cosmid ZK938 and extending through Y53C12C(data not shown). These data indicate that *ebDf1* deletes ~125-150 kb on chromosome II, including the C. elegans Myt1 ortholog, *wee-1.3* (see www.wormbase.org). The *wee-1.3* candidate gene was sequenced from each ENU induced *wee-1.3* ([q89];*sup*) suppressor. When suppressor homoyzygotes were viable, individual nonGFP *wee-1.3* ([q89];*sup*) *unc-4* animals were picked from *wee-1.3* ([q89];*sup*); *unc-4*/*mln1* GFP+ parents and used to prepare template DNA (Williams, 1995). For lethal *wee-1.3* null mutants, nonviable embryos were used to prepare template DNA (Williams, 1995). At least four independent Taq-generated PCR products were fractionated by agarose gel electrophoresis and purified using the GeneClean system (Bio101, Vista, CA). Sequencing was performed at the Iowa State University (Ames, IA) DNA sequencing facility by primer walking using standard ABI automated sequencing. Sequence was analyzed using the DNASTAR software package (DNASTAR, Madison, WI).

To sequence *wee-1.3* ([q89]) mutants, hermaphrodites of genotype *wee-1.3* ([q89]) *unc-4/mln1* GFP+ were crossed to *dpy-2 ebDf1/mln1* males.
The non-GFP F1 hermaphrodites [wee-1.3(gf) unc-4/dpy-2 ebDf1] were picked to verify the dominant Spe phenotype. Individual animals were prepared for PCR as described for deficiency mapping. Multiple PCR reactions were pooled and sequenced, as described above, to identify the molecular lesions associated with wee-1.3(gf) mutants. All mutations were verified by sequencing the wild-type N2 strain (var. Bristol).

A wee-1.3 rescuing transgene was prepared by high-fidelity PCR (Advantage2, Clontech Laboratories, Palo Alto, CA). A sense primer (TL49 – 5’-ATGTATAGATCTTTTTAAAACCCCAAACC-3’) just outside the predicted 3’ end of Y53C12A.1 ORF and an antisense primer (TL50 – 5’-CAACTGACATGCTCGGAACTCCCAAAAAG-3’) just outside the predicted 5’ end of Y53C12A.6 were used to amplify a 4.3 kb fragment from N2 genomic DNA. This PCR fragment was microinjected with the dominant rol-6 (su1006) encoding plasmid pRF4 (Mello et al., 1991) into wee-1.3(gf) ebDf88 unc-4/+/mnl1GFP+ hermaphrodites. F1 rollers were isolated and a determination pathway (Doniach, 1986). Further analysis isolated in a screen for dominant mutants in the sex study of the dominant sterile mutant either the hermaphrodite’s own sperm (self-fertility) or sperm produce oocytes. Ovulated eggs are fertilized internally by germline that first produces sperm and then switches to sterile but outcross fertile. As oocytes can be fertilized in just outside the predicted 3’-primer (TL55 – 5’-GCAAGAAAATAAGAAGGGCCGAACAAAG-3’) and TL50 (5’-CAACTGACATGCTCGGAACTCCCAAAAG-3’) allowed 1 kb of sequence 5’ to the transcriptional start plus the first wee-1.3 exon to be amplified from N2 genomic DNA. These primers introduce SpeI (TL49) and Kpn1 (TL50) sites into the resulting PCR product, which was restriction digested with these enzymes. This fragment was ligated into the SpeI/Kpn1-digested GFP-encoding plasmid pPD95.77 (A. Fire, S. Xu, J. Ahnm and G. Seydoux, personal communication) to create pSTL1. pSTL1 (10 ng/m) was ligating it to the green fluorescent protein (GFP)-coding sequence (Chalfie et al., 1994). High-fidelity PCR (Advantage2, Clontech) with primers TL49 (5’-ATGTATAGATCTTTTTAAAACCCCAAACC-AT-3) and TL50 (5’-CAACTGACATGCTCGGAACTCCCAAAAAG-3) were picked to verify the dominant Spe phenotype. Individual animals containing PCR fragments frequently died as embryos (data not shown).

The tissue specificity of the wee-1.3 promoter was analyzed by ligating it to the green fluorescent protein (GFP)-coding sequence (Chalfie et al., 1994). High-fidelity PCR (Advantage2, Clontech) with primers TL49 (5’-ATGTATAGATCTTTTTAAAACCCCAAACC-AT-3) and TL50 (5’-CAACTGACATGCTCGGAACTCCCAAAAAG-3) were picked to verify the dominant Spe phenotype. Individual animals containing PCR fragments frequently died as embryos (data not shown).

Light and electron microscopy
Light microscopy and immunofluorescence were performed as previously described (Arduengo et al., 1998). Post-acquisition image analysis was performed using ImagePro (Media Cybernetics, Silver Springs, MD) and VolumeScan (Vaytek, Fairfield, IA) software. Electron microscopy was performed as previously described (L’Hernault and Roberts, 1995). Figures were assembled using Adobe Photoshop 5.0 (Adobe Systems, San Jose, CA) or Canvas 7 (Deneba Systems).

RESULTS

All dominant spe mutants map close to unc-4 on chromosome II
The C. elegans hermaphrodite is somatically female and has a germline that first produces sperm and then switches to produce oocytes. Ovulated eggs are fertilized internally by either the hermaphrodite’s own sperm (self-fertility) or sperm inseminated by a male (cross-fertility). This work began as a study of the dominant sterile mutant e1947+/+, which was isolated in a screen for dominant mutants in the sex determination pathway (Doniach, 1986). Further analysis of e1947+/+ hermaphrodites revealed that this mutant is self-sterile but outcross fertile. As oocytes can be fertilized in e1947+/+ hermaphrodites and the mutant displays defective spermatogenesis rather than a sex-determination phenotype, it behaves like a dominant spermatogenesis-defective (spe) mutant (L’Hernault, 1997) and was initially named spe-37(gf). Three factor mapping of spe-37(e1947) placed it between dpy-2 and unc-4 on chromosome II. Five other independently derived dominant spe mutants also mapped to chromosome II (e89, eb95, eb104, hc144 and hc145) and four of these were mapped between dpy-2 and unc-4 (data not shown). Dominant sterility is not the result of haploinsufficiency because many deficiencies extend through this region and heterozygotes bearing these deficiencies are self-fertile (Shen and Hodgkin, 1988; Sigurdson et al., 1984).

Spermatogenesis in all dominant spe mutants arrest at an early stage
Sperm from the dominant spe mutants were analyzed by light microscopy. Wild-type spermatocytes progress through meiosis and differentiation in an invariant manner (Fig. 1A-D), with each male producing hundreds of haploid spermatids. By contrast, all dominant spe mutant males accumulate arrested primary spermatocytes and no spermatids are observed (Fig. 1E-H). These mutant spermatocytes have a condensed nucleus that is often asymmetrically located, which are characteristic
of later stages of spermatogenesis. Spermatocyte cytokinesis and condensed chromosomes aligned on the metaphase plate have not been observed in any of the dominant spe mutants. The same spectrum of cellular defects was observed when a dominant spe mutation was in trans to a non-complementing deficiency (data not shown), so this phenotype is neither dependent on the presence of the wild-type allele nor improved by its absence. No dominant spe mutant shows obvious evidence of earlier germline defects (data not shown).

**Dominant spe mutants partially differentiate in the absence of cell division**

The defects associated with spe-37(e1947)/+ and q89/+ were examined by electron microscopy and both show identical ultrastructural phenotypes. Initially, spe-37(e1947)/+ (Fig. 1I) and q89/+ (not shown) appear similar to wild type. Specifically, these mutants contain an uncondensed, centrally located nucleus that is surrounded by a nuclear envelope and normal ER/Golgi-derived fibrous body-membranous organelles (FB-MOs). Terminal spe-37(e1947)/+ spermatocytes contain condensed chromatin inside an intact nuclear envelope, FBs are not observed and MOs are highly vacuolated (Fig. 1J). These terminal spermatocytes polarize and place their nucleus and FB-MOs on opposite sides of the cell, as if attempting to differentiate. However, this attempt at differentiation occurs without karyokinesis or cytokinesis, suggesting that these dominant spe mutations affect a gene that coordinates the meiotic divisions with spermatocyte differentiation.

**Spermatocytes in dominant spe mutants have phosphorylated histone H3**

Although spermatocytes in dominant spe mutants do not divide, they do enter pachytene of meiotic prophase I (data not shown). Therefore, we postulated that they are unable to complete M phase. To test this hypothesis, wild-type and q89/+ sperm were stained with an antibody that recognizes phosphorylated histone H3, a well-characterized marker for M phase/chromosome condensation (Boxem et al., 1999; Golden, 2000; Hsu et al., 2000; Strahl and Allis, 2000). In wild-type, phospho-histone H3 staining is observed in dividing spermatocytes but not in spermatids (Fig. 2A-D). In q89/+ males, all spermatocytes contain phospho-histone H3 (Fig. 2E-H). This suggests that dominant spe spermatocytes arrest with condensed chromosomes, possibly at the G2/M phase boundary of meiosis I.

**Establishing that spe-37(e1947)/+ and q89/+ are allelic**

Dominant mutants can be subjected to mutagenesis and screened for a new recessive mutation that suppresses the dominance caused by the original mutation (Greenwald and Horvitz, 1980; Conradt and Horvitz, 1998). When the dominant mutation and its recessive suppressor show tight linkage, both mutations frequently reside in the same gene. An allelic series of such second site intragenic suppressor mutations often reveals the null phenotype of that gene. Using this technique, nine ethyl nitrosourea (ENU) induced suppressors of q89 and one γ-ray-induced suppressor of spe-37(e1947) were recovered. All suppressor mutations genetically map very close to q89 and could be unambiguously placed into one of three distinct phenotypic classes (Table 1). Each suppressor mutation functions in cis to q89, suggesting that suppression is intramolecular in nature. Furthermore, suppressors derived from q89/+ fail to complement the suppressor derived from spe-37(e1947)/+, indicating that q89 and e1947 are both spe-37 alleles.

**spe-37(q89)/+ G2/M meiotic arrest is partially alleviated by Class 1 suppressors**

The class 1 suppressor mutant spe-37(q89 eb61)/+, spe-37(q89 eb62)/+ and spe-37(q89 eb94)/+ hermaphrodites, unlike spe-37(q89)/+, are self-fertile. Class 1/+ hermaphrodites have brood sizes that are significantly smaller than those produced by wild type, and they also produce an unusually high percentage of males (Table 1). C. elegans males are XO and usually arise in a wild-type hermaphrodite population by X chromosome nondisjunction at a rate of ~0.1% (Brenner, 1974). The higher frequency of males suggests that an increased rate of X chromosome nondisjunction could be occurring in Class 1/+ hermaphrodites. Alternatively, Class 1/+ suppressor mutants might produce a high incidence of males because some gametes lose the X chromosome by another type of meiotic aberration. All three Class 1 suppressor homozygotes, in addition to spe-37(q89 eb62)/Df and spe-37(q89 eb94)/Df hemizygous hermaphrodites are self-sterile, laying unfertilized oocytes and inviable embryos. Given the increased frequency of X chromosome segregation abnormalities, autosomal segregation might also be aberrant and thus contribute to the observed embryonic lethality. Unlike self-sterile spe-37(q89 eb61) homozygous hermaphrodites, spe-37(q89 eb61)/Df hemizygotes produce a few viable progeny (mean=11 progeny; n=63); therefore two copies of spe-37(q89 eb61) results in a more severe phenotype than one copy. All Class 1 heterozygous, hemizygous and homozygous hermaphrodites produce large numbers of cross progeny after mating to wild-type males. Thus, Class 1 homozygotes and hemizygotes have defective sperm but are largely or completely unaffected in somatic development or other aspects of germline development (Table 1).

While spe-37(gf)/+ males never complete spermatogenesis (Fig. 1E-H), the modest self-fertility shown by Class 1/+
suppressor hermaphrodites suggests that spermatogenesis is occasionally completed in these animals (Table 1). Class 1/+ mutant males contain many spermatid-like cells. Therefore, some spermatocytes in Class 1/+ suppressor mutants complete the meiotic divisions, something that never occurs in spe-37(q89)+/+ mutants. However, Class 1/+ mutant testes contain many cells that show features never observed during wild-type spermatogenesis, such as 2-4 nuclei within the same cell (Fig. 3). Homozygous Class 1 mutant males contain very few sperm, but those that are present have a phenotype similar to the sperm observed in heterozygous Class 1/+ mutant males (data not shown). Overall, these data indicate that Class 1 suppressor mutants partially alleviate the G2/M meiotic block shown by spe-37(q89)+/+ mutants. Perhaps Class 1 mutants reduce, but do not eliminate, the gain-of-function effects of spe-37(q89) and this explains why hermaphrodite brood size and male spermatogenesis are both different from wild type.

Maternal and zygotic functions of spe-37 are revealed by hypomorphic suppressors

The Class 2 heterozygous spe-37(q89 eb60)+, spe-37(q89 eb93)+/+ and spe-37(q89 eb87)+/+ mutants completely suppress spe-37(q89)+/+ and such suppressed hermaphrodites exhibit wild-type self-fertility (Table 1). Like Class 1 suppressors, homozygous spe-37(q89 eb60) and spe-37(q89 eb93) Class 2 suppressors are self-sterile, but they produce many more inviable embryos than Class 1 suppressors. Homozygous spe-37(q89 eb87) hermaphrodites are self-sterile and lay many unfertilized oocytes but do not produce inviable embryos. All Class 2 mutant hermaphrodites produce large numbers of normal progeny after they are mated to wild-type males. These results suggest that Class 2 alleles are not competent to support embryonic development and at least one wild-type copy of spe-37 is required for a zygote to develop normally.

The Class 2 suppressor mutant phenotype is partly dependent on the maternal genotype. When Class 2/+ heterozygous hermaphrodites are crossed to deficiency(Df)/+ males (where Df removes the spe-37 gene), the resulting Class 2/Df hemizygous hermaphrodites mature into adults that lay a

![Fig. 3. Light microscopic phenotypes of spe-37(q89) Class 1 semidominant suppressors. (A-C) DIC and (D-F) DAPI fluorescence light microscopy. (A,D) Sperm from spe-37(q89 eb61)+/+ males; (B,E) spe-37(q89 eb62)+/+ males; (C,F) spe-37(q89 eb94)+/+ males. Arrowheads indicate abnormal sperm with multiple nuclei. Scale bars: 5 μm.](image-url)
mixture of oocytes and inviable embryos, which is like Class 2/Class 2 homozygous hermaphrodites. However, crossing sterile homozygous Class 2/2 hermaphrodites to Df/+ males, results in inviable Class 2/Df hemizygous embryos that show no morphogenesis. These data suggest that a Class 2/+ mother can maternally supply enough wild-type spe-37 activity to allow her homozygous Class 2/Class 2 progeny to complete development and begin germline formation. This germline does not function normally, presumably because there is insufficient maternal endowment. When little maternal endowment occurs (Class 2/2 hermaphrodites) and sperm provide no wild-type spe-37 gene, Class 2 alleles are not able to support embryonic development. These phenotypic properties are not as severe as Class 3 suppressor mutants (see below), which suggests that Class 2 suppressor mutations are loss-of-function but not-null (hypo/hypomorphic) alleles.

The many inviable embryos produced by Class 2/Class 2 homozygous hermaphrodites suggests that Class 2 mutants contain spermatozoa that are competent for fertilization. Microscopic examination of dissected heterozygous Class 2/+ or homozygous Class 2/Class 2 mutant males revealed that they contained cytologically wild-type spermatids (data not shown). These spermatids can form functional spermatozoa because hemizygous Class 2/Df mutant males sire viable progeny when crossed to wild-type hermaphrodites. As Class 2 mutants are hemizygous, these data suggest that reduced spe-37 gene activity still allows apparently normal spermatogenesis.

Two Class 2 suppressor mutants (eb60 and eb93) are affected when the SMG surveillance system is genetically disabled (see Materials and Methods). The wild-type SMG surveillance system degrades mRNAs that are defective, and a loss-of-function mutation in any of seven smg genes eliminates this ability to detect and degrade defective mRNAs (Mango, 2001). Heterozygous spe-37(q89 eb60)+ or spe-37(q89 eb93)+, which are fertile in a wild-type SMG background, become dominant Spe when the SMG surveillance system is genetically disabled. Similarly, homozygous spe-37(q89 eb60) or spe-37(q89 eb93), which produce inviable embryos in a wild-type SMG background, become dominant Spe when the SMG surveillance system is genetically disabled. In both cases, the observed dominant Spe phenotype is highly similar to spe-37(q89). These results suggest that spe-37(q89)-associated dominance is suppressed because eb60 or eb93 can, respectively, trigger spe-37(q89 eb60)- or spe-37(q89 eb93)-encoded mRNA degradation by the wild-type SMG surveillance system. Manipulation of the SMG surveillance system in the Class 2 suppressor double mutant background also provided a way to create what are effectively spe-37(q89) homzygotes, and spe-37(q89)/+, spe-37(q89)/spe-37(q89) and spe-37(q89)/Df (using deficiencies that remove the spe-37 gene) all exhibit the same phenotype. These data suggest that the spe-37(q89) mutation is insensitive to gene dose and thus may be constitutively active or neomorphic gain-of-function in nature [activity at an inappropriate place or time (Muller, 1932)].

The null phenotype of spe-37 is embryonic and larval lethal

Like Class 2 mutants, Class 3 spe-37(q89 eb88)+, spe-37(q89 eb90)+ and spe-37(q89 eb91)+ mutant heterozygous hermaphrodites all exhibit wild-type self-fertility (Table 1). Class 3 homozygotes derived from a Class 3/+ mother receive a maternal endowment of SPE-37 but, unlike Class 1 and 2 mutants, Class 3 homozygous mutants always die. Usually, Class 3 homozygous mutant embryos die after limited morphogenesis but, occasionally, they hatch into abnormal larvae that die. These data suggest that in wild-type, there is limited maternal SPE-37(+) and that the zygotic embryonic lethality of Class 3 homozygotes is the result of Class 3 alleles that provide little or no functional SPE-37 activity.

Among individual Class 3 mutants, spe-37(q89 eb91) causes the least severe phenotype because it produces fewer inviable embryos and more inviable L1 or L2 progeny than do either spe-37(q89 eb88) or spe-37(q89 eb90) (data not shown). The spe-37(q89 eb88)/Df or spe-37(q89 eb90)/Df (where the Df removes the spe-37 gene) -associated phenotypes are identical to that shown by the respective mutant homozygotes. This suggests that these Class 3 double mutants exhibit the spe-37 null phenotype because lowering the gene dose has no effect on the observed phenotype.

A candidate gene approach reveals that spe-37 is wee-1.3

The spe-37(e1947) γ-ray induced suppressor failed to complement the chromosome II deficiencies mnDf63, mnDf58, mnDf29, mnDf57 and edDf21 with regard to embryonic lethality and its phenotype is similar to the Class 3 spe-37(q89 eb88) or spe-37(q89 eb90) suppressor double mutants. The spe-37(e1947) γ-ray-induced suppressor also failed to complement mab-3 and all ENU-induced spe-37(q89) suppressors, so it behaved genetically like a deficiency and was named ebDf1. As ebDf1 mapped to the same location as spe-37(e1947), we hypothesized that it deleted the spe-37 gene and other adjacent genes. This hypothesis proved correct and the breakpoints of ebDf1 define a region that includes the spe-37 gene.

Although there are at least 26 predicted genes in the ~125-150 kb interval deleted by ebDf1, the only obvious cell cycle regulatory gene in this region is wee-1.3 (see www.wormbase.org). As the spe-37 associated phenotype suggests it encodes a cell cycle regulatory protein, we hypothesized that spe-37 and wee-1.3 were the same gene. This hypothesis was confirmed by showing that transgenes containing the wild-type wee-1.3 genomic sequence could rescue Class 3 spe-37(q89 eb88) homozygotes from embryonic lethality. Although viable, rescued transgenic Class 3 mutants are sterile because they do not form a germline. Prior work has shown that C. elegans transgenes frequently express in somatic tissues but show no germline expression because of epigenetic silencing (Kelly and Fire, 1998; Kelly et al., 1997). Although inconvenient, this co-suppression phenomenon permits an assessment of whether a gene must be expressed in the germline for normal germline development. The co-suppression phenotype suggests that wee-1.3 expression is required for establishment and/or proliferation of the germline.

Each of the six dominant spe-37 mutants proved to have a point mutation in the wee-1.3 gene (Fig. 4). Three of these dominant alleles (q89, e1947 and eb104) contained the same G1957A mutation that changes the encoded glycine at position 560 to an arginine. hc144 also affects glycine 560 but this mutation changes it so that glutamate (nucleotide change: G1958A) is encoded. eb95 converts the encoded glycine at position 558 to an arginine (nucleotide change: G1951A) and hc145 converts the encoded aspartic acid at position 561 to an
asparagine (nucleotide change, G1960A). Remarkably, all six spe-37(gf) mutations affect a four amino acid region in the C-terminal region of wee-1.3.

Each of the nine ENU induced spe-37(q89) suppressor double mutants had two mutations in wee-1.3 (Fig. 4). Each suppressor double mutant had the G1957A point mutation (see Table 1) present in the parental spe-37(q89) mutant. The second mutation was unique to each suppressor mutant (Fig. 4; Table 1). These DNA sequencing results and wee-1.3 transgenic rescue unambiguously show that spe-37 is the C. elegans Myt1 ortholog wee-1.3 (Wilson et al., 1999).

spe-37 suppressor mutations affect key residues in this Wee1-like kinase and an alignment of WEE-1.3 (=SPE-37) to its orthologs helps in interpreting some of the suppressor mutations (Fig. 5). The Class 1 suppressors include two missense mutations (eb62 and eb94) and one mutation (eb61) in a donor site (Fig. 4; Table 1). The eb62 suppressor mutation is the weakest Class 1 suppressor and it allows hermaphrodites to produce broods of approx. five progeny (Table 1). eb62 is an I160N missense mutation in an amino acid that shows weak conservation between wee-1.3 and its vertebrate orthologs (Fig. 5). The eb94 suppressor mutation allows hermaphrodites to produce broods of ~72. The eb94 F103I missense mutation affects a residue that is conserved among Myt1 orthologs (Fig. 5) but not among other members of the Wee1p kinase family (Wilson et al., 1999). The eb61 mutant permits the largest brood (~95; Table 1) of any of the Class 1 suppressors, and it converts the fourth intron splice donor into an in-frame codon (Fig. 4 and Table 1). The Genemarker HMM program (Borodovsky, 1998) suggests that other upstream consensus splice donor sites are present (data not shown), and the incompleteness of suppression suggests that such sites might be used.

The Class 2 suppressors include two premature stop mutations (eb60 and eb93) and one splice acceptor mutation in intron 4 (eb87; Fig. 4A). The molecular natures of eb60 and eb93 are consistent with their ability to be suppressed by mutants in the SMG mRNA surveillance system, as some smg-suppressible mutations are premature stop codons (Mango, 2001). Both eb60 and eb93 cause UAA ochre stop codons that would truncate the polypeptide sequence near the C terminus. The eb87 mutation alters a conserved splice acceptor site and RT-PCR of mutant animals shows that exon 5, which contains the transmembrane domain for WEE-1.3, is skipped (Fig. 4B). Additionally, the mechanism of eb87 suppression is not through a SMG-mediated reduction in mRNA levels, as eb87 is unchanged in a smg mutant background.

The Class 3 suppressors include two missense mutations (eb88 and eb90) and one small deletion (eb91) (Fig. 4; Table 1). The eb88 suppressor results in a G245E missense mutation and eb90 results in a H163P missense mutation; each of these mutations affects an amino acid that is within a strongly conserved region (Fig. 5) found in all Wee1p kinases from yeast to humans (Wilson et al., 1999). The eb91 suppressor mutation is a 545 bp deletion that removes the first 465 bp of coding sequence and 80 bp 5’ to the start codon. It is unlikely that wee-1.3 is transcribed in eb91 suppressor mutants because part of the promoter and the entire 5’ untranslated region, including the intron trans-splice acceptor for SL1 (A. Golden, personal communication), are missing. Consequently, its molecular features indicate that eb91 is a wee-1.3 null mutation.

wee-1.3 is widely expressed during C. elegans development

The recessive lethality shown by the Class 3 spe-37(q89) suppressors indicates that transcription of this gene is required outside the testes. Northern hybridization experiments reveal that the wee-1.3-encoded 2.4 kb mRNA was found in fem-1(hc17lf) hermaphrodites, which make sperm but no oocytes (data not shown). In contrast, spe-37(q89) null mutation shows no expression. Tissue specificity of wee-1.3 was further examined by fusing the promoter and first exon to the coding sequence for GFP and using this construct to create transgenic worms. Seven stable transgenic lines all consistently showed GFP during early embryonic development (Fig. 6A,B), in the distal region of the larval, but not adult, germline (Fig. 6C,D), and in some larval neurons and hypodermal cells (Fig. 6E,F). These data confirm that the wee-1.3 promoter is active during both germline and embryonic development.

DISCUSSION

wee-1.3(gf) has allowed a genetic analysis of the Myt1 kinase family

We have analyzed a series of six dominant mutations in wee-1.3 that affect the G/M transition during spermatogenesis but do not affect either the somatic cell cycle, germline...
proliferation or oogenesis. Each of these dominant mutations affects a residue within a four amino acid region, suggesting that this region is important for regulation of WEE-1.3 during spermatogenesis. Alleviation of the dominant Spe self-sterile phenotype allowed isolation of 10 self-fertile intragenic wee-1.3 suppressors. These suppressor mutations all reduce WEE-1.3 activity, indicating that the dominant mutants have excess WEE-1.3 activity during spermatogenesis. The suppressor mutations also reveal that wee-1.3 is an essential gene, required during embryogenesis and for germline proliferation.


**Fig. 5. Alignment of *C. elegans*, *C. briggsae*, *Xenopus*, mouse and human WEE-1.3 kinases.** Red boxes indicate identical residues among all WEE-1.3 orthologs. Black boxes indicate identity to the *C. elegans* sequence. Asterisks indicate the sites of mutation in *C. elegans* spe-37(gf), missense and nonsense suppressors. The phenotypic class of each missense or nonsense suppressor is indicated in parentheses (see Table 1). Alignment was performed using the ClustalX program (Thompson et al., 1997). The *C. briggsae* WEE-1.3 sequence is publicly available unpublished data from the *C. briggsae* Genome Project (The Sanger Institute Cambridge, UK and The Genome Sequencing Center, Washington University, St Louis, MO). *C. briggsae* WEE-1.3 cDNA was predicted using GeneMark hmm (Borodovsky, 1998) and sequence around the spe-37(gf) region was verified by examination of ABI sequence traces (see http://trace.ensembl.org). ATP, ATP-binding domain; kinase, Wee1p-like kinase domain; TM, predicted transmembrane domain. The GenBank Accession Number for *C. elegans* wee-1.3 is NP_496095, *C. briggsae* wee-1.3 is based on the GeneMark HMM prediction from contig C000100543, *Xenopus* Myt1 is A57247, Mouse Myt1 is NP_075545 and Human Myt1 is NP_004194.
The self-sterile phenotype exhibited by wee-1.3(q89 eb87) homozygotes could indicate that membrane localization is required for WEE-1.3 to function during spermatogenesis. Alternatively, perhaps the wee-1.3(q89) dominant mutation can still affect spermatogenesis in homozygous wee-1.3(q89 eb87) suppressor mutants, but only when it does not have to compete with wild-type WEE-1.3.

**Mechanism of wee-1.3(gf) dominance**

The six wee-1.3(gf) mutations described in this paper specifically affect spermatogenesis and do not require a wild-type copy of wee-1.3 to have their effect. wee-1.3(gf)/Df animals only produce the dominant mutant form of WEE-1.3 and exhibit the same phenotype as wee-1.3(gf)/+ animals. As the wee-1.3(gf) mutant phenotype is not different when wee-1.3 gene dose is reduced, the dominance is probably neomorphic/gain of function (gene activity in an inappropriate time or place) in nature. Furthermore, these data indicate that WEE-1.3(gf) can substitute for wild-type during embryonic and oocyte development, but not during spermatogenesis. The WEE-1.3 protein appears to be negatively regulated during spermatogenesis because strong hypomorphic suppressor double mutants, like Class 2 wee-1.3(q89 eb60), mimic negative regulation and restore spermatogenesis in both heterozygous and homozygous mutant animals. The wee-1.3(gf) phenotype is tissue specific, and perhaps there is a negative regulator expressed only during spermatogenesis that specifically regulates male meiosis.

Gamete-specific cell cycle regulation has been observed in other organisms and two cases are especially relevant to our study. The *Drosophila* twine(lf) mutant fails to complete the G2/M transition during spermatogenesis but still differentiates, which is similar to spermatogenesis in wee-1.3(gf) mutants (Alphey et al., 1992). Twine encodes a Cdc25p phosphatase, so *cdc25(lf)* and *wee-1.3(gf)* mutants would both shift cyclinB1/Cdc2p phosphorylation towards the inhibitory state. Deletion of the mouse Cdc25b gene has no somatic effects but results in female sterility because oocytes cannot exit meiosis I prophase arrest (Lincoln et al., 2002). These data suggest that metazoan regulation of the gamete cell cycle is fundamentally different from regulation of the somatic cell cycle, and that studies of WEE-1.3 function in *C. elegans* are likely to be applicable to Myt1 function in higher vertebrates.

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