Rapid and Efficient Identification of Caenorhabditis elegans Legacy Mutations Using Hawaiian SNP-Based Mapping and Whole-Genome Sequencing

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ABSTRACT The production of viable embryos requires the coordination of many cellular processes, including protein synthesis, cytoskeletal reorganization, establishment of polarity, cell migration, cell division, and in Caenorhabditis elegans, eggshell formation. Defects in any of these processes can lead to embryonic lethality. We examined six temperature-sensitive mutants as well as one nonconditional mutant that were previously identified in genetic screens as either embryonic lethal (maternal-effect or zygotic lethal) or eggshell defective. The responsible molecular lesion for each had never been determined. After confirmation of temperature sensitivity and lethality, we performed whole-genome sequencing using a single-nucleotide polymorphism mapping strategy to pinpoint the molecular lesions. Gene candidates were confirmed by RNA interference phenocopy and/or complementation tests and one mutant was further validated by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 gene editing. This approach identified new alleles of several genes that had only been previously studied by RNA interference depletion. Our identification of temperature-sensitive alleles for all of these essential genes provides an extremely useful tool for further investigation for the C. elegans community, such as the ability to address mutant phenotypes at various developmental stages and the ability to carry out suppressor/enhancer screens to identify other genes that function in a specific cellular process.

KEYWORDS whole-genome sequencing Hawaiian SNP mapping CRISPR/Cas9 essential genes cdc-25

Early studies using the model organism Caenorhabditis elegans sought to elucidate complex biological processes by modeling genetic studies performed in bacteriophage, E. coli, yeast, and Drosophila (Edgar et al. 1964; Edgar and Lielausis 1964; Wright 1970; Wright and Shaw 1970; Hartwell et al. 1974; Hereford and Hartwell 1974). The reasoning behind this approach was to use mutagenesis to accumulate developmental intermediates that could be classified and ordered on the basis of their phenotypes. C. elegans is a particularly useful model for studying developmental processes of metazoaens because of its small size, amenable genetics, anatomical simplicity, invariant cell lineage, and transparent body and embryos. The early studies using C. elegans applied this strategy to analyze nervous system development, behavior, and embryonic/zygotic development (Brenner 1973; Hirsh and Vanderslice 1976; Miwa et al. 1980; Schierenberg et al. 1980; Wood et al. 1980; Cassada et al. 1981). With the goal of reaching genetic saturation to provide a clear picture of a given developmental pathway, these studies generated about 600 embryonic (emb)/zygotic (zyg) lethal mutants and 54 maternal-effect (mel) mutants. Through linkage analysis and complementation tests, this large data set of mutants was distilled down to approximately 60 emb/zyg genes and 32 mel genes (Hirsh and Vanderslice 1976; Miwa et al. 1980; Schierenberg et al. 1980; Wood et al. 1980; Cassada et al. 1981; Kemphues et al. 1988). Since these early studies, more screens, both conditional and nonconditional, have been conducted, some in hopes of saturating the genome for essential genes (Rose and Baillie 1980; Meneely and Herman 1981; Sigurdson et al. 1984; Johnsen and Baillie 1991; Johnson et al. 2000).
and thus the number of genes that can be mutated to reveal essential embryonic phenotypes is now much greater. Mutants identified in these early screens are referred to as legacy mutants.

Several of these early screens focused on isolating temperature-sensitive mutations of developmental processes (Hirsch and Vanderslice 1976; Miwa et al. 1980; Schierenberg et al. 1980; Wood et al. 1980; Cassada et al. 1981; Kempfes et al. 1988). In these original screens, temperature-sensitive mutants made it possible to recover and maintain potentially lethal mutations at the permissive temperature and, as a tool, to understand the temporal control of gene function. Another advantage of temperature-sensitive mutants is that temperature-shift experiments could be performed at various times during development revealing new functions for a particular gene during different developmental stages. This is a big advantage over current methods of gene deletion such as RNA interference (RNAi), which usually only uncovers the earliest phenotypes. Temperature-sensitive mutants also are particularly useful in genetic suppressor screens. Suppressor screens allow for an unbiased approach in the identification of second-site extragenic mutations that ameliorate the phenotype of the original mutation. The use of temperature-sensitive alleles has the advantage of being able to perform mutagenesis on homozygous lines of mutations that are otherwise lethal.

The molecular identities of several of the legacy mutants have been determined through traditional cloning methods. However, these methods often are difficult and time-consuming, leaving the identities of hundreds of the legacy mutant alleles unknown. Recent advances in next-generation, whole-genome sequencing (WGS) has made it a rapid and cost-effective technique for the identification of molecular lesions causing a given phenotype (Minevich and Costello 2010) developed a one step protocol that combines WGS and single-nucleotide polymorphism (SNP) mapping that further eases mutation identification in C. elegans. This method backcrosses the mutant of interest (normally generated in the N2 Bristol isolate) to the polymorphic Hawaiian isolate of C. elegans generating recombinants that are sequenced in a single pool. The genomic region linked to the mutation of interest is identified as the region with a decrease in Hawaiian SNPs. This region is then analyzed for molecular lesions.

With the intention of identifying additional reagents that could help expand our knowledge of different aspects of embryonic development including meiotic chromosome segregation and eggshell formation, we performed a literature search for legacy mutants that were previously identified in genetic screens and were preliminarily characterized as temperature sensitive. Of the more than 500 temperature sensitive mutants isolated in early screens, we focused on mutants that were characterized as embryonic lethal or reported to have eggshell defects (osmotic sensitivity, irregular shape, and unshelled or thinly shelled embryos). We chose 12 mutants from publications at least 25 yr old in which the molecular lesion producing the phenotype had not been determined. Of those 12 legacy mutants, only six fit the criteria of being highly penetrant temperature-sensitive mutants. We chose an additional mutant, mel-2(t20), that was not temperature-sensitive but fit the criteria of being a legacy mutant and maternal-effect embryonic lethal. These seven were subjected to WGS/SNP mapping. The molecular lesion causing the phenotype of each mutant was confirmed by RNAi phenocopy and/or by complementation tests. Our analysis revealed that all seven of these legacy alleles bore mutations in genes that had only been studied previously in nonconditional mutants or by RNAi (tyms-1, chaf-1, tofu-6, fasn-1, mus-101, and cdc-25.2). Two of the temperature-sensitive mutations [zyg-2(b10)] and [emb-10 (k126)], thought to define distinct genes, were shown to be allelic; both had mutations in the mus-101 gene. The identification of emb-29 (g52ts) as an allele of cdc-25.2 was further confirmed by CRISPR/Cas9 gene editing where we rescued the mutation and recreated the temperature-sensitive phenotype by editing the single mutation in a wild-type background. We have demonstrated the ease and utility of one-step WGS/SNP mapping to identify legacy mutations. The strategies presented here could be applied to any temperature-sensitive mutant that remains to be identified molecularly.

MATERIALS AND METHODS

C. elegans strains and culture

The following strains were used: N2 (Bristol), GG36: emb-6(g36) I, MJ65: emb-6(kc65) I, GG43: emb-14(g43) I, GG20: emb-17(g20) I, MJ63: emb-10(k12) I, DH10: zyg-2(b10) I, GG52: emb-29(g52) V, YH525: cdc-25.2(ok597Δ) (V)nc11[glS31] (IV;V), VC2252: npp-6 (ok2821Δ) (IV)hT2[bl4(e937) let-6872] qIs48(Pmyo-2::gfp, Pges-1::gfp) [I;III], CV87: ysp-4(m2713Δ) (IV)hT2[bl4(e937) let-6872] qIs48 [I;III], VC3196: smgl-1(ok2423Δ) (IV)hT2[bl4(e937) let-6872] qIs48 [I;III], VC1878: lpd-3(ok2138Δ) I, VC150: ekl-1(ok11978) [I;III], VC(hT2[bl4(e937) let-6872] qIs48) [I;III], MT20434: chaf-1(n5453Δ) [I;III], VC(hT2[bl4(e937) let-6872] qIs48) [I;III], CB4856: wild-type Hawaiian isolate, KK359: mel-2(t20) unc-4(e120)/mnc1 [dpy-10(e128) unc-52(444)] II, K1373: tofu-6(y12) II, yEx100, AG247: tyms-1(tm2429Δ) (IV)hT2[bl4(e937) let-6872] qIs48 [I;III], AG248: mus-101(tm1761Δ) [I;III] hT2[bl4(e937) let-6872] qIs48 [I;III]. All tm deletion alleles were obtained from the National Bioresource Project as heterozygotes and were balanced with hT2. The presence of the deletion allele was monitored by polymerase chain reaction. All legacy, ok deletion, and tm deletion alleles are available from the Caenorhabditis Genetics Center.

Quantification of embryonic viability

Single L4 hermaphrodites of the indicated genotype were placed on individual plates at 15°C, 20°C, or 24°C. Adult worms were transferred to new plates daily until the sperm was exhausted. Percent hatching is calculated as the number of hatched larvae divided by the total number of eggs laid. For all quantifications, the number of broods scored is indicated in the figure legends.

RNAi phenocopy and complementation tests

RNAi phenocopy: Gene candidates were obtained from either the Ahringer RNAi feeding library or the OpenBiosystems RNAi feeding library (Timmons et al. 2001; Kamath and Ahringer 2003). Bacteria expressing double-stranded RNA were seeded onto MYOB plates containing 25 μg/mL carbenicillin and 1 mM IPTG and allowed to grow at 22°C for 48 hr. L4 hermaphrodites were fed bacteria expressing double-stranded RNA specific to a C. elegans gene (as indicated in text and figures) and scored for the number of dead embryos vs. live progeny. Bacteria expressing smd-1 and cdk-1 were used as negative and positive controls respectively.

Complementation tests: Homozygous (or balanced heterozygotes) L4 hermaphrodites of mutant X were mated to homozygous L4 males of mutant Y at 15°C. L4 cross progeny were picked to individual plates, incubated at 24°C, and examined for embryonic viability as described previously.

mel-2(t20) rescue: mel-2(t20) unc-4(e120)/mnc1 hermaphrodites were mated to N2 males. mel-2(t20) unc-4(e120)/+ heterozygous males were then mated to tofu-6(y12) hermaphrodites carrying the
Whole-Genome Sequencing

Identification of the molecular lesion responsible for each mutant was determined using a SNP-based mapping strategy combined with WGS (Doitsidou et al. 2010). Hermaphrodites from mutant strains were crossed with Hawaiian males (strain CB4856). Approximately 200 F2 progeny were single-sorted to fresh 3.5-cm plates and shifted to 24°C for 16 hr to reveal the mutant phenotype. The plates were returned to 15°C and allowed to produce progeny for one to two generations. The animals from 20 to 50 mutant plates were then pooled and genomic DNA isolated and sheared by sonication. Libraries were prepared using TruSeq reagents and sequenced for each library. Variants were identified using a pipeline of BFAST for alignment (Homer et al. 2009), SAMtools for variant calling (Li et al. 2009), and ANNOVAR for annotation (Wang et al. 2010) with C. elegans reference genome version WS220 (www.wormbase.org). Hawaiian SNP density was plotted against chromosome position using R (R Development Core Team 2014), and the mapping interval delimited by the absence of Hawaiian SNPs. Mutations within the mapping interval were filtered to remove variants derived from the Hawaiian and pre-mutation strain backgrounds. Candidate genes were defined by homozygous (>85% variant call), nonsynonymous mutations.

CRISPR/Cas9-mediated single-nucleotide rescue of emb-29(g52ts)

Rescue of the cytosine 716 to thymine mutation (codon 239) in cdc-25.2 in emb-29(g52ts) was performed by editing the single nucleotide 716 from T to a wild-type C using the CRISPR/Cas9 technology. An additional silent nucleotide change was made to prevent possible recutting of the rescue oligo or the target site (Kim et al. 2014). This additional change created an Eead recognition site (underlined to follow), which was used to distinguish the rescued line from N2,cdc-25.2 was targeted for Cas9 cleavage using the guide sequence (ATGTCTCTCAATGTTTCGG) in pDD162 (Dickinson et al. 2013), targeting the non-coding strand. emb-29(g52ts) young adults were injected with plasmids containing a dominant rescue transgene. F1 rescue progeny were selected, and co-conversion screening purposes (Arribere et al. 2014). Seven N2 worms were successfully injected and kept at 15°C. Thirty-five F1 rollers were single-sorted and allowed to lay embryos. Of the F1 rollers, eight contained the C681→G silent mutation in the PAM site by restriction digest (confirmed by sequencing), but only one of those eight had the C716→T edit when sequenced. We believe the greater frequency of the silent mutation may be attributable to the Cas9 cut site being only one nucleotide away from the silent mutation, whereas the C716→T site was 34 bases away. To obtain multiple lines, two lines of mutant adults were injected with the same constructs as described previously and 34 roller F1s were single-sorted at 15°C. Twelve F2 L4 larvae from each of the 34 mothers were single-sorted and shifted up to 24°C. After laying embryos, F2s were returned to the permissive temperature (15°C) for recovery. After 24 hr, plates were examined for hatching embryos at 24°C. In two of the 34 cases (~25%) of the animals had a temperature-sensitive embryonic lethal phenotype. Homozygous non-dpy-10 embryonic lethal lines were recovered from these lines. Sequencing confirmed the C716→T change and both the lines also contained the C681→G silent mutation. These lines were given the allele designation av40.

RESULTS

The selection and characterization of C. elegans mutants for WGS

Twelve mutants that had been identified previously in genetic screens but whose molecular identity remained unknown were chosen for analysis and WGS (Table 1). Each mutant was tested for temperature sensitivity and for embryonic lethality by counting the number of dead embryos vs. live progeny at both the permissive (15°C) and restrictive (24°C) temperatures (Figure 1A). One mutant, mel-2(t20), was not temperature sensitive and was maintained by picking balanced heterozygous mel-2(t20) unc-4(e120)mm1 (Figure 1B). These animals were scored for embryonic lethality by picking Unc homozygotes at 20°C and counting the number of dead embryos vs. live progeny produced. For six mutants, embryonic lethality was temperature-sensitive and strongly penetrant at 24°C (Figure 1A). Although all mutants were preliminary characterized as embryonic lethal in the original studies, some mutants showed weak penetrance of the embryonic lethal phenotype at the restrictive temperature and others did not appear lethal at any temperature tested (Figure 1A). The lack of an embryonic lethal phenotype could be due to several reasons such as loss of the original mutations during the 30-plus years of maintaining the strain.

We chose the six mutants with strong temperature-sensitive embryonic lethality, as well as mel-2(t20), for WGS using the single step SNP mapping strategy of Doitsidou et al. (2010) (WGS/SNP mapping) (Doitsidou et al. 2010; Wang et al. 2014). All seven mutants were recessive. After a single outcross into the Hawaiian strain, CB4856,
homzygous F2 embryonic-lethal mutants were reisolated and allowed to grow another one to two generations at the permissive temperature (15°C). Pooled populations of F3/F4 animals were harvested for WGS and SNP analysis (see the section Materials and Methods). A loss or reduction of Hawaiian SNPs defined the mapping interval containing the mutant allele. A representative graph plotting the Hawaiian SNPs along the chromosome from a population of F2 recombinants is shown in Figure 2A. This strategy produced an average mapping interval of 3.0 Mb (Figure 2B). The mapping interval was then analyzed for mis-
vocates of F2 recombinants shown in Figure 2A. This strategy produced an average mapping interval of 3.0 Mb (Figure 2B). The mapping interval was then analyzed for mis-

List of mutants analyzed in this work

| Mutant       | Preliminary Phenotypic Characterization                                                                 | References                                      |
|--------------|----------------------------------------------------------------------------------------------------------|------------------------------------------------|
| emb-6(g36ts) | Maternally or zygotically required for embryogenesis. Arrest at the 14- to 19-cell stage.                | (Schierenberg et al. 1980; Miwa et al. 1980; Denich et al. 1984) |
| emb-10(k12ts)| Arrested embryonic development.                                                                          | (Herman et al. 1980)                           |
| emb-11(g4ts) | Osmotically sensitive. 94% embryonic arrest during early proliferation.                                   | (Cassada et al. 1981)                         |
| emb-12(g5ts) | Osmotically sensitive. 72% embryonic arrest during early proliferation.                                    | (Cassada et al. 1981)                         |
| emb-14(g43ts)| Osmotically sensitive. 100% arrest.                                                                      | (Cassada et al. 1981)                         |
| emb-17(g20ts)| Eggs irregular in shape. Arrest at the lima bean stage.                                                   | (Cassada et al. 1981; Denich et al. 1984)      |
| emb-21(g31ts)| 90% arrested embryonic development at the 26- to 30-cell stage. Eggs round in shape.                     | (Cassada et al. 1981; Denich et al. 1984)      |
| emb-29(g52ts)| Maternally required for embryogenesis. 100% arrest at the 150- to 200-cell stage. Also affects gonadogenesis.| (Cassada et al. 1981; Denich et al. 1984)      |
| zyg-2(b10ts)| Arrested embryonic development at the 25- to 50-cell stage. Also required for gonadogenesis and male fertility. | (Wood et al. 1980; Hecht et al. 1982)           |
| zyg-3(b18ts)| Maternal and zygotic expression required for embryonic development. Arrest during proliferation. Eggs lyse in vitro. | (Wood et al. 1980)                            |
| zyg-7(b18ts)| Maternal and zygotic expression required for embryonic development.                                      | (Wood et al. 1980)                            |
| mel-2(it20)| Maternal-effect lethal. No male rescue.                                                                     | (Kemphues et al. 1988)                        |

emb-6(g36ts) is an allele of tyms-1

Emb-6 had been mapped previously to chromosome I at -0.47 cM. The WGS and SNP-based (WGS/SNP) method of mutant mapping also mapped the molecular lesion to chromosome I and to a 2.8-Mb interval. Analysis of that interval produced an average mapping interval of 3.0 Mb (Figure 2B). The mapping interval was then analyzed for mis-

In embryonic lethality (98% embryonic viability). Because RNAi knockdown of both tyms-1 and npp-6 phenocopied the embryonic lethality of the emb-6(g36ts) mutant, further validation was required.

To determine which of these two mutations is responsible for the emb-6(g36ts) embryonic-lethal phenotype, complementation tests were performed. Males of emb-6(g36ts); him-8(c1489) were mated to either npp-6(ok2821Δ)/hT2 or tyms-1(tm2429Δ)/hT2 hermaphrodites at 15°C (hT2 is a translocation balancer with a pharyngeal GFP insertion). Single non-green F1 cross progeny were picked to individual plates, incubated at 24°C and scored for the number of dead embryos vs. viable progeny. emb-6(g36ts) and npp-6(ok2821Δ) complemented each other with 94% of the progeny of F1 trans-heterozygotes hatching at 24°C (Table 2 and Figure 3). We conclude that npp-6(ok2821Δ) and emb-6(g36ts) are not alleles of the same gene. On the other hand emb-6(g36ts) and tyms-1(tm2429Δ) fail to complement (0% hatching, Table 2 and Figure 3) suggesting that tyms-1 and emb-6 are the same gene. The g36ts allele has a G→A transition (Gly42 to Arg) in tyms-1. As further confirmation, we sequenced the tyms-1 gene in another emb-6 allele, hcs5ts, and found a missense mutation in tyms-1 (C→T), which changes proline 71 to a serine.

Even though smgl-1 RNAi did not phenocopy emb-6, a deletion mutant allele of smgl-1 was available from the Caenorhabditis Genetics Center that was embryonic lethal. To further rule out smgl-1 as a candidate for emb-6, we performed complementation tests between emb-6(g36ts) and smgl-1(ok2423Δ). The trans-heterozygotes complemented at 24°C (95% hatching, Table 2 and Figure 3) ruling out smgl-1 as an emb-6 candidate. Because tyms-1 is the only candidate that both phenocopied the emb-6 embryonic lethality and failed to complement, we conclude that emb-6 is an allele of tyms-1, an ortholog of thymidylate synthase. Thymidylate synthase is involved in the pyrimidine biosynthesis pathway and is a major target of 5-fluorouracil, an anticancer drug (Kim et al. 2008). The Gly42 to Arg mutation found in
**emb-6(g36ts)** and the Pro71 to Ser mutation of **emb-6(nc65ts)** are located in the pyrimidine hydroxymethylase domain.

**emb-17(g20ts)** is an allele of **chaf-1**

Previous mapping of **emb-17(g20ts)** placed it on chromosome I but its position along the chromosome was not determined. WGS/SNP mapping found a reduction in the number of Hawaiian SNPs on chromosome I within a 7.2-Mb interval. This interval contained 10 nonsynonymous sequence variants (Table 3). Of the 10 candidates, four were reported to have some degree of embryonic lethality by RNAi (rab-10, ekl-1, smgl-1, and chaf-1). RNAi clones were available for ekl-1 and smgl-1. To determine whether either DNA lesion found in ekl-1 or smgl-1 resulted in the same phenotype as **emb-17(g20ts)**, we performed RNAi phenocopy experiments. As stated in the previous section, RNAi knockdown of **smgl-1** did not result in embryonic lethality but RNAi knockdown of ekl-1 resulted in 42% embryonic lethality (Table 3).

Previous analysis of deletion alleles of three of the 10 candidates found them to be sterile [ekl-1(ok1197Δ)] or embryonic lethal [**smgl-1** (ok2423Δ) and **chaf-1** (n5453Δ)]. For the remaining candidates, deletion alleles were either not available (**met-1**, **M04F3.2**, **rde-2**, **F55H12.4**, and **B0205.1**) or not characterized as sterile or embryonic lethal (**ldp-3** and **rab-10**). To further validate the mutant candidate responsible for the **emb-17(g20ts)** embryonic lethal phenotype, we performed complementation tests between **emb-17(g20ts); him-8(e1489)** males and **chaf-1** males with **emb-17(g20ts)**; **him-8(e1489)** males and **chaf-1** males crossed with **lad-3(hok2143Δ)** hermaphrodites. **lad-3(hok2143Δ)** complemented **emb-17** (**g20ts**), 97% and 87% hatching of F2 progeny at 24°C, Table 3 and Figure 4, suggesting that neither of these genes are allelic to **emb-17(g20ts)**. Although **lad-3(hok2143Δ)** was not characterized to be sterile or embryonic lethal, a deletion allele existed. Therefore, we also performed complementation tests between **emb-17(g20ts); htk-8(e1489)** males and **chaf-1** males crossed with **lad-3(hok2143Δ)** hermaphrodites. **lad-3(hok2143Δ)** complemented **emb-17** (**g20ts**), 98% hatching of F2 progeny at 24°C, Table 3 and Figure 4. Only **trans**-heterozygous **F1s** from **emb-17(g20ts); htk-8(e1489)** males crossed with **chaf-1(n5453Δ); htk-2** hermaphrodites produced dead embryos (4.9% and 2.8% hatching at 24°C, Table 3 and Figure 4). Furthermore, **F1 trans**-heterozygotes were not healthy at the permissive temperature of 15°C (Figure 4), suggesting that these two alleles do not make enough active gene product at the permissive temperature to support development. Taken together these data show that **chaf-1(n5453Δ)** fails to...
complement *emb-17(g20ts)*, demonstrating that they are alleles of the same gene that codes for the chromatin assembly factor chromatin assembly factor-1 p150 subunit. Chromatin assembly factor-1 is a three-subunit protein complex (p150, p60, and p48) required for the deposition of histones H3-H4 into nucleosomes during DNA replication (Nakano et al. 2011; Smith and Stillman 1989). The g20ts allele is a C→T transition that changes Arg436 to His in the p60 binding domain of the p150 subunit.

**emb-10(k12ts) and zyg-2(b10ts) are alleles of mus-101**

Mapping data of *emb-10* placed it on chromosome I at 5.74 cM and zyg-2 was previously mapped to chromosome I at 6.24 cM. WGS/SNP mapping placed *emb-10* in a 0.9-Mb interval on chromosome I and zyg-2 within a 5.2-Mb interval on chromosome I. Only two nonsynonymous sequence variants were identified for both *emb-10* (*mus-101* and *F59C6.8*, Table 4) and zyg-2 (*mus-101* and *lrk-1*, Table 5). Previous studies had not tested whether these two mutants might be alleles of the same gene. However, the WGS/SNP mapping analysis found *mus-101* lesions as candidates for both mutants. In addition, *mus-101* was the only candidate reported to be embryonic lethal by RNAi. To validate whether the *mus-101* lesions were responsible for the *emb-10(k12ts)* and zyg-2(b10ts) phenotypes, we performed RNAi phenocopy experiments. However, *mus-101* RNAi was not embryonic lethal in our hands.

Mutant analysis had previously determined that *mus-101 (tm1761Δ)* was sterile and/or lethal. To validate whether the *mus-101* genetic lesions identified from WGS of *emb-10(k12ts)* and zyg-2 (b10ts) were indeed alleles of *mus-101*, complementation tests were performed. Matings between *emb-10(k12ts)* males or *zyg-2(b10ts); him-8* males and *mus-101(tm1761Δ); hT2* hermaphrodites were performed. Single F1 trans-heterozygous cross progeny were analyzed for the number of dead F2 embryos vs. hatched F2 progeny at 15° and 24°. *mus-101(tm1761Δ)* failed to complement both *emb-10(k12ts)* and zyg-2(b10ts) (1% and 0% hatching at 24°, Table 4, and Table 5, and Figure 5) demonstrating that all three were allelic. In addition, embryonic viability of *mus-101(tm1761Δ); zyg-2(b10ts)* F1 trans-heterozygotes at the permissive temperature of 15° was only 3.9% (Figure 5) suggesting that the nonsense allele of *zyg-2(b10ts)* is stronger than the missense allele of *emb-10(k12ts)* when combined with the deletion allele of *mus-101(tm1761Δ)*. To further validate that *emb-10(k12ts)* and zyg-2(b10ts) are two alleles of the same gene, we crossed *emb-10(k12ts)* males with zyg-2(b10ts) hermaphrodites and analyzed the progeny of single F1 trans-heterozygous animals. *emb-10(k12ts)* and zyg-2(b10ts) fail to complement with only 3.6% hatching at 24° (Figure 5, Cross 1). The reciprocal mating [zyg-2(b10ts); him-8* males crossed with *emb-10(k12ts)* hermaphrodites] also failed to complement (3.5% hatching, Cross 2). These data demonstrate that *emb-10 (k12ts)* and zyg-2(b10ts) are temperature-sensitive alleles of *mus-101*, a member of the Mus101 gene family involved in DNA damage repair and cell-cycle checkpoint control. In *C. elegans* the *mus-101* homolog is a 1227 amino acid protein that plays a role in both DNA replication and in the response to DNA damage (Holway et al. 2005). *emb-10 (k12ts)* is a G→A change resulting in Cys268 to a Tyr in one of the six BRCT domains, which mediate protein–protein and protein–DNA interactions. zyg-2(b10ts) is a G→A transition that changes Thr1062 to a premature stop codon. The 165-bp region removed by the zyg-2 (b10ts) premature stop is not predicted to contain a functional domain. However, an oligomerization domain resides in the C-terminus of the human homolog TOPBP1 (Sokka et al. 2010).

**mel-2(it20) is an allele of tofu-6**

*mel-2(it20)* was originally identified in a screen for maternal-effect lethal mutants on linkage group II and was mapped to the left of *dpy-10* (Kemphues et al. 1988). WGS/SNP mapping found a 1.3-Mb interval with a reduction in Hawaiian SNPs on chromosome II (Table 6). Seven homozygous, nonsynonymous sequence variants were identified within that interval; two variants were identified within the same gene, *T14B4.2*. Two of the candidates were not reported to have an embryonic lethal phenotype by RNAi (*C25H3.8* and *F41G3.20*), and one was reported to have a low penetrance of embryonic lethality (*cyp-4*) and were not investigated further (Table 6). The other three candidates were reported to have a range of embryonic lethality by RNAi (tofu-6, *C56C10.10*, and *T14B4.2*).

Because *tofu-6(yt2)* was identified as an allele that caused 100% maternal-effect embryonic lethality in a screen for altered expression of an early embryonic reporter construct (Minasaki and Streit 2007), it was a likely candidate. A combination of classical mapping, SNP mapping, and sequencing identified the *yt2* allele as a premature stop codon. An extrachromosomal array (*ytEx100*) containing a 3245-bp genomic fragment including wild-type *tofu-6* rescued the embryonic lethality of *tofu-6(yt2)* (Minasaki and Streit 2007). To validate whether *mel-2(it20)* is an allele of *tofu-6*, we crossed *mel-2(it20) unc-4(e120)*/+ heterozygous males with rolling *tofu-6(yt2); yEx100* (*ytEx100* is marked with a dominant marker rol-6(R1006)) hermaphrodites. The RolUnc progeny of F1 rollers were picked and scored for hatched progeny. All *mel-2(it20) unc-4(e120); YtEx100* animals produced live progeny whereas Unc non-Rol animals that segregate from these lines exhibit...
Table 2  emb-6(g36ts) is an allele of tyms-1

| Strain            | Candidate | Mutation     | Description                        | RNAi Phenocopy-Reported on Wormbase | RNAi Phenocopy-This Study | Complementation Test |
|-------------------|-----------|--------------|------------------------------------|-------------------------------------|---------------------------|----------------------|
| emb-6(g36ts)      | tyms-1    | G->A, Gly42Arg | Thymidylate synthase ortholog       | Embryonic lethal                    | 89% embryonic lethal      | Fails to complement tyms-1(tm2429Δ) at 24°C (9% hatching, N = 16) |
| npp-6             |           | G->A, Gly861Arg | Nuclear pore complex               | Embryonic lethal                    | 99% embryonic lethal      | Complements npp-6(ok2821Δ) at 24°C (94% hatching, N = 14) |
| fm-4              |           | C->T, Pro241Ser | FERM domain protein                | Not embryonic lethal                | Not embryonic lethal      | NP                   |
| Ipd-2             |           | G->A, Ala14Thr | Lipid depleted Capping, ARp2/3, Myosin I linker protein | Not embryonic lethal                | Not embryonic lethal      | NP                   |
| FzdF4.3           |           | C->T, Ser66Leu | No information available           | Not embryonic lethal                | Not embryonic lethal      | NP                   |
| F27D4.6           |           | G->A, Arg980Lys | No information available           | Not embryonic lethal                | Not embryonic lethal      | NP                   |
| smgl-1            |           | C->T, Pro698Ser | SMG-associated and lethal           | ~50% embryonic lethal               | Not embryonic lethal      | Complements smgl-1(oc2423Δ) at 24°C (95% hatching, N = 8) |

List of gene candidates with genomic DNA sequence variants identified by WGS/SNP mapping of emb-6(g36ts), the missense mutation identified and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. N is the number of F1 trans-heterozygous animals shifted to 24°C for F2 hatching analysis. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.

emb-14(g43ts) is an allele of fasn-1

emb-14 was previously mapped to chromosome I at 1.71 cM. Analysis of the sequence data generated by WGS/SNP mapping of emb-14(g43ts) found a reduction of Hawaiian SNPs on chromosome I within a 2.2 Mb interval. This interval contained seven nonsynonymous sequence variants (Table 7). Of the seven candidates, only one was reported to have an embryonic lethal phenotype by RNAi (fasn-1) and one was reported as lethal or sterile by mutant analysis (syp-4). fasn-1 RNAi phenocopied the embryonic lethal phenotype of emb-14(g43ts), however, no deletion mutant was available for further validation by

Figure 3 emb-6(g36ts) fails to complement tyms-1 (tm2429Δ). The following complementation tests were performed: emb-6(g36ts); him-8(e1489) males crossed with tyms-1(tm2429Δ)/hT2, npp-6(ok2831Δ)/hT2, smgl-1(oc2423Δ)/hT2, and emb-6(hc65ts) hermaphrodites. The progeny of single F1 trans-heterozygous hermaphrodites were scored for the number of dead embryos vs. hatched larvae and the results calculated as the average percent embryonic viability at 15°C or 24°C. The broods of at least seven hermaphrodites were scored for each cross at 15°C. The broods of at least eight hermaphrodites were scored for each cross at 24°C. F1 trans-heterozygous hermaphrodites from the emb-6(g36ts); him-8(e1489) males crossed with emb-6(hc65ts) produced only one hatched larva out of the 1906 embryos scored. *P < 0.0001. Error bars indicate SD. N = total number of embryos and hatched larvae scored. At least 1100 embryos were scored for each cross.
complementation tests. The deletion allele, syp-4(tm2713Δ), was used for complementation tests with emb-14(g43ts). syp-4(tm2713Δ)/+ males were crossed with emb-14(g43ts) homozygous hermaphrodites and the progeny of trans-heterozygous F1 were examined for their ability to hatch at 15°C and 24°C. All F1 hermaphrodites produced viable progeny (81% hatching, Table 7). To further validate the identity of

| Strain        | Candidate | Mutation     | Description               | RNAi Phenocopy—Reported on Wormbase | RNAi Phenocopy—This Study | Complementation Test |
|---------------|-----------|--------------|---------------------------|-------------------------------------|---------------------------|----------------------|
| emb-17(g20ts) | lpd-3     | C->T, Ala467Thr | Lipid depleted           | Not embryonic lethal                | NP                        | Complements lpd-3(ok2138Δ) but some larval lethality in F2 at 24°C (98% hatching, N = 10). |
| met-1         | C->T, Arg1167Cys | Histone methyltransferase | Not embryonic lethal | NP                                   | NP                        |                      |
| M04F3.2       | G->T, Glu17stop | No information available | Not embryonic lethal | NP                                   | NP                        |                      |
| rab-10        | C->T, Ala82Thr | Rab-like GTPase | Embryonic lethal          | 42% embryonic lethal                | NP                        | Complements ekl-1(ok1197Δ) at 24°C (97% hatching, N = 9) |
| ekl-1         | C->A, Leu582Ile | Enhancer of Ksr-1 Lethality | Range of embryonic lethal | NP                                   | NP                        |                      |
| rde-2         | G->T, Thr148Asn | RNAi defective | Not embryonic lethal      | ~50% embryonic lethal               | NP                        |                      |
| smgl-1        | C->T, Asp1141Asn | SMG-associated and lethal | Not embryonic lethal     | not embryonic lethal                | NP                        |                      |
| F55H12.4      | T->C, Lys170Arg | No information available | Not embryonic lethal     | NP                                   | NP                        |                      |
| chaf-1        | C->T, Arg436His | Chromatin Assembly Factor | Embryonic lethal | NP                                   | NP                        | Fails to complement chaf-1(n5453Δ) at 24°C (4.9% hatching, N = 46) |
| B0205.1       | C->T, Ser268Asn | No information available | Not embryonic lethal     | NP                                   | NP                        |                      |

List of genes with genomic DNA sequence variants identified by WGS/SNP mapping of emb-17(g20ts), the missense mutation identified, and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. N is the number of F1 trans-heterozygous animals shifted to 24°C for F2 hatching analysis. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.

Figure 4 emb-17(g20ts) fails to complement chaf-1 (n5453Δ). Complementation tests were performed by crossing emb-17(g20ts); him-8(e1489) males with lpd-3(ok2138Δ), ekl-1(ok1197Δ)/hT2, smgl-1(ok2423Δ)/hT2, or chaf-1(n5453Δ)/hT2 (Cross 1) hermaphrodites. In addition, emb-17(g20ts); him-5(e1490) males were crossed with chaf-1(n5453Δ)/hT2 hermaphrodites (Cross 2). The progeny of single F1 trans-heterozygous hermaphrodites were scored for the number of dead embryos vs. hatched larvae and the results calculated as the average percent embryonic viability at 15°C or 24°C. The broods of at least seven F1 hermaphrodites were scored for each cross at 15°C. The broods of at least five F1 hermaphrodites were scored for each cross at 24°C. *P < 0.0001. Error bars indicate standard deviation. At least 900 embryos were scored for each cross.
**Table 4** emb-10(k12ts) is an allele of mus-101

| Strain          | Candidate | Mutation       | Description                                                                 | RNAi Phenocopy—Reported on Wormbase | RNAi Phenocopy—This Study | Complementation Test |
|-----------------|-----------|----------------|------------------------------------------------------------------------------|-------------------------------------|---------------------------|----------------------|
| emb-10(k12ts)   | **mus-101** | G->A, Cys268Tyr | Chromosomal protein for DNA metabolic processes                                | Range of embryonic lethal            | Not embryonic lethal     | Fails to complement mus-101(tm1761Δ) at 24° (1% hatching, N = 12) |
| F59C6.8         | C->T, Thr446Met | No information available | Not embryonic lethal                                                         | Not embryonic lethal                |                           | NP                   |

List of genes with genomic DNA sequence variants identified by WGS/SNP mapping of emb-10(k12ts), the missense mutation identified, and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. N is the number of F1 trans-heterozygous animals shifted to 24° for F2 hatching analysis. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.

**emb-14(g43ts)** seven Dpy non-Unc and seven Unc non-Dpy recombinants were isolated from emb-14(g43ts)/dpy-5 unc-13 heterozygotes (Supporting Information, Figure S1A). After generating homozygous recombinants for the dpy-5 or unc-13 markers, these recombinants were shifted to 24° to test for the presence of emb-14(g43ts). Each line was then subjected to polymerase chain reaction and sequencing of the fasn-1 and syp-4 genes and the sequences analyzed for the mutations identified by WGS. All seven Dpy non-Unc recombinants carried emb-14(g43ts) (i.e., exhibited embryonic lethality at 24°). These seven lines also contained the fasn-1 mutation (G→A, Ala1425 to Thr) and one of the lines was wild type for syp-4 (Figure S1B). None of the seven Unc non-Dpy were embryonic lethal at 24°, suggesting that they did not contain emb-14(g43ts). In addition, one of the Unc non-Dpy (not embryonic lethal) carried the syp-4 mutation (Figure S1B). These genetic data combined with the sequencing data supports the conclusion that emb-14 is allelic with the fasn-1 gene, fasn-1 is the *C. elegans* fatty acid synthase, which synthesizes the conversion of malonyl CoA into palmitate in the fatty acid biosynthetic pathway. Depletion of maternal fasn-1 by RNAi causes a loss of anterior-posterior polarity in the progeny and osmotic sensitivity (Rappleye et al. 2003). A previously identified allele, fasn-1(fr8), has also been found to play a role in the innate immune response (Lee et al. 2010). The emb-14(g43ts) mutation changes Ala1425 to Thr in a conserved beta-ketoacyl reductase domain, which is needed for reduction of the growing fatty acid chain.

**emb-29(g52ts)** is an allele of cdc-25.2 and is rescued by CRISPR gene editing

Previous characterization placed *emb-29* on chromosome V at −18.34 cM and another group previously implicated *emb-29(g52ts)* as a complex allele of *cdc-25.2* (Nair et al. 2013). Using a different method of WGS, Nair et al. (2013) found a Ser239 to Phe change in both *emb-29(g52ts)* and *emb-29(b262)*. However, no definitive proof such as rescue experiments has ever been reported. Using the method of WGS/SNP mapping, we found a reduction of Hawaiian SNPs in a 1.3-Mb interval on chromosome V, consistent with previous characterization. Within this interval there were two nonsynonymous sequence variants, *nas-32* (Ala77 to Thr) and *cdc-25.2* (Ser239 to Phe) (Table 8). Only *cdc-25.2* was reported to be embryonic lethal by RNAi, but we never observed embryonic lethality by RNAi feeding.

Further validation by complementation has proven difficult. A deletion allele of *cdc-25.2* is homozygous sterile (Kim et al. 2010). We performed complementation tests by crossing homozygous *emb-29(g52ts)* males to *cdc-25.2(ok597A)/hT2* hermaphrodites and analyzed single non-green *trans*-heterozygous progeny for the number of dead F2 embryos vs. live F2 progeny. Similar to Nair et al. (2013), we found that at 24° only ~21% of progeny from *emb-29(g52ts)/cdc-25.2 (ok597A)* F1s die as embryos but 91% of the surviving progeny are sterile (Table 8). These data suggest that *emb-29(g52ts)/cdc-25.2 (ok597A)* complements the embryonic lethal phenotype of *emb-29(g52ts)* but fails to complement the sterility of *cdc-25.2 (ok597A)*.

Because there appears to be a complex genetic interaction between these two alleles making the complementation test results ambiguous, we rescued *emb-29(g52ts)* using the CRISP/Cas9 gene editing system. *emb-29(g52ts)* was rescued by a single nucleotide conversion at nucleotide 716 of *cdc-25.2* with CRISPR/Cas9 gene editing and oligonucleotide-mediated recombination. We recovered three independent rescued lines. While embryonic viability of *emb-29(g52ts)* was 0% (1039 embryos) at 24°, the single-nucleotide change (T→C)

**Table 5** zyg-2(b10ts) is an allele of mus-101

| Strain          | Candidate | Mutation       | Description                                                                 | RNAi Phenocopy—Reported on Wormbase | RNAi Phenocopy—This Study | Complementation Test |
|-----------------|-----------|----------------|------------------------------------------------------------------------------|-------------------------------------|---------------------------|----------------------|
| zyg-2(b10ts)   | **mus-101** | G->A, Trp1062stop | Chromosomal protein for DNA metabolic processes                                | Range of embryonic lethal            | Not embryonic lethal     | Fails to complement mus-101(tm1761Δ) at 24° (0% hatching, N = 9) |
| Irk-1           | G->A, Gly1244Asp | Leucine-rich repeat kinase | Not embryonic lethal                                                         | Not embryonic lethal                |                           | NP                   |

List of genes with genomic DNA sequence variants identified by WGS/SNP mapping of zyg-2(b10ts), the missense mutation identified, and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. N is the number of F1 trans-heterozygous animals shifted to 24° for F2 hatching analysis. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.
led to an average of 99% hatching (2511 embryos) among the three rescued lines at 24°C, similar to N2 (99.8% hatching; 1618 embryos, Figure 6). To further validate that this single nucleotide change in cdc-25.2 is the cause of the emb-29(g52) temperature-sensitive embryonic lethal phenotype, we edited the genome of wild-type (N2) animals to recreate the emb-29(g52ts) allele. Although 99.8% (1618 embryos) of N2 progeny hatched at 24°C, the percentage hatching of the recreated emb-29(g52ts) (C>T) mutation in three independent lines drastically decreased hatching to 0% (1094 embryos) at this temperature, similar to emb-29(g52ts) (0%; 1039 embryos, Figure 6). The rescue of emb-29(g52ts) and recreation of the temperature sensitivity by changing only a single nucleotide confirms that embryonic lethality in this line is in

| Table 6 mel-2(it20) is an allele of tofu-6 |

| Strain          | Candidate | Mutation | Description                                      | RNAi Phenocopy—Reported on Wormbase | RNAi Phenocopy—This Study | Complementation Test |
|-----------------|-----------|----------|--------------------------------------------------|-------------------------------------|---------------------------|----------------------|
| mel-2(it20) unc-4(e120) | tofu-6    | C>T, Gln52stop | SRA stem-loop interacting RNA binding protein | Range of embryonic lethal | NP | ytEx100 rescues embryonic lethality of mel-2(it20). Two independent lines. |
| cyp-4           |           | C>T, Thr157Ile | Cyclophilin divergent member                     | Low-range embryonic lethal | NP | NP |
| C25H3.8         |           | G>A, Ser281Asn | No information available                        | Not embryonic lethal            | NP | NP |
| C56C10.10       |           | G>A, Arg118Gln | No information available                        | Range of embryonic lethal       | NP | NP |
| T14B4.2         | A>C       | Asn94His   | No information available                        | Range of embryonic lethal       | NP | NP |
| T14B4.2         | A>C       | Lys95Gln   | No information available                        | Range of embryonic lethal       | NP | NP |
| F41G3.20        | Insertion of a C between nucleotide 207 and 208; frameshift | No information available | Not embryonic lethal | NP | NP |

List of genes with genomic DNA sequence variants identified by WGS/SNP mapping of mel-2(it20) unc-4(e120), the missense mutation identified, and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.
fact caused by the C716→T nucleotide change in cdc-25.2, cdc-25.2 codes for one of four protein phosphatases (cdc-25.1-cdc-25.4) that promote cell cycle progression (Ashcroft et al. 1998). The Ser239 to Phe change of emb-29(g52ts) falls within a semi-conserved Rhodanese homology domain (amino acids 223-341). Members of the Rhodanese homology superfamily activate cell division kinases throughout the cell cycle.

**DISCUSSION**

Generating and characterizing an allelic series of mutants defective in a particular developmental process is a common strategy to understand how genes control that particular process. From classical work in phage and yeast, this approach has helped define complex biological processes from metabolic pathways to cell cycle division (Hartwell et al. 1974; Edgar et al. 1964). Pioneering work in the nematode, *C. elegans*, also applied a genetic approach to define nervous system development and behavior (Brenner 1973). In early attempts to understand the genetic control of embryonic development, many groups isolated thousands of mutants with an embryonic lethal phenotype (Hirsh and Vanderslice 1976; Vanderslice and Hirsh 1980; Schierenberg et al. 1980; Wood et al. 1980; Cassada et al. 1981). Many of these legacy mutants have only been partially characterized and in many cases the causative molecular lesion remains unknown.

The traditional method for the identification of the phenotype-causing mutation is a long and laborious process. Mutagenized strains contain a substantial number of sequence variants. Even with the identification of sequence variants by WGS, it is often necessary to first map the mutation to a chromosomal interval via three-point mapping or a SNP mapping strategy. Both of these strategies require

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### Table 7  *emb-14(g43ts)* is an allele of *fasn-1*

| Strain     | Candidate | Mutation | Description                                      | RNAi Phenocopy-Reported on Wormbase | RNAi Phenocopy-This Study | Complementation Test |
|------------|-----------|----------|--------------------------------------------------|-------------------------------------|---------------------------|----------------------|
| *emb-14(g43ts)* | *pqn-20* | C→T, Ser51Phe | Prion-like-(Q/N-rich)-domain-bearing protein | Not embryonic lethal | NP | NP |
| *syf-4* | C→T, Thr354Ile | Synapsis in meiosis abnormal | Not embryonic lethal | NP | Complements *syf-4(tm2713ts)* at 24°C (81% hatching, N = 9) |
| T21G5.1 | C→A, Pro144Gln | No information available | Not embryonic lethal | NP | NP |
| *smg-1* | G→A, Trp652stop | Phosphatidylinositol kinase-related protein kinase | Not embryonic lethal | NP | NP |
| *lrp-1* | C→T, Pro645Leu | Low-density lipoprotein (LDL) receptor-like protein | Not embryonic lethal | NP | NP |
| *F26A3.1* | G→A, Glu276Lys | Fatty acid synthase | Not embryonic lethal | NP | NP |
| *fasn-1* | G→A, Ala1425Thr | Fatty acid synthase | 100% embryonic lethal | NP | See text for genetic analysis of recombinants |

List of genes with genomic DNA sequence variants identified by WGS/SNP mapping of *emb-14(g43ts)*, the missense mutation identified and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. N is the number of F1 trans-heterozygous animals shifted to 24°C for F2 hatching analysis. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.

### Table 8  *emb-29(g52ts)* is an allele of *cdc-25.2*

| Strain     | Candidate | Mutation | Description                                      | RNAi Phenocopy-Reported on Wormbase | RNAi Phenocopy-This Study | Complementation Test |
|------------|-----------|----------|--------------------------------------------------|-------------------------------------|---------------------------|----------------------|
| *emb-29(g52ts)* | *nas-2* | G→A, Ala77Thr | Astacin family zinc metalloprotease Cdc25 phosphatase protein family homolog | Not embryonic lethal | NP | NP |
| *cdc-25.2* | C→T, Ser239Phe | Cdc25 phosphatase protein family homolog | Embryonic lethal penetrance 40-90% | not embryonic lethal | *cdc-25.2(ok597ts)* complements embryonic lethality at 24°C (78.6% hatching, N = 20), but 91% of F2s are sterile (N = 34) |

List of genes with genomic DNA sequence variants identified by WGS/SNP mapping of *emb-29(g52ts)*, the missense mutation identified and corresponding amino acid change, a description of the gene function, RNAi phenotypes reported on Wormbase, the percent embryonic lethality by RNAi experiments performed in this study, and the results of complementation tests performed in this study. NP, not performed. N is the number of F1 trans-heterozygous animals shifted to 24°C for F2 hatching analysis and the number of F2 animals with observed sterility. RNAi, RNA interference; WGS, whole-genome sequencing; SNP, single-nucleotide polymorphism. Bold indicates the gene, when mutated, that is responsible for the embryonic lethal phenotype.
reiterative mapping to narrow down the relevant interval, which is a time-consuming process. We applied the WGS/SNP mapping strategy originally devised by Doitsidou et al. (2010) to identify the molecular lesions responsible for the embryonic lethal phenotype of mutants isolated 30 years ago. In this work, we have demonstrated that this is a relatively fast and efficient method to map and identify the molecular lesion of legacy mutants. The entire process, from the first outcross with Hawaiian males, to the picking of F2 progeny, making the library, and WGS can take place in 3–4 wk. The list of candidates can be generated in as little as 2 d. The complementation tests take about 7–10 d once all necessary strains have been obtained. The total protocol can be accomplished in less than 2 mo. We applied the WGS/SNP mapping strategy to seven legacy mutants and found that all seven of the mutants carried a single-point mutation resulting in a nonsynonymous amino acid change that caused the mutant phenotype, six of which were temperature-sensitive.

A recent paper used a non-SNP-based method of WGS to identify the molecular lesion of 64 mutants of essential genes on chromosome I (Chu et al. 2014). Although five of the mutants we identified had mutations in genes on chromosome I, none of these were identified in the work by Chu et al. (2014). In this work, the newly identified alleles corresponded to genes that have been studied by other groups ranging in processes from cell cycle regulators to components of the fatty-acid biosynthetic pathway. Many of the newly identified mutations are either in functional domains or in conserved regions of the corresponding protein. Because they are essential genes, mutations result in embryonic lethality precluding extensive characterization. With the identification of temperature-sensitive alleles, further studies are possible simply by changing the growth temperature.

In summary, we demonstrated that the WGS/SNP-based mapping strategy can be used to identify the unknown molecular lesions from the hundreds of legacy mutants that were isolated from early studies of C. elegans development, and that ts mutants are ideal targets for this approach. We have identified additional alleles of known genes and alleles of essential genes, which, previously, could only be studied by RNAi. All but one of these alleles is temperature-sensitive. We believe that these new temperature-sensitive alleles of essential genes are a valuable resource for the further investigation of essential developmental processes.

ACKNOWLEDGMENTS

We thank S. Kim for generating and analyzing emb-14 recombinants and comments regarding the manuscript. We also thank A. Walters and K. O’Connell for their advice and comments on the manuscript. This research was supported by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Diabetes and Digestive and Kidney Diseases. Some nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Some deletion allele strains were provided by the National Bioresource Project in Japan lead by S. Mitani.

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Figure 6 emb-29(g52ts) embryonic lethality is caused by a point mutation in cdc-25.2. Percent embryonic viability for control lines N2 (wild type) and emb-29 (g52ts) and of converted lines emb-29(g52ts) to emb-29(g52av38[T716C])(rescued) and N2 to cdc-25.2(av40[C716T])(recreated). *P < 0.0001. Error bars indicate standard deviation. N = total number of embryos and hatched larvae scored. At least 860 embryos were scored for each genotype at each temperature.
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Communicating editor: D. S. Fay