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—Review—
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Nematode, an Experimental Animal in the National BioResource Project

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Abstract: The nematode *C. elegans* is a small and simple animal, which is easy to culture and store. Many detailed descriptions, biological resources, and methods for genetical and biochemical analyses have accumulated due to past research activities, and *C. elegans* is readily useful for molecular analyses with transgenic, RNAi, and mutants. Thus, *C. elegans* is an ideal model organism for detailed molecular analyses and functional genomics.
Key words: *C. elegans*, deletion mutants, functional genomics

Nematode *Caenorhabditis elegans* as an Ideal Model Organism

The nematode *Caenorhabditis elegans* (*C. elegans*) was introduced as a model organism by Dr. Sydney Brenner at the Laboratory of Molecular Biology, Medical Research Council at Cambridge about 40 years ago [2]. It is a close descendent of the organism evolved from unicellular organisms and is the simplest multicellular model animal. *C. elegans* is a small animal whose size is about 1 mm long at the adult stage and it proliferates hermaphroditically. It can be easily cultivated on agar plates fed with *E. coli* as a food. It has about 1,000 somatic cells whose morphology and cell lineage have been completely described [13, 16]. It grows to adulthood in 2 days at 25°C and its brood size is 200–300, allowing fast proliferation. It can be stored for years in deep freezers or liquid nitrogen.

The genome of *C. elegans* was physically mapped and sequenced for the first time as an animal in 1998 [14]. Also, ESTs (expressed sequence tags) have been extensively collected and analyzed by the Kohara's group and gene structures have been deduced and depicted in databases, such as WormBase (http://wormbase.org). The genome analysis has revealed that *C. elegans* has about 20,000 genes and about 60% of them are homologous to genes found in other organisms including humans. Thus, in spite of its simple structure, *C. elegans* has many genes which are similar to those of other higher eukaryotes, suggesting many similar functions in cellular and molecular mechanisms. Because of its ease of handling, *C. elegans* has been used for systematic and comprehensive analyses, such as the description of phenotypes by RNA interference [8, 9]. Only small model organisms such as *C. elegans* can be used for such experiments. By exploiting these useful characteristics, researchers have been analyzing the gene-phenotype relationships of many biological processes as outlined elsewhere [12].

It should be stressed that these systematic analyses

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resulted in very precise descriptions of biological phenomena and eventually high quality research achievements. It is well known that *C. elegans* researchers have been awarded with Nobel prize on three occasions (6 persons) in the 21st century, for the discoveries of the apoptosis pathway [4], RNA interferences [5] and the first application of the green fluorescent protein in life sciences [3].

Deletion Mutants of *C. elegans* Are Useful to Analyze Gene Functions

While sequencing of the *C. elegans* genome, the *C. elegans* genome sequencing consortium made the sequences available to researchers. Many genes, which have homologous structures found in other organisms including humans, were identified in the course of the project. So, *C. elegans* researchers began to try to develop reverse genetics methods at an early phase of genome sequencing. However, it was difficult to isolate a large number of mutants by reverse genetics, because the methods were not very efficient. Contributing to these efforts, we developed an efficient method for isolating deletion mutants of *C. elegans* genes [6]. Our method is based on the chromosomal cross-link created by ultraviolet-activated trimethylpsoralen (Fig. 1A). We also developed an efficient screening system from frozen mutant library construction to actual isolation of deletion mutants. Deletion mutant alleles have DNA fragments shorter than those of wild-type animals. We developed a very sensitive PCR protocol, the so-called "stringent PCR", which can preferentially amplify shorter targets over abundant longer (wild-type) targets even from very small amounts, such as 1 in 10,000 compared to wild-type DNA (Fig. 1B). After selective amplification of deletion DNA fragments and actual isolation of mutants as clones (*C. elegans* reproduces hermaphroditically), researchers can easily handle the mutants, because the genotype can be identified by PCR and agarose gel electrophoresis of samples from a single animal.

Genetical and biochemical analyses of deletion mutants of the nematode would elucidate the molecular mechanisms underlying various phenomena seen in multicellular animals. It is very attractive for researchers to work with transgenic analyses with *C. elegans* because it is transparent and suitable for expression analyses with fluorescent proteins [3]. Many phenotypes can be quickly described in live nematodes with fluorescent protein reporters. Because there are detailed descriptions of its morphology and visible phenotypes, and it is easy to perform transgenic analyses with *C. elegans* [10, 16], it is one of the most suitable model animals for functional genomics with any gene or mutant. Describing the phenotypes of mutants would contribute to the understanding of how genes work in the nematode. For example, some mutant phenotypes are shown in Fig. 2.

Researchers can conduct transgenic rescue experiments, once mutant phenotypes have been described. Wild-type and mutant DNAs can be used to examine whether they are functional *in vivo* in the nematode and capable of rescuing the mutant phenotypes. Transgenes with a domain swapped with other genes including human cDNA may reveal the structure-function relationship of the molecules of interest. Extrachromosomal transgenes which rescue the mutant phenotypes would be useful for mosaic analyses to show where the products work.

Mutants with described phenotypes can be used for general genetics; researchers may screen for more mutants that enhance or suppress the phenotypes. Also, the feeding RNAi (RNA interference) method, which only needs the double stranded RNA expressing *E. coli* as a food, greatly enhances this screening [15]. By using ready-made feeding RNAi clones [8], researchers don't even need positional cloning of genes of particular enhancers and silencers. This way, researchers can quickly get insights into the possible other members which would work on the related genetic pathways.

When, researchers wish to analyze mutants that show subtle phenotypes, deletion mutants would help statistical analyses to discriminate between wild-type and mutant animals, where the RNAi method is not helpful.

Representative Strains Provided by the Core Facility of NBRP—*C. elegans*

The core facility of National BioResource Project for the nematode has more than 3,200 strains posted on its website. We distribute more than 1,000 mutants per year to many laboratories world-wide. Because the gene

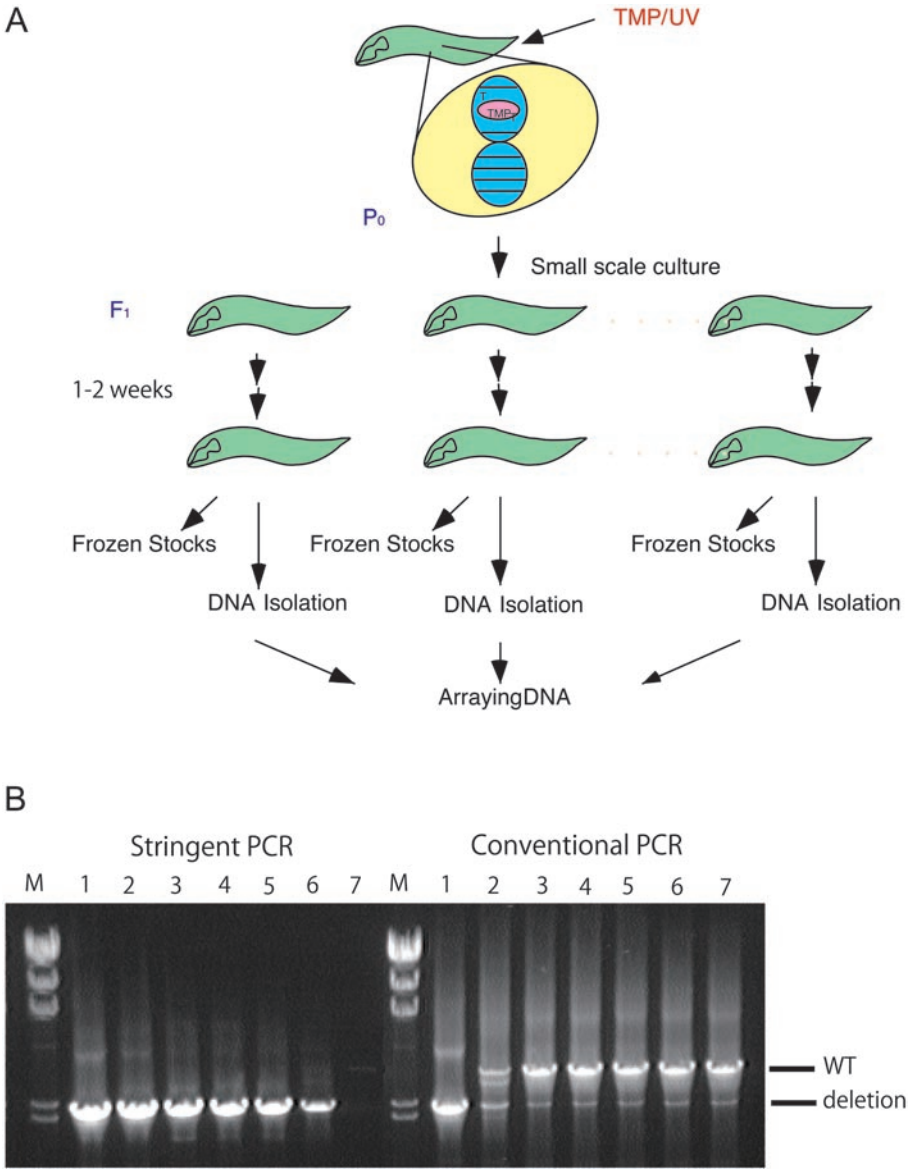

Fig. 1. Strategy for mutant isolation. (A) A schematic drawing of frozen mutant bank construction. P_0 animals are treated by UV-activated trimethylpsoraren and cultured in 3.5 ml dishes. After 1–2 weeks, some of the full-grown animals are frozen and the rests are used for DNA extraction in 96-well format PCR tubes. (B) Representative figures of high sensitive mutant detection by agarose gel electrophoresis. The left panel shows PCR products generated with the "stringent PCR", whereas the right panel shows PCR products generated with conventional PCR. Lanes for size markers are shown by "M". Lanes labeled by numbers 1–7 show amplified products from the template DNAs, which have different ratios of deletion mutant wild-type to DNAs of 1:0, 1:100, 1:1000, 1:2000, 1:5000, 1:10000, and 0:1, respectively.

families whose mutations have been isolated are broad, genes of interest are not restricted to any particular field of biological phenomena. Among subjects enthusiastically studied using *C. elegans* are mechanisms of longevity. An example of popular mutants requested is *ins-7* (*tm1907*), an insulin homologue, which is a key molecule for the *daf-2-daf-16* (an insulin receptor and FoxO transcription factor homologue) signaling pathway and lon-

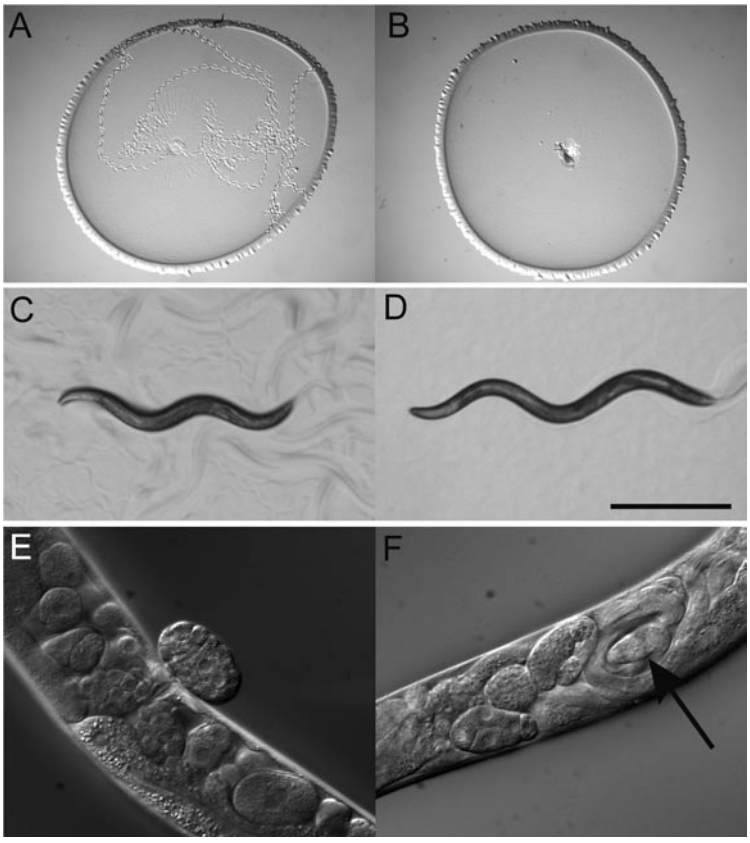

Fig. 2. Examples of mutant phenotypes. Unc (uncoordinated movement) phenotype: tracking pictures of free moving for one hour are shown in A and B. Body length phenotype is shown in C and D (scale bar =0.5 mm). Egl (egg-laying defective) phenotype: appearances near vulva are shown in E and F. The *unc-18* (*tm3212*) mutant animal cannot move (B) while many traces of movement are observed for the wild-type animal (A). The *lon-2* (*tm3050*) mutant animal (D) is much longer than the wild-type animal (C). The *egl-26* (*tm1244*) mutant animal has grown embryos and hatched larvae in the uterus (F), while the wild-type animal lays eggs of early embryonic stages (E).

gevity [11]. Because *C. elegans* has a much shorter life span (normally 2 weeks) than higher vertebrates like mammals, it is very easy to examine the effects of mutations on longevity. The method is straightforward and simple: visible inspection of dead or live organisms among the population time [7].

The *pdr-1* (*tm598*) mutant is also popular among researchers. This gene is homologous to human parkin, an E3 ubiquitin ligase and a causal gene of hereditary Parkinson's disease. Its popularity suggests that *C. elegans* is actively used as a model organism not only for basic biology but also for medical sciences.

How to Obtain *C. elegans* Strains from the Core Facility of NBRP

Deletion mutant strains can be requested by sending an MTA (material transfer agreement) to the core facility, if the necessary strains have been isolated and posted on the mutant database. Researchers can download the template form from the project homepage and fill in the name, address, and the affiliation etc. of the principal investigator. The MTA can be sent to the core facility by courier or postal services, or as a scanned PDF attachement to e-mail.

Table 1. An enhanced freezing solution for *C. elegans*

NaCl	100 mM
potassium phosphate (pH 6.0)	50 mM
trehalose	1 M

The solution is autoclaved and is added to a concentration of $MgSO_4$ 0.3 mM after cooling, then the solution is stored at room temperature. The solution is used by adding to the same volume of worm suspension (such as M9 solution).

If the strain of interest can not be found in the database, researchers can make "screening requests" through the same website. After receiving a screening request, the core facility prepares screening primers and isolates new mutants from its frozen mutant library and posts new strains on the website periodically.

How to Culture and Store the Mutant Strains

When researchers receive mutant strains from the core facility, they need to culture and store the mutants for their analyses. Sometimes, mutants appear completely normal in appearance and homozygous viablity. In such cases, researchers can culture by simple passage to fresh NGM (nematode growth medium) dishes with a lawn of *E. coli* as a food. After the animals grow confluent and young larvae are enriched, they can be frozen for permanent storage [2]. We modified the freezing solution by replacing glycerol included in the original protocol with trehalose (Table 1). Using trehalose as a preservative, we routinely find better recovery after thawing even sick mutants, and this freezing solution sometimes allows even adult worms to survive.

When mutant animals are not homozygous-viable, we need to culture them as heterozygotes. There are a number of balancers for *C. elegans* genetics. Some balancers with fluorescent markers are available and they are useful for maintaining heterozygous animals as they make them easy to identify under a dissecting microscope equipped with epifluorescence.

Examples of Published Papers

Papers, which have used mutants from the NBRP core facility, are being constantly being published, perhaps more than 100 articles per year. So, it is difficult to select a representative one, but the paper introduced below is among the papers, which have greatly contributed to life sciences. Boulton *et al*. discovered the essential factor involved in the homologous recombination process in eukaryotic cells [1]. It was known that Srs2 helicase in yeast is important for the elimination of inappropriate recombination, but orthologues in the higher eukaryotes were elusive. By analogy to yeast Srs2 function, Boulton *et al*. examined *C. elegans* helicases, which show a synthetic lethal phenotype to *him-6* mutant (Bloom's Syndrome gene homologue). They found that the *rtel-1* mutant (*tm1866*) is an Srs2 homologue; a schematic drawing of the strategy is shown in Fig. 3. The

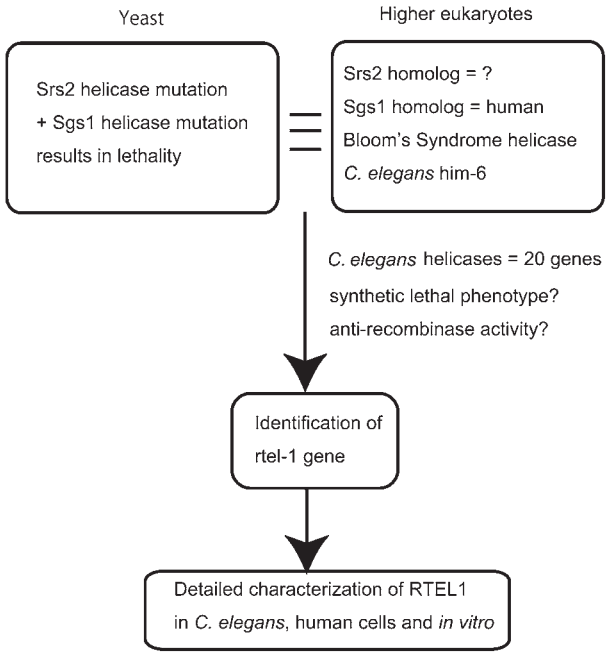

Fig. 3. Strategy of identification of functional homolog by using *C. elegans*. In yeast, Srs2 and Sgs1 double mutation shows synthetic lethal phenotype (upper left). Amino acid sequence alone could not identify the homolog of Srs2 helicase in higher eukaryotes. On the other hand, yeast Sgs1 is homologous to human Bloom's Syndrome and *C. elegans him-6* helicases (upper right). Boulton *et al*. examined 20 *C. elegans* helicases that were potential functional homologs of Srs2. They identified the *rtel-1* mutant (*tm1866*) as the synthetic lethal phenotype with *him-6*. They further examined the role of RTEL1 by using *C. elegans* phenotype, human cells, and *in vitro* DNA biochemistry to demonstrate that RTEL1 is indeed the anti-recombination protein (lower part of the chart).

rtel-1 (*tm1866*) mutant meets other criteria as an Srs2 homologue. Boulton *et al*. also showed that the human homologue, RTEL1, has the similar defect when down-regulated by siRNA. In addition to the discovery of the human Srs2 homologue, they also revealed the mechanisms how RTEL1 plays its role in preventing inappropriate recombination; it inhibits D loop formation in an ATP-dependent manner. Thus, *C. elegans* research may be used to address essential issues, which are common to many organisms and are important to the understanding of the molecular mechanisms of many biological phenomena.

Relevant URL

Our website is http://www.shigen.nig.ac.jp/c.elegans/index.jsp.

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Refereces

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