Minireview

High-throughput reverse genetics: RNAi screens in *Caenorhabditis elegans*

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Abstract

Two recent chromosome-wide screens for phenotypes caused by RNA-mediated interference (RNAi) in *Caenorhabditis elegans* have increased our understanding of essential genes in nematodes. These papers represent a major advance in functional genomics.

With the elucidation of complete genomes comes the need to learn the functions of many new genes. About a third of all yeast genes were already under study when yeast was sequenced, but for metazoan genomes the fraction of characterized genes is much smaller. Only about 1,000 of the 19,000 *Caenorhabditis elegans* genes were characterized before the genome was sequenced, and perhaps twice as many *Drosophila* genes. In these animals, in *Arabidopsis*, and in mammals, knowledge lags far behind the information that the genome provides. An essential goal for the near future is whole-genome functional analysis, and an exciting step toward that goal was achieved in two recent papers in *Nature* by the Ahringer and Hyman groups [1,2]. Each group analyzed the function of an entire *C. elegans* chromosome (covering a total of a third of the genome), providing tremendous new understanding of genes that are required for worm development and viability.

Central to both papers was the use of double-stranded (ds) RNA-mediated interference, or RNAi. Exposing *C. elegans* to dsRNA causes a reduction in the level of mRNA for the corresponding endogenous gene, a phenomenon related to post-transcriptional gene silencing in plants and quelling in *Neurospora* [3]. In *C. elegans,* injection of dsRNA into the syncytial gonad of an adult hermaphrodite leads to a high frequency of the appropriate mutant phenotypes in her progeny. This injection method was scaled up to genome size - 2,174 genes on chromosome III, injected in pools of two genes - by Gonczy and colleagues [2]. In addition, *C. elegans*

is so susceptible to dsRNA that merely soaking animals in the dsRNA or feeding them bacteria that produce dsRNA can lead to a mutant phenotype [4,5]. The feeding strategy was used by Fraser and colleagues, who made 'RNAi bacterial strains', each expressing a specific RNA, for 2,416 predicted genes on chromosome I [6]. The beauty of the RNAi screens is that a mutant phenotype can immediately be related to the sequence of the gene that induced it. Unlike a genetic screen, where positional cloning of the mutated gene can take months or years, the molecular nature of genes identified in an RNAi-based reverse genetics screen is known at the onset. Moreover, the RNAi injection method leads to the synchronized depletion of both maternal and zygotic mRNAs around the time of fertilization. This has advantages for cell biological studies, because all gene functions are interrogated at the same developmental point, whereas early developmental defects can be missed with conventional mutations because of persistent maternal mRNAs.
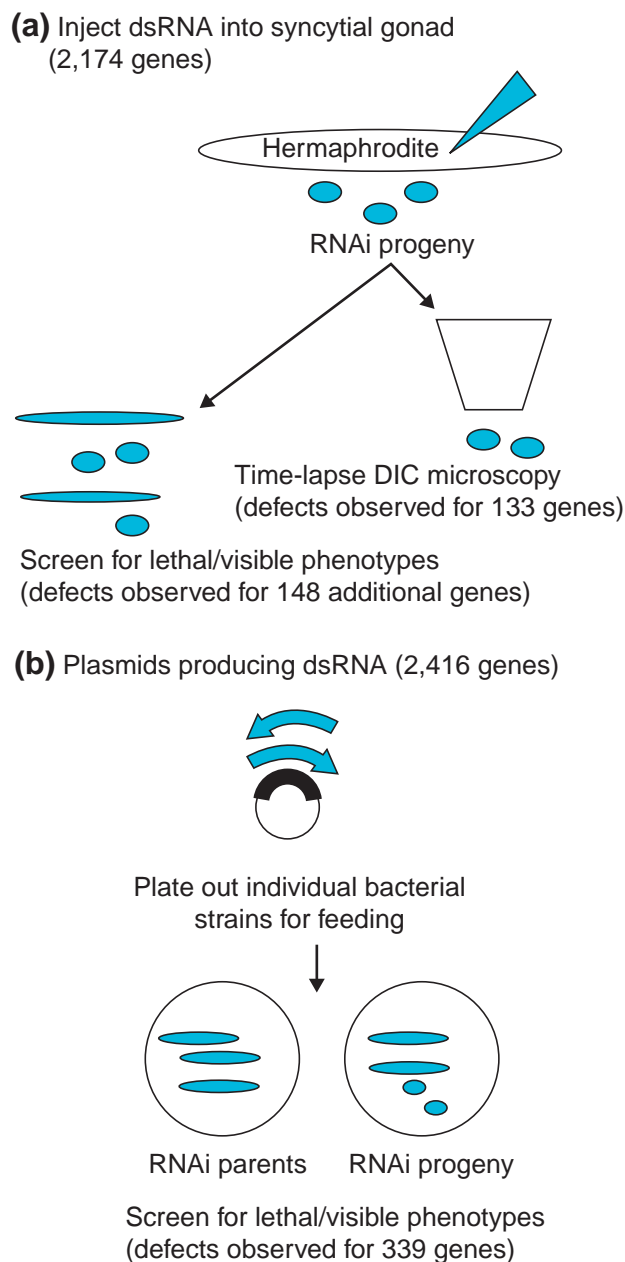
To screen for mutant phenotypes, Gonczy *et al.* [2] focused on the first events of embryogenesis by conducting time-lapse microscopy of the RNAi-treated embryos from fertilization to the four-cell stage. Early *C. elegans* development is highly stereotyped and a trained eye can follow individual cells and subcellular structures using differential interference contrast (DIC) microscopy. The DIC time-lapse images were scanned for defects in cellular processes including meiosis, pronuclear appearance and migrations, spindle assembly, mitosis, and cytokinesis, yielding 133 genes from

this high-resolution screen of chromosome III (Figure 1). A secondary dissecting-microscope screen for lethality or visible phenotypes later in development yielded 139 additional genes on chromosome III. In their chromosome I screen, Fraser *et al.* [1] used a dissecting-microscope screen to look for lethality, visible phenotypes, and sterility.

The increase in understanding provided by these studies is enormous. On chromosome I, 339 genes with a phenotype were found, whereas only 70 genes with both mutations and clones were known before the screen. On chromosome III, 281 genes with a phenotype were found (86 previously known). In total, 12.9-13.9% of tested genes yielded a phenotype in the two screens, suggesting that the methods were about equally efficient in capturing gene function.

As one might expect, genes that are conserved among species prove to be essential genes in *C. elegans*, but the magnitude of the bias was impressive. A *C. elegans* gene with a highly conserved homolog in *Drosophila*, mammals, or yeast was at least five times more likely to yield an RNAi phenotype than a nematode-specific gene. Genes with an RNAi phenotype also had a higher propensity to be represented in expressed sequence tag (EST) libraries, suggesting that high expression either is correlated with essential function or itself makes genes vulnerable to RNAi. Genes required in the post-embryonic stages or adult were far more likely to be novel genes without known functions than genes required in the embryo, which were again more likely to be novel than genes required for the germ line [1]. A recent genome-wide expression study [7] supports this observation; larval stages express a higher fraction of nematode-specific genes than do embryos. Thus, the conserved 'core' genome is essential to the earliest stages of development, with an increasing component of unknown (and nematode-specific?) functions as the animal reaches maturity.

In the high-resolution DIC screen of the first few cell divisions, a large number of different phenotypes could be matched with genes of related functions [2]. RNAi of 27 genes required for protein synthesis disrupted the fidelity of meiotic divisions; this reveals that, by this developmental stage, maternal RNAs have been destroyed by RNAi and protein synthesis is required for any further development. Progression of meiosis required mRNA for genes encoding the proteasome and anaphase-promoting complex; spindle formation required gamma tubulin and a novel gene; pronuclear migration required beta tubulin, dynein, dynactin, the lissencephaly-associated protein LIS-1 and a novel gene; cytokinesis required Rho-GTPase-activating protein (GAP), actin, myosin light chain, and a formin homology protein. The phenotypes for RNAi of DNA replication factors suggested the existence of two novel cell-cycle checkpoints, one in nuclear reassembly and one in cell division. Most of the genes required for the spindle, cytokinesis, and so forth were expected from other species but had not previously been

(a) Inject dsRNA into syncytial gonad
(2,174 genes)

Hermaphrodite

RNAi progeny

Time-lapse DIC microscopy
(defects observed for 133 genes)

Screen for lethal/visible phenotypes
(defects observed for 148 additional genes)

(b) Plasmids producing dsRNA (2,416 genes)

Plate out individual bacterial
strains for feeding

RNAi parents   RNAi progeny

Screen for lethal/visible phenotypes
(defects observed for 339 genes)

Figure 1
RNAi screening strategies. **(a)** Gonczy *et al.* [2] injected dsRNA into the gonads of hermaphrodites (each injection targeted two genes) and scored their progeny for mutant phenotypes in two screens: a time-lapse DIC microscopy screen of meiosis and the first two embryonic cell divisions; and a dissecting-microscope screen for lethal or visible phenotypes. A total of 281 genes with phenotypes were identified; 130 of the 133 genes identified in the DIC screen were also lethal. A similar strategy, using a lethal screen followed by a DIC time-lapse screen, was followed by Piano *et al.* [12], who identified 81 genes with phenotypes in a screen of 350 cDNAs expressed in the ovary. **(b)** Fraser *et al.* [1] generated bacterial strains that produced dsRNA, fed these bacteria to worms, and scored progeny in a dissecting-microscope screen for lethal or visible phenotypes.

studied in *C. elegans*. The most intriguing candidates are the 14% of genes that were novel but now have phenotypes that suggest their functions. The dissecting microscope phenotypes were not as diagnostic as the DIC phenotypes, but some, such as the 'high incidence of males' phenotype associated with defects in chromosome segregation, suggest a specific function (chromosome mechanics) [1].

What about the genes that didn't have phenotypes in these RNAi screens? RNAi doesn't always work. Only 50% of genes with known phenotypes that should have been detected were detected in the RNAi screens. An additional 12% of genes gave an unexpected phenotype and could be false positives, which are known to occur sometimes. The good news is that the known mutant phenotypes of about 80% of embryonic genes were reliably phenocopied by RNAi in control experiments. There has been a general concern that some tissues are more resistant to RNAi than others, and these papers confirm that: neurons and sperm are resistant to RNAi, to this author's sorrow, and indeed, postembryonic tissues in general have a high failure rate, with well under half of known genes giving the expected phenotype. Taking all these issues into account, the true fraction of genes with visible phenotypes is probably about 25%, not the 12.9-13.9% found. Furthermore, the screens in these papers were biased toward genes required for viability: they were not geared toward subtle neuronal, muscular, or developmental phenotypes. More refined assays are needed to detect these important functions.

These papers represent a breakthrough advance for *C. elegans* researchers and others interested in the results that can emerge from this system. Between the two approaches, there is likely to be a first-pass whole-genome set of RNAi data in the near future. Like many microarray papers, these deceptively short papers are associated with masses of supplementary information on publicly accessible websites [8-10], following the full-disclosure, complete-dataset spirit of genome data. Hyman and colleague's site [8] includes time-lapse images of embryonic phenotypes, allowing the possibility that a reader with a sharp eye may detect phenotypes that they missed. The RNAi-by-feeding approach is particularly useful because a library of bacterial strains for RNAi can be passed between groups for future studies with more specialized phenotypes. One large library could ultimately be used for dozens of different genetic screens, including enhancer-suppressor analysis.

What are the future prospects outside the worm? Whole-genome RNAi screening should be practical for the fly and plant genomes, with comparable yields of data. The vertebrate genome may not be as accessible to RNAi - its track record is still limited - but similar technologies such as morpholino-oligonucleotides are being used in fish and could ultimately be scaled up the same way [11]. Another exciting prospect is the idea of bringing RNAi to non-model organisms. RNAi can be

used to inactivate genes in organisms as diverse as *Hydra, Planaria,* and trypanosomes, and perhaps throughout the fungal, invertebrate and plant worlds. Many of these organisms may be sequenced over the next few years. Even for those that aren't, RNAi can be scaled up to ask genome-wide questions. For example, a recent paper [12] describes RNAi on a set of *C. elegans* cDNAs expressed in ovaries, leading to the identification of 81 essential genes in another time-lapse DIC screen. Similar library-based approaches could be used in any RNAi-sensitive organism. Since an extensive infrastructure isn't needed for RNAi studies, the diversity of biology is open for exploration.

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