Studying gene function in *Caenorhabditis elegans* using RNA-mediated interference

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Advance Access publication date 28 April 2008

Abstract

The RNA interference (RNAi) method for targeted gene silencing is widely used in *Caenorhabditis elegans* for large-scale functional genomic studies, analysis of limited gene sets and detailed analysis of individual gene function. The application of RNAi has identified genes that participate in various aspects of development, physiology and cell biology. In addition, RNAi has been used to identify interacting genes and to study functionally redundant genes. This review discusses the various applications of RNAi in *C. elegans*, focusing particularly on the analysis of developmental processes.

Keywords: *Caenorhabditis elegans*; RNA; functional genomics; gene networks; gene interaction

INTRODUCTION

RNA interference (RNAi) is a process whereby the introduction of double-strand (ds) RNA into cells or tissues triggers degradation of cognate mRNA. As a consequence of mRNA degradation, the corresponding protein is depleted ('knocked down'), leading to defects associated with the loss of protein function. In the literature, the term ‘RNAi’ is used to describe both the laboratory method for silencing gene expression and, often, the cellular mechanism by which silencing occurs. RNAi is effective in many contexts including cell culture and the analysis of organisms not amenable to traditional genetic analysis. In addition, major efforts are underway to adapt RNAi for clinical applications in the treatment of disease [1–3].

Thanks to intense research efforts over the last decade or so, the core mechanism of RNAi is now understood. This mechanism is briefly outlined here; readers are referred to recent comprehensive reviews for more details [1, 4]. Upon introduction into the cell, dsRNA is cleaved by Dicer, a type III endonuclease, into 21–23 nt small-interfering (si) RNAs. SiRNA associates with the RNA-induced silencing complex (RISC) and guides it to target mRNAs that are then cleaved by RISC enzymatic activity. The core component of RISC is an RNA-binding protein of the Argonaute/PIWI/PAZ family; additional proteins are included in certain circumstances. In *Caenorhabditis elegans*, as in plants and fungi, RNA-directed RNA polymerase (RdRP) activity can synthesize additional (secondary) siRNAs using the target mRNA as template, thus amplifying the RNAi response. RNAi is typically more robust in organisms that contain an RdRP than in those that do not.

The above mechanism is part of a network of interrelated cellular pathways that repress gene expression at post-transcriptional and transcriptional levels. Studies in many organisms have implicated small non-coding RNA in the regulation of chromatin structure, genome stability, mRNA stability and mRNA translation [5–9]. The inter-relationships among these mechanisms are not yet clear. Best understood is the role of microRNA (miRNA) in post-transcriptional gene silencing. MiRNAs are 21–23 nt non-coding RNAs that base pair with mRNA and either repress translation or target the mRNAs for degradation [5, 6, 9]. Plant miRNAs generally match their mRNA targets perfectly and trigger mRNA degradation, whereas most animal miRNAs base pair imperfectly and block translation without apparently altering mRNA
stability. MiRNAs are encoded by cellular genes, production of mature miRNA relies on Dicer, and the interaction of an miRNA with its target mRNA involves an Argonaute protein. RNAi-related mechanisms that rely on endogenous siRNAs have been implicated in transposon silencing [10, 11] and defence against infection by RNA viruses in a variety of organisms [1, 6], and in meiotic silencing of unpaired DNA in *Neurospora crassa* [12].

RNAi-related mechanisms participate in transcriptional regulation by promoting formation of repressive chromatin structures. In certain circumstances, DNA methylation and the accumulation of specific histone modifications are directed by processes that utilize components of the RNAi machinery, including RdRP, Dicer, Argonaute proteins and/or putative RNA helicases [7, 8]. At present, the data suggest that small RNAs participate in multiple chromatin regulatory mechanisms [e.g. in *Schizosacharomyces pombe* versus *C. elegans* versus *Drosophila*; 13–15]. Non-coding RNAs are also implicated in the process of DNA elimination (chromosome diminution) in the macronucleus of protists, such as *Tetrahymena*. Although the details of this process are unclear, dsRNA has been shown to trigger the loss of complementary DNA sequences in a mechanism involving small non-coding RNA and an Argonaute protein [16].

**STRATEGIES FOR USING RNAI AS A TOOL TO STUDY GENE FUNCTION**

RNAi was first described in *C. elegans* [17, 18] in the 1990s and quickly became an important laboratory tool for investigating gene function. While RNAi is effective in many eukaryotes, *C. elegans* is particularly amenable to RNAi, as dsRNA can be easily administered and off-target effects are rare. Moreover, the availability of the genome sequence helped to make RNAi the reverse genetic tool of choice, particularly for genome-wide studies of developmental processes. Meanwhile, the extensive genetic tools available in *C. elegans* were used to analyse the RNAi machinery itself. This approach eventually led to the recovery of mutant strains with an enhanced RNAi response (an Eri phenotype) that greatly increased the effectiveness of RNAi as a laboratory tool.

RNAi-based studies have now become an integral part of the effort to map genotype to phenotype (see Figure 1). Although major efforts are underway to recover loss-of-function or null deletion mutations in every *C. elegans* gene (The *C. elegans* Knockout Consortium, http://celeganskoconsortium. omrf.org/; National Bioresource Project, http:// shigen.lab.nig.ac.jp/c.elegans/index.jsp), the process is cumbersome and it seems likely that several more years of work are needed before the goal is met. In contrast, (near) genome-wide RNAi has been a reality for several years, and most genes have been evaluated as to whether they are required for viability or fertility. Researchers are identifying components/regulators of specific biochemical pathways and defined cellular/developmental processes using both RNAi-based genome-wide surveys and the analysis of candidate gene sets. To facilitate such goals, RNAi is often performed with a tester strain that carries a visually tagged reporter transgene or a weak mutation that sensitizes the genetic background. The latter approach is especially useful in studying essential genes whose function is required for multiple aspects of development and, conversely, in cases where RNAi-mediated knockdown in a wild-type background does not produce visible defects.

From the start, a major consideration in using RNAi to study gene function has been how to optimize the degradation of mRNA and, consequently, protein knock-down. Genome-wide surveys using wild-type animals observed visible defects (inviability, sterility, gross alterations in body morphology or movement) with RNAi directed against ~10% of genes tested [19–22]; overall, this number is ~50% less than what is expected based on analysis of genetic mutants. Clearly, RNAi is less effective than mutagenesis at disrupting gene expression. However, RNAi is generally more effective at silencing gene expression required for embryonic development than for post-embryonic development. The detection of expected phenotypes increases ~25% when the tester strain carries a mutation in the *rf-3* gene [23, 24] (see subsequently); detection of post-embryonic phenotypes is particularly enhanced in *rf-3* mutants. Although efforts have been made to optimize the RNAi procedure, there apparently is no overall optimal set of conditions, and the final design of one’s RNAi-based assay depends on technical considerations as well as the biological question one wants to address. Fortunately, the rate of false positives is very low (<1%) because off-target effects are unusual. Technical considerations have been discussed elsewhere [25–28] and will be considered here only in brief.
The primary considerations for RNAi in *C. elegans* are (i) dsRNA delivery method, (ii) growth temperature, (iii) the specific tester strain to use and (iv) the developmental stage at which to treat. (i) *In vitro* transcribed dsRNA can be injected into the animal, animals can be soaked in a dsRNA solution or animals can be fed bacteria that have been engineered to express dsRNA. In these cases, the dsRNA triggers a systemic RNAi response in most tissues throughout the body. Some cells, notably neurons, do not respond well to systemic RNAi. To circumvent this problem, transgenic strains can be generated that express hairpin (ds) RNA *in vivo* in the desired cells and tissues. Delivery by feeding is generally the method of choice today (see subsequently). (ii) RNAi against many genes is more effective at higher temperatures (e.g. 25°C) than at lower. However, 25°C may not be optimal for the desired tester strain. (iii) Sensitized genetic backgrounds can facilitate the identification of genes of interest. Mutations in genes such as *rrf-3* (RNA-direct RNA polymerase family) and *eri-1* (enhanced RNAi) produce an enhanced RNAi response in many tissues [23, 29]; mutations in components of the retinoblastoma pathway preferentially enhance RNAi in nervous tissue [30]. A drawback to using Eri strains is that many are unhealthy, particularly at higher temperatures, and thus may not be suitable for some screens. (iv) Selective application of dsRNA during a specific developmental stage can allow one to examine the function of a gene that is active at multiple developmental stages.

The systemic response to dsRNA delivered by feeding or injection indicates that the *C. elegans* intestine can export dsRNA (or siRNAs) and that most *C. elegans* tissues can import dsRNA. Genetic analysis has identified mutants that are specifically defective in RNAi in response to feeding (i.e. they respond to dsRNA delivered by injection or soaking) [31]. These systemic RNA mutants presumably are defective in the export, transport or import of dsRNA, and their analysis should provide insight into the mechanism(s) and biological function(s) of cell-to-cell RNA transport.

**ANALYSIS OF SPECIFIC DEVELOPMENTAL PROCESSES AND BIOCHEMICAL PATHWAYS USING LARGE-SCALE RNAI-BASED SCREENS**

RNAi-based screening has at least two advantages compared with genetic screens: it provides a method
for comprehensively assaying the genome; and one immediately knows the molecular identity of each positive gene. Most researchers who take the RNAi-based approach have chosen to use a standardized protocol where animals of the desired genotype are provided with bacterial food sources that contain dsRNA corresponding to the series of genes to be tested [21, 32]. Large-scale surveys have also been done by injecting in vitro transcribed dsRNAs [20, 22] or soaking animals in dsRNA solution [33]. However, as it is technically simpler to deliver dsRNA by feeding, efforts were made to generate libraries of bacterial ‘feeding’ plasmids that represent each predicted gene [21] or a substantial portion of expressed genes [34]. Many researchers have chosen to use the commercially available library generated by the Ahringer laboratory [21, 32].

Essentially, any assay that is amenable to genetic screening can be adapted for use in an RNAi-based screen. Details of the protocol depend on the tissue, developmental stage and/or process one wants to study. Initial genome-wide studies catalogued the deleterious effects of RNAi on viability, fertility and morphology during embryonic and larval development [19–21, 33, 35, 36]. As RNAi-based surveys became more common, more detailed sets of phenotypic data were recorded, and subsequent detailed surveys continued this trend [22, 24]. These data provide a general resource for analysis of gene function and have been well discussed in previous reviews [28]. This review will focus on current trends, particularly the burgeoning use of RNAi to carry out synthetic interaction (suppressor/enhancer) screens and to examine gene interaction networks.

**General surveys**

Many RNAi-based surveys have been conducted to identify proteins whose knockdown causes specific developmental or cellular defects. Such surveys have identified proteins that regulate longevity/ageing [37–42], fat metabolism [43], transposon silencing [44], the DNA damage response [45], pronuclear migration during fertilization [46], germ cell apoptosis [47], co-suppression [48], the nonsense-mediated mRNA decay pathway [49], RNAi [50] and those that protect against mutation [51]. Two other recent studies that address the regulation of cell migration and axon guidance are discussed here.

Cram et al. [52] identified proteins that function in cell migration. The authors devised a low-magnification visual screen for cell migration defects based on displacement of the intestine, which often reflects abnormal migration of the distal tip cell, a somatic gonadal cell responsible for gonad morphology; candidate migration defective animals were then examined at high magnification using differential interference contrast (DIC) optics to identify those with distal tip cell migration defects. It was suspected that cell migration would involve genes essential for embryogenesis, thus RNAi was performed by placing newly hatched (L1) larvae onto feeding plates and screening for the migration defect in late (L4) stage larvae or adults. In this way, maternal effect lethality was avoided by treating animals at a developmental stage when the maternal product was no longer needed. Approximately 0.6% of the genes tested (from a library representing ~80% of predicted open reading frames) were consistently positive in the visual screen. The 99 gene products fell into several different functional classes, and many of them had not been previously implicated in the regulation of cell migration (e.g. cell cycle regulators and nucleic acid-binding proteins). To identify genes that might participate in common regulatory mechanisms, the authors combined their set of migration–related genes and a pre-existing functional interaction network constructed by Zhong and Sternberg [53] to build a gene interaction network. They constructed a network containing 59 of the cell migration genes, within which are two sub-clusters of genes implicated in (i) cell adhesion and migration and (ii) tubulin interactions.

Schmitz et al. [54] sought to identify genes that function in axon outgrowth. Because neurons tend to be resistant to systemic RNAi, the authors first isolated a mutant strain with an enhanced RNAi response in neurons. This strain carried mutations in two genes: *lin-15B* (previously identified as Eri by Wang et al. [29]) and a gene that Schmitz and colleagues named *me-1*, for neuronal RNAi efficient. They visualized axons with UNC–119::GFP expression and screened a set of ~4600 genes (on chromosomes I and III) for effects on axon guidance. Consistent axonal guidance defects were associated with RNAi-mediated knockdown of ~2% of the tested gene products; guidance defects were grouped into several distinct classes. The guidance-associated genes fell into a wide range of known/putative functional classes, and many had not previously been implicated in the regulation of neural development.
Synthetic interaction screens and gene interaction networks

RNAi is potentially a powerful tool in the search for gene interactions, and increasing numbers of such surveys have been reported in the last few years. One approach is to perform RNAi in a sensitized mutant background as a means to identify genes whose knockdown will either enhance or suppress a specific phenotype of interest. A second approach is to search more broadly for synthetic interactions as a means to map gene interactions on a large (ultimately, genome-wide) scale.

As an example of the former approach, Labbé et al. [55] sought to identify factors that function in establishing embryonic polarity. PAR (partitioning) proteins were known to participate in the establishment of anterior–posterior polarity during early embryogenesis. Labbé et al. screened for suppression of the embryonic lethality associated with loss of par-2 function. They identified eight strong suppressors of par-2 lethality and placed them into two classes: regulators acting independently of PAR-2 (e.g. the Nanos family Zinc-finger protein, NOS-3) and regulators acting via PAR-2 (e.g. the proteasome regulatory subunit, RPNI-12). The identity of certain suppressors led the authors to revisit related genes (which had not been strong suppressors in the RNAi-based screen) and identify additional regulators of PAR-2 activity. For example, two known nos-3 co-regulators, FBF-1 and FBF-2, act redundantly to regulate PAR-2 activity.

Parry et al. [56] devised an interaction screen to identify components of the microRNA pathway. The authors first demonstrated that the phenotype of a weak mutation in the miRNA gene, let-7, could be enhanced by knockdown of Dicer activity. Next, they conducted a systematic screen for enhancement of a let-7 developmental defect (vulval bursting) and identified 213 candidate genes. Follow-up biochemical and genetic studies ultimately identified a subset of 44 new genes whose products function in the let-7 pathway. Among these were 19 general miRNA pathway genes, most of which act downstream of miRNA biogenesis. Analysis of these 19 genes should provide insight into poorly understood aspects of miRNA regulation and function, such as how the miRNA–mRNA duplex is sensed and ultimately how it represses translation.

In addition to identifying components of distinct biochemical pathways or networks, synthetic interaction screens are also providing information about genetic interactions on a genome-wide scale. Two recent studies have taken a similar approach to identifying synthetic interactions between known developmental pathway components and a battery of tester genes [57, 58]. Interaction data were used to build gene interaction maps that revealed overarching patterns. It is not currently feasible to assay all possible gene pairs; therefore, both studies by necessity evaluated only a relatively small subset of the possible interactions.

Lehner et al. [57] chose weak mutations in 31 ‘query’ genes encoding components of EGF, Notch and Wnt signalling pathways, several other cell surface receptors and signalling components, and several chromatin regulators. Mutants were cultured on each of 1744 feeding ‘library’ bacterial RNAi strains, and gene-x(mutant); gene-y(RNAi) animals were visually scored for a range of phenotypes. Three hundred and fifty pair-wise interactions between query mutations and RNAi strains were identified. Interaction mapping revealed that most library genes, called ‘specific modulators’, interacted with a single query gene. An exponentially smaller number of library genes interacted with more than one query gene, and a very few so-called ‘hub’ genes interacted with many query genes representing multiple signalling pathways. Lehner et al. hypothesized that hub genes act to buffer development against (minor) fluctuations in protein levels. Interestingly, the six hub genes are all predicted to encode chromatin proteins.

Byrne et al. [58] chose a set of 11 ‘query’ mutants representing six cell-signalling pathways and 858 target genes; population growth was assayed as a measure of genetic interaction. Genes were scored as interacting if the population of gene-x(mutant); gene-y(RNAi) animals grew more slowly than gene-x(mutant) or gene-y(RNAi). This assay apparently was more sensitive than that of Lehner et al.: among 1165 gene pairs tested in both studies, 78.5% were negative in both, 1.5% were positive in both, 0.85% were positive only for Lehner et al., and 19.1% were positive only for Byrne et al. However, the overall pattern of interactions was similar (many specific modulators, few hub genes) and the two gene interaction maps appear equally valid when compared with other C. elegans interaction networks that are based on protein interactions, gene expression patterns, phenotypes and functional characteristics ([53, 59]; see WormBase, http://www.wormbase.org). This approach provides information on multiple...
levels ranging from the organization of eukaryotic genomes to the relationships among subsets of genes that act in concert during development.

Numerous other large-scale gene interaction screens have been performed. These include screens designed to identify: modifiers of retinoblastoma pathway activity [33], genes redundant with PTEN/DAF-18 [60], modifiers of Unc-induced tau phenotype [61], regulators of lifespan [62], genes redundant with the glycopeptide hormone-like receptor, FSHR-1, in germ line development [63], regulators of meiotic maturation [64], genes that are synthetically lethal in combination with loss of Ras-like GTPase RAP-1 activity [65], genes that participate redundantly with SynMuv A or B genes [66] or antagonize SynMuv activity [67] to regulate vulval development, synthetic lethality among candidate targets of PAL-1 homeodomain protein activity [68], negative regulators of the mitochondrial unfolded protein response [69] and modifiers of the developmental phenotype associated with reduced activity of MUS-101 (mutagen sensitive), a protein implicated in DNA stability [70]. Additional examples of the gene interaction approach are included subsequently.

USING RNAI TO STUDY TARGETED SETS OF GENES

Many researchers use RNAi to study subsets of genes that have been identified via various criteria. It was recognized early on that RNAi could target individual members of repetitive gene families whose members might be (at least partially) redundant for function. RNAi can be extremely helpful for assigning biological function to genes that have been identified by various biochemical or molecular criteria, such as candidate targets of transcription factor activity identified by microarray analysis. Illustrative examples of such studies are outlined subsequently. For the purposes of this review, studies are categorized as analysing redundant gene families, candidate gene sets or tissue- or developmental-stage-specific gene sets; however, there is clearly some overlap among these categories.

Redundant gene families

Simonet et al. [71] addressed functional redundancy among a set of 27 SET domain proteins to identify those whose loss of function would enhance or suppress developmental defects associated with hpl-1 and hpl-2 mutations. The SET domain (initially described in Drosophila suppressor of variegation, enhancer of zeste and trithorax proteins) is characteristic of histone methyltransferases and is required for their enzymatic activity. HPL-1 and HPL-2 are the two C. elegans members of the heterochromatin protein 1 (HP1) family. HP1 proteins are implicated in heterochromatin assembly and transcriptional regulation (both positive and negative) in diverse metazoan species. Simonet et al. demonstrated that hpl-2 sterility was enhanced by knockdown of MET-2 (methyltransferase), and the hpl-2 growth defect was suppressed by knockdown of four other SET domain proteins. Additional examples include the analysis of 20 T-box family transcription factors [72] and 129 putative RNA/DNA helicases [73]. A particularly good example is provided by Lublin and Evans [74]. This study identified the RNA-binding protein, PUF-5 (Pumilio family) as a regulator of maternal glp-1/Notch mRNA translation. Caenorhabditis elegans has nine Pumilio-related RNA-binding proteins. Using RNAi, the authors evaluated the role of each PUF protein, and demonstrated that PUF-5 and its closest family members, PUF-6 and PUF-7, function redundantly during late oogenesis to regulate expression of some (but not all) maternal mRNAs.

Recently, Tischler et al. [75] developed a combinatorial RNAi assay to address functional redundancy on a genome-wide scale. Pairs of genes were targeted by RNAi, and the resulting defects were compared with those caused by RNAi targeted against each single gene. A major concern in this study was that dilution of feeding strain bacteria would lead to false negatives, hence the authors carefully tested for this effect. They estimated their protocol as successfully detecting ~50% of pair-wise gene interactions. As a proof of concept, the authors examined 143 sets of duplicated genes whose Saccharomyces cerevisiae or D. melanogaster orthologues are not duplicated. Combinatorial RNAi of 16 of these gene sets revealed synergistic defects in brood size and/or embryonic survival. Interestingly, 14/16 of these gene duplications are also present in C. briggsae. Classically, one thinks of duplicated genes as free to diverge in function, yet functional redundancy of these 14 gene pairs has been (at least partially) maintained for >80 million years since C. elegans and C. briggsae diverged.

Candidate gene sets

Sieburth et al. [76] sought to identify genes required for acetylcholine secretion using a candidate gene
approach. They first chose a set of 2072 candidate genes based on predicted function, such as involvement in cell signalling or membrane trafficking, localization to synapses or regulation of cytoskeletal structure/function. Using the neuronal enhanced RNAi strain, *eri-1;lin-15B* [30; see above], they tested whether RNAi-mediated knockdown of any of the 2072 gene products rendered animals insensitive to an acetylcholinesterase inhibitor, aldicarb. A secondary assay tested whether knockdown could suppress the enhanced aldicarb sensitivity of *dgk-1* (diacylglycerol kinase) mutant animals. These assays identified 185 genes required for a normal level of acetylcholine secretion, of which 132 were not previously known to influence synaptic transmission. In subsequent studies, functional analysis of 60 genes identified subsets whose products function in the synaptic vesicle cycle or neuropeptide signalling. Follow-up studies included determining the subcellular localization pattern of 100 of the 132 proteins and further evaluating the role of 19 genes in synapse formation/structure using genetic mutants. This study greatly expanded the number of proteins known to influence synaptic function, and provides a rich resource for future studies.

In another example, Srayko et al. [77] built on data accumulated in previous RNAi studies to select a candidate list of genes that were known to influence microtubule-associated processes and might, therefore, function in microtubule nucleation or growth. They developed a sensitive visual assay based on distribution of an EBP-2::GFP transgenic marker that normally labels the growing (plus) end of microtubules. An RNAi-based survey of the candidate genes identified proteins whose function either increased or decreased microtubule nucleation rate, promoted microtubule growth or limited the retrograde movement of microtubule plus ends. One interesting finding was that nucleation and growth rate are regulated by largely independent sets of proteins, suggesting these are independent processes. Moreover, few proteins function to regulate microtubule polymerization; the authors propose that, instead, growth depends mainly on the availability of tubulin subunits.

A number of studies examining other problems have taken the same general approach as those above. The sizes of the candidate gene sets vary widely. Examples include analysis of: the role of predicted transcription factors in regulation of vulval development [78]; predicted kinases that may mediate the response to oxidative stress [79]; proteins that localize to the mid-body and may function in formation and movement of the cleavage furrow [80]; candidate targets of the DAF-16 transcriptional regulator [81]; candidate regulators of phosphatidylserine exposure during apoptosis [82] and regulators of mRNA translation during oogenesis [74].

**Tissue- and developmental-stage-enriched transcripts**

RNAi can provide information as a complement to biochemical, molecular and proteomic data. For example, early gene expression profiling studies identified genes whose transcripts are enriched in the germ line relative to the soma [83, 84]. Colaiacovo et al. [85] searched these microarray data to identify genes whose transcript pattern mirrored that of known meiotic genes, and then used RNAi to investigate as to which of these germ line-enriched transcripts might, in fact, function in meiosis. Fifty-two of 192 genes assayed (27%) were required for aspects of meiosis and/or for other aspects of germ line development, such as proliferation. Piano et al. [35] took a related approach to identify genes required for oogenesis and/or embryogenesis. Starting with a (random) set of cDNAs made using adult ovarian mRNA, they identified 81 genes as required for embryonic viability; 36 of these genes were also required for oogenesis. In a follow-up study, Piano et al. [36] evaluated a much larger set of (~750) genes whose expression was described as ovary-enriched based on microarray analysis. This study provided information about gene expression trends in the germ line (for example, X-linked genes are rarely expressed in the germ line). Piano et al. [36] defined 47 patterns of defective embryogenesis associated with RNAi-mediated knockdown of different genes and grouped these genes into ‘phenoclusters’ that might reflect related gene functions. Later, Sonnichsen et al. [22] applied a similar approach to classify a far larger set of genes into phenoclusters based on a set of 23 embryonic phenotypes. Investigation of the relationships among gene products in individual phenoclusters should provide insight into developmental processes during embryogenesis.

**RNAI AS A COMPLEMENT TO MUTATIONAL ANALYSIS**

*Caenorhabditis elegans* researchers use RNAi to complement mutational analysis in many contexts. As described above in the context of RNAi-based
screens, focused analysis of a regulatory pathway or developmental process can benefit from combining RNAi with traditional genetic analysis. For example, RNAi can silence gene expression at a specific developmental stage (helpful in the absence of an available temperature-sensitive allele) or be used in combination with genetic mutations to simultaneously silence multiple gene products. In many cases, gene-x(-) gene-y(RNAi) animals may be far easier to generate than gene-x(-) gene-y(-) double mutants [86]. Another common use of RNAi is in gene cloning, particularly in tissues where DNA-mediated transformation rescue is problematic (e.g. the germ line). Once a gene of interest is mapped to a discrete chromosomal interval, candidate genes located within the interval can be assayed by RNAi to identify any of those whose knockdown mimics the mutant phenotype of the gene of interest [87].

Tissue-specific RNAi can also function as a sort of poor man’s genetic mosaic analysis. In particular, rrf-1 (RNA-dependent RNA polymerase family) mutants disrupt RNAi in the soma but not the germ line, allowing one to distinguish between germ line and somatic gene expression [88]. If dsRNA treatment of wild-type animals produces a defect that does not arise when rrf-1 mutants are treated with the same dsRNA, then the standard interpretation is that the defect depends on gene silencing in the soma. This approach has been used extensively to distinguish germ line versus soma as the tissue site of action for many genes that promote germ line development [e.g. 87, 89–94, among others]. Similarly, comparative RNAi in wild-type versus Rb pathway mutants may be useful for distinguishing the tissue site of action for genes that regulate development of specific neurons.

DATA ANALYSIS AND STORAGE

As researchers have generated increasing amounts of data from RNAi-mediated gene-silencing studies, one challenge has been how to best store the data for ease of retrieval and comparison. Data from many of general RNAi surveys have been compiled in the C. elegans online database, WormBase (http://www.wormbase.org). WormBase includes the following information: the phenotypic terms that were scored; the results (positive and negative) and the portion of each open reading frame used to produce dsRNA. Piano and colleagues [35, 36] developed an online repository of DIC photomicrographs and time-lapse imagery of embryonic defects called RNAi Database (http://nematoda.bio.nyu.edu:8001/cgi-bin/index.cgi). A similar database of germ line defects is now being assembled and will be incorporated into RNAi Database (J. Hubbard, personal communication). Likewise, videomicroscopy data from Sonnichsen et al. [22] are available online at Phenobank (http://www.worm.mpicbg.de/phenobank2/cgi-bin/MenuPage.py). These RNAi data complement other large-scale efforts to characterize C. elegans biology, including: mRNA in situ hybridization data (The Nematode Expression Pattern Database, http://nematode.lab.nig.ac.jp/); nematode anatomy and morphology (Wormatlas, http://www.wormatlas.org/); mutant phenotypes (Wormbase and The National Bioresource Project, URL listed above) and gene expression profiling (microarray) data (Wormbase; GermOnline, http://www.germonline.org/index.html). See Piano et al. [95] for an excellent discussion of the various global approaches to the study of C. elegans biology and compilation of C. elegans databases.

SUMMARY

RNAi-mediated gene silencing is a common tool for the analysis of gene function in C. elegans, and often used as a complement to mutational, molecular and biochemical approaches. The RNAi approach has provided information about numerous aspects of development and cell/biochemical processes. Increasingly, gene interaction data obtained via RNAi surveys (alone or in combination with other data) are providing material for the construction of gene interaction networks.

Key Points

- RNAi-mediated gene silencing is a versatile method for studying gene function.
- RNAi-based gene interaction screens are identifying components of known biochemical and cellular pathways.
- RNAi-based screens are identifying proteins that function in defined developmental and cellular processes.
- RNAi-mediated gene silencing is providing information for the assembly of gene interaction networks.

Acknowledgements

The author thanks Jane Hubbard for communicating data prior to publication, and Xingyu She and two anonymous reviewers for comments on the manuscript. The author apologizes to all whose work is not cited here due to space limitations. The study was funded by the National Science Foundation.
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