Decoding the Riddle: The Dawn of RNAi for the Study of Gene–Gene and Gene–Environment Interactions


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The advent of RNA interference (RNAi) technology has truly revolutionized modern biology. The ability to scrutinize biological function by knocking out virtually any gene using small interfering (si)RNAs now allows genetic studies to be completed at a genome-wide level. As such, RNAi in combination with transcriptional profiling and other molecular technologies provides a powerful tool to unravel the complex interactions that mediate environmental pathogenesis.

Transcriptional profiling has been used successfully to study mechanisms of cellular injury in response to toxic chemicals, identify molecular targets for environmental exposure, and elucidate biological pathways underlying the pathogenesis of environmental disease. Consequently, DNA microarrays are now a mainstay in the elucidation of molecular signatures of the response triggered by foods and biological and chemical agents encountered in the environment. Changes in mRNA profiles follow chemical binding and activation/inactivation of transcription factors, as seen with hormone receptors or aryl hydrocarbon receptor (AhR), or modifications of macromolecules and organelles that disrupt cellular homeostasis. Once a pattern of gene expression is identified and associated with a biological phenomenon, the central issue becomes the validation of putative associations.

Such analyses have greatly benefited from the application of mathematical and computational approaches to translate the complex biological interactions into units that can be resolved with greater ease. However, even when the functional interactions of thousands of genes are minimized to hundreds of genes within critical nodes of biological activity, the reciprocal, synergistic, collateral, dependent, or antagonistic interactions that mediate a given biological response cannot be ascertained. Classic gene knockout experiments involving deletion or mutational inactivation of target genes can be used successively to address such questions of certainty.

However, this technology is costly and time consuming and is of limited value if embryonic viability, developmental programming, and/or reproducibility are compromised. Because conventional knockout methodology often involves a single gene, the approach is clearly not suitable for high-throughput analysis of gene functions. Consequently, one major goal in functional genomics has been the development of tools that allow easy manipulation of gene expression levels in cell culture, tissue, and whole animal and that would be suitable for high-throughput analysis. RNAi has emerged as one of the preferred approaches to achieve this goal.

RNAi is an important biological mechanism in the regulation of gene expression in plants, fungi, and animals [for review see Dykxhoorn et al. (2003)]. While the ribosomal and transfer RNAs participate in protein synthesis on ribosomes, a new class of functional RNAs can prevent protein synthesis through interference with translating mRNAs. RNAi, also referred to as posttranscriptional gene silencing (PTGS), selectively ablates the effect of a gene by destroying messenger (m)RNA. RNA regulatory molecules either reduce or eliminate target gene expression by binding mRNA and targeting it for degradation by cellular enzymes. Genetic and biochemical data indicate that siRNAs [19- to 25-nucleotide double-stranded (ds)RNAs] are produced from much larger RNAs by Dicer. Upon binding through base-pairing to target mRNA, siRNAs recruit RNases to a protein complex called the RNA-induced silencing complex (RISC) that degrades the targeted sequence. RNAi is critical in viral defense and transposon silencing (Sijen and Plasterk 2003; Voinnet 2001), heterochromatin formation in *Schizosaccharomyces* (Volpe et al. 2002), RNA-dependent DNA and histone methylation in plants (Matzke et al. 2001; Zilberman et al. 2003), and programmed DNA deletion in *Tetrahymena* (Yao et al. 2003). While it remains unclear whether endogenous RNAi mechanisms operate in mammalian cells as described in other organisms, a recent study shows that loss of Dicer in mice leads to lethality early in development due to depletion of stem cells (Bernstein et al. 2003). This observation suggests that Dicer, a conserved component of the RNAi machinery, is required for vertebrate development. Previous studies in plants and worms have also pointed to a developmental role for the RNAi machinery (Hannon 2002).

The nematode *Caenorhabditis elegans* is the first organism in which dsRNA was used to silence RNA (Fire et al. 1998). Because of the availability of a complete genomic sequence and the ease of genetic manipulation (simply soaking the worm in a solution containing dsRNA or by feeding the worm with bacteria expressing specific dsRNA), large-scale functional analysis of *C. elegans* genes has been performed in the whole animal. Recently, Kamath et al. (2003) presented the results of an investigation of more than 16,000 genes for loss-of-function phenotypes in *C. elegans*. This is the first study of its kind and could provide insights into human gene function, given that more than half the genes in *C. elegans* are homologous to human genes. Most studies to date indicate that RNAi occurs in the cytoplasm by acting on translating polyribosomes. However, recent studies have shown that in the yeast *Schizosaccharomyces pombe*, silencing of centromeric repeats is mediated by dsRNA and Dicer/RISC (Volpe et al. 2002). Another study in trypanosomes directly demonstrates that siRNAs can attack small nucleolar (sno)RNAs, non-mRNAs involved in the biosynthesis of ribosomal RNA in the nucleolus (Liang et al. 2003).

The availability of searchable genetic databases such as BLAST has greatly accelerated progress in the field of functional genomics. Of great potential impact to the field is the availability of the first RNAi database, RNAiDB, for archiving, distribution, and analyses of phenotypic data derived from large-scale RNAi experiments in *C. elegans* (Gunsalus et al. 2004). RNAiDB can be searched using combinatorial queries and the novel tool PhenoBlast, which ranks genes according to their overall phenotypic similarity. Thus, RNAiDB could serve as a model database for distributing and navigating *in vivo* functional information from large-scale systematic phenotypic analyses in different organisms.

RNAi has also emerged as a powerful genetic tool for analyses of gene function in mammalian cells [reviewed by Dykxhoorn et al. (2003)]. Cell culture–based assays of gene function are already conducted in a 96- or 384-well plate format. For example, a recent investigation based on silencing of 510 genes identified new cellular components that modulate the

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apoptotic response to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Aza-Blanc et al. 2003). Moreover, recent studies have used microarray-based cell transfection platforms to achieve large-scale measurable RNAi-induced knockdowns and phenotypes in mammalian cells (Kumar et al. 2003; Mousses et al. 2003). In these experiments, siRNAs are first arrayed on glass slides, overlaid with a monolayer of cells, and incubated to allow transfection to occur. A fluorescent end point is then used as a quantitative measure of RNAi. A significant advantage of this platform when compared to a plate format is the uniformity of experimental design and conditions that allow for all processes to take place on the same surface. Potentially, any assay that can be conducted using cells growing on a glass slide can be applied to the RNAi microarray platform. This should enable analysis of phenotypic end points such as toxicity, differentiation, and cell cycle effects to directly evaluate gene function.

Although RNAi experiments rely on high target specificity, nonspecific effects can and do occur. Recent reports demonstrate that under certain conditions mammalian cells treated with 21-bp siRNAs may also activate components of the interferon system (Jackson et al. 2003; Persengiev et al. 2004; Sledz et al. 2003). This suggests that when introduced into cells, siRNAs have broad and complex effects beyond the selective silencing of target genes. Therefore, investigators must proceed with caution when interpretating data using RNAi technology.

Clearly, the application of RNAi to environmental health research will help address critical questions at a genome-wide level. A recent study demonstrated that downregulation of AhR by siRNA inhibits functional interactions between dioxin and estrogen, and modulates transition of MCF-7 breast cancer cells through the cell cycle (Abdelrahim et al. 2003). These findings not only ratified the critical role of AhR in the regulation of mammalian functions but also lend credence to the usefulness of siRNA methodology in evaluating the mammalian response to environmental injury. Allele-specific silencing of acetylcholine receptor subunit mutants has also being recently reported (Abdelgany et al. 2003), suggesting that this approach may find applications in the study of dominant genetic disorders associated with environmental exposures. Finally, siRNAs are now being used to facilitate target selection at various stages of drug development (Lavery and King 2003). We are currently applying RNAi technology to the study of four-gene interaction networks involved in the regulation of AhR-dependent functions and phenotypic control of mammalian cells. Clearly, the door to RNAi has just opened for environmental health researchers. The best is yet to come.

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