CHAPTER 26
Gene Silencing

INTRODUCTION

The phenomenon of gene silencing mediated by short double-stranded RNA molecules was only discovered a few years ago, and many of the underlying molecular mechanisms involved remain to be elucidated. Nevertheless, a variety of techniques have been developed that exploit the use of short interfering RNAs (siRNAs) to study gene function in a variety of eukaryotic hosts. siRNAs were discovered in the course of experiments that involved the use of antisense RNA molecules to specifically knock down the expression of a particular target gene. Now it appears that siRNA-mediated gene silencing is an important component of the eukaryotic immune response to viral infection that has been highly conserved in evolution. Short double-stranded RNA molecules also appear to play an important role in gene regulation in plants, animals, and fungi. This high level of evolutionary conservation presumably explains the broad applicability of RNA interference technology (referred to as RNAi) to modulate gene expression in both readily manipulable genetic hosts such as Caenorhabditis elegans, Drosophila melanogaster, and Arabidopsis thaliana, as well as in mammalian tissue culture cells.

RNAi technology has a particularly important role in genome-wide studies of gene function. Given the genome sequence of a target eukaryotic organism, RNAi libraries corresponding to every open reading frame in a genome can be generated, thereby allowing genome-wide screens of gene function. This approach is particularly powerful for C. elegans and D. melanogaster. In C. elegans, RNAi can be accomplished by the relatively straightforward approach of constructing an E. coli-based plasmid vector that directs the synthesis of a dsRNA molecule and then simply feeding this plasmid-containing E. coli strain to C. elegans. Thus, genome-wide C. elegans RNAi libraries are being generated which can be systematically fed to C. elegans, one clone at a time. Given a particular phenotype, many C. elegans genes affecting the phenotype can be readily identified. In D. melanogaster, genome-wide RNAi analysis can be carried out by exposing tissue culture cells to short ds RNAs. In contrast, identifying genes that correspond to a particular C. elegans or D. melanogaster mutant generated by traditional forward genetic approaches is often an arduous process, involving time-intensive map-based cloning strategies.

This chapter begins with an overview of the brief history of RNAi techniques and the underlying science concerning the role of siRNAs in pathogen defense and gene regulation (UNIT 26.1). The overview is followed by a set of protocols for carrying out RNAi in different hosts. The chapter currently has protocols for mammalian cells (UNIT 26.2), C. elegans (UNIT 26.3), D. melanogaster (UNIT 26.5) and for cloning small endogenous RNA (UNIT 26.4); in future supplements, we anticipate including RNAi protocols for plants and perhaps other hosts as well. Each of these protocols is highly species-specific, focusing on the most efficient methods for delivering dsRNA molecules to a particular host. As more is understood about the underlying molecular machinery involved in mediating RNAi, we anticipate that much more efficient RNAi protocols will be developed. We will therefore update the protocols to keep them current with new advances in this rapidly evolving field.

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