CHAPTER 6

Transgenesis in *C. elegans*

Vida Praitis[†] **and Morris F. Maduro**[*]

[*]Department of Biology, University of California Riverside, Riverside, California, USA

[†]Biology Department, Grinnell College, Grinnell, Iowa, USA

Abstract

The ability to manipulate the genome of organisms at will is perhaps the single most useful ability for the study of biological systems. Techniques for the generation of transgenics in the nematode *Caenorhabditis elegans* became available in the late 1980s. Since then, improvements to the original approach have been made to address specific limitations with transgene expression, expand on the repertoire of the types of biological information that transgenes can provide, and begin to develop methods to target transgenes to defined chromosomal locations. Many recent, detailed

METHODS IN CELL BIOLOGY, VOL 106

0091-679X/10 $35.00
DOI 10.1016/B978-0-12-544172-8.00006-2

protocols have been published, and hence in this chapter, we will review various approaches to making *C. elegans* transgenics, discuss their applications, and consider their relative advantages and disadvantages. Comments will also be made on anticipated future developments and on the application of these methods to other nematodes.

I. Introduction

Following the generation and characterization of chromosomal mutations (Brenner, 1974), the ability to generate transgenic lines in *C. elegans* (Fire, 1986; Mello *et al.*, 1991; Stinchcomb *et al.*, 1985) opened the system for the rapid genetic characterization of many diverse biological phenomena. The assembly of the genome sequence (*C. elegans* sequencing consortium, 1998) accelerated the rate of gene identification because candidate genes could be identified by first correlating the genetic and physical maps and then transforming easily obtained subclones of the genome into mutants to look for complementation rescue. The advent of green fluorescent protein (GFP) as a reporter seemed destined for this system, as *C. elegans* animals are essentially transparent at all life stages and exhibit little autofluorescence (Chalfie *et al.*, 1994). The discovery of RNA-mediated interference (RNAi) (Fire *et al.*, 1998) expanded further on this set of tools, and the vast majority of work published in the *C. elegans* field uses a combination of all three approaches: genetics, transgenes, and RNAi.

Early approaches for transgenesis in *C. elegans* involved microinjecting DNA into either the hermaphrodite gonad or into unfertilized oocytes for the generation of transgenic animals (Fire, 1986; Mello *et al.*, 1991). In contrast with other systems, *C. elegans* embryos are not used for injection, because it is technically much more challenging and less efficient than gonadal injection, which typically produces many transformed F_1 animals per hermaphrodite. Unlike other systems in which transgenic DNA is generally integrated into chromosomal DNA in single copy (Ringrose, 2009; Ziemienowicz, 2010), *C. elegans* transgenes obtained following microinjection assemble into multicopy extrachromosomal arrays that are transmitted to progeny at 5–95% fidelity (Mello and Fire, 1995). While an extrachromosomal transgene is sufficient or even required for many purposes, arrays can be made to integrate following treatment of a transgene strain with ionizing radiation or chemical mutagenesis (see Evans, 2006).

The ease of producing transgenics in *C. elegans*, and the general reliability of transgene-expression patterns, have permitted rapid characterization of gene expression and often function without the use of *in situ* hybridization or antibodies (Fig. 1). To a first approximation, genes in arrays are expressed similarly to endogenous genes, although the relative expression may be increased due to a higher gene dosage or reduced due to silencing of repetitive sequences (Fire and Waterston, 1989; Kelly *et al.*, 1997; MacMorris *et al.*, 1994; Okkema *et al.*, 1993; Stinchcomb *et al.*, 1985). For many years it was observed that promoters normally active in the germ line fail to

function when present in transgene arrays, whether they are integrated or extrachromosomal. The inclusion of complex DNA in the injection mixtures was found to overcome this problem (Kelly *et al.*, 1997), although such transgene strains require careful maintenance to avoid silencing. Microparticle bombardment, a technique used for many years to make transgenic plant cells (Sanford, 1989), was found to

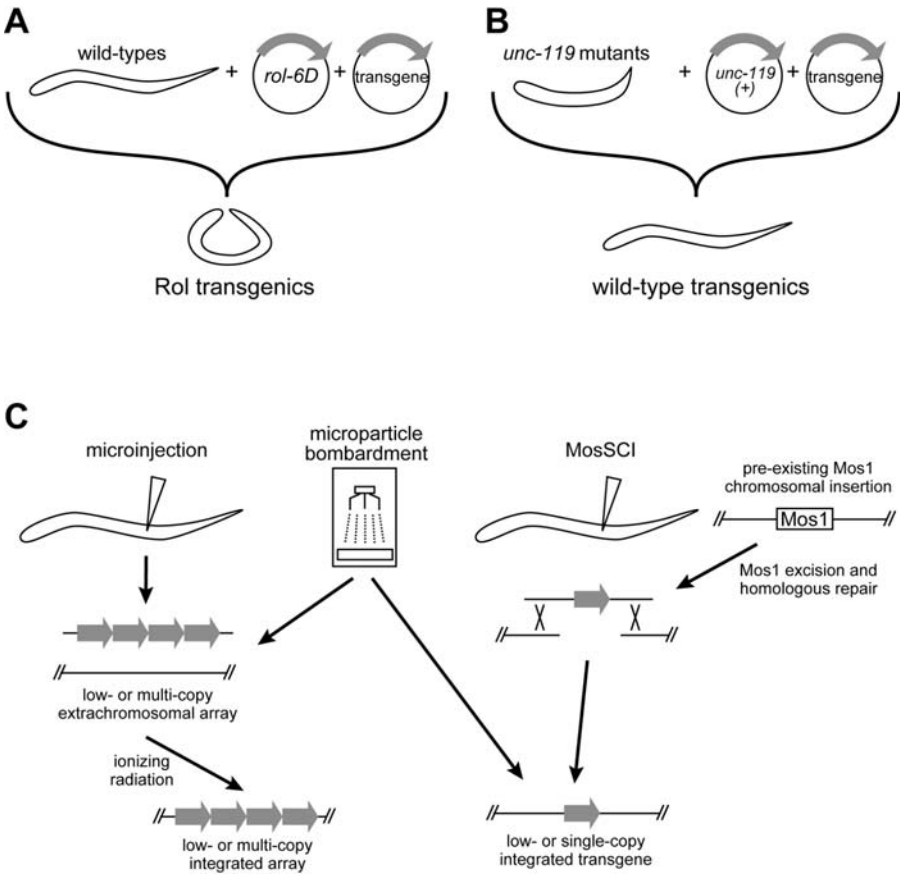

Fig. 1 Basic strategies for marking transgenics and delivering DNA to *C. elegans*. (A) Transgenic animals can be marked by an induced gain-of-function phenotype in a wild-type background, such as by the presence of the *rol-6D* allele in the transgene array, or through rescue of a mutant to a wild-type phenotype, as with rescue of *unc-119* mutants (B). (C) Delivery of transgenes is achieved primarily by microinjection, but also by microparticle bombardment and a modification of injection, Mos Single Copy Insertion (MosSCI). Each approach produces a different spectrum of extrachromosomal and/or integrated transgene types. Higher copy number arrays (generated by strategies in A and B) give higher transgene expression, but can undergo silencing (particularly for maternally expressed genes), while lower copy number transgenes (generated by strategies in C) show weaker expression that is less prone to silencing.

be capable of generating transgenes in *C. elegans,* some of which integrate randomly into the genome at low or even single copy (Praitis *et al.*, 2001). These types of transgenes generally overcome the limitations of high-copy arrays and are able to express more efficiently in the germ line.

What has lagged behind in the *C. elegans* field is a robust method for single-copy gene insertions and targeted chromosomal modifications. Such modifications would, by their nature, permit expression of maternal and zygotic genes under the control of endogenous regulatory elements and allow generation of custom-made alleles.

Two general methods to generate homologous recombinants, both of which depend on either microinjection or microparticle bombardment to generate transgenic lines, have been developed in the last few years (Fig. 1C). One approach takes advantage of the excision of a transposable element to create a double-stranded (ds) break in DNA, which can be used to promote gene conversion or direct insertion of transgenic sequences directly into the chromosome (Frokjaer-Jensen *et al.*, 2008; Plasterk and Groenen, 1992; Robert *et al.*, 2009). Genomewide screens that have produced thousands of Tc1 and Mos1 transposon insertion lines have significantly increased the applicability of this approach (Bazopoulou and Tavernarakis, 2009; Boulin and Bessereau, 2007; Duverger *et al.*, 2007; Williams *et al.*, 2005). In a second approach, scaled-up methods for microparticle bombardment have been used to produce integrations targeted at the endogenous locus (Berezikov *et al.*, 2004) and recent work using a positive- and negative-selection strategy promises to dramatically improve the efficiency of this process (Vazquez-Manrique *et al.*, 2010).

Comprehensive protocols for generating transgenics by microinjection and microparticle bombardment are available online, in the *WormMethods* section of *WormBook* and in a variety of other excellent published sources (Evans, 2006; Green *et al.*, 2008; Hope, 1999; Kadandale *et al.*, 2009; Mello and Fire, 1995; Praitis, 2006; Praitis *et al.*, 2001; Rieckher *et al.*, 2009). What follows are brief descriptions of the uses of transgenes in *C. elegans* research, general considerations for constructing transgenes and delivering them to *C. elegans*, an assessment of methods in other nematodes, and a brief discussion of what future developments may lie ahead.

II. Uses for Transgenes in *C. elegans*

A. Analysis of Gene Expression

The most frequent use of transgenes in *C. elegans* is for the assessment of endogenous gene-expression patterns of protein-coding genes. The simplest approach for making a transcriptional reporter is to clone the 5′ regulatory sequence from a gene of interest, fuse it to a reporter gene whose activity can be easily assayed, and include a 3′UTR, usually that of the *unc-54* gene (Fire *et al.*, 1990). Because of

the transparency of the animal at all life stages, the reporter of choice is GFP or other fluorescent proteins such as the GFP variants YFP and CFP (Miller *et al.*, 1999), or the red fluorescent proteins dsRed and its faster-folding, monomeric variant, mCherry (Shaner *et al.*, 2004).

The most important consideration in constructing a transcriptional reporter is the amount of predicted regulatory sequence to include, as this will be the primary determinant of transcriptional regulation of most genes. Because the genome sequence of *C. elegans* is completely known, researchers can examine the physical map around an uncharacterized gene on WormBase and select as large a region as is practical, typically some 3–10 kbp or to the next upstream gene (Dupuy *et al.*, 2004b; Mounsey *et al.*, 1999). Comparison of noncoding sequences in orthologous genes across sequenced genomes has also been helpful in identifying key regulatory elements (Elemento and Tavazoie, 2005; Kuntz *et al.*, 2008). If it is found that the nearest upstream gene is a very short distance (100–400 bp) from the start of the gene of interest, it is possible that the two genes are in an operon (Zorio *et al.*, 1994), in which case the promoter sequences will lie upstream of the most 5′ transcript. Regulatory sequences can also be found in introns, so transcriptional fusions may need to include these sequences (Okkema *et al.*, 1993). Common methods of constructing reporters will be described later.

To aid in determining the timing and tissue- or cell-specificity of gene expression, it is useful to include sequences that direct the transgene product to the nucleus. Both the SV40 nuclear-localization signal (NLS) and the coding sequence for a histone have been used to concentrate signals in nuclei to facilitate cell identification (Fire *et al.*, 1990; Strome *et al.*, 2001). The histone tags have the advantage that they stay with chromosomes during mitosis. When combined with other transgenic lines for which expression patterns are well characterized, a precise cell-expression pattern can be determined. The advent of new software combined with four-dimensional imaging using confocal microscopy has made this type of analysis technically simpler and more sophisticated (Murray *et al.*, 2008).

B. Analysis of Protein Localization

Where the subcellular localization of a protein is being studied, the transgene can be engineered to carry most (or all) of the coding region for the gene of interest, tagged to a reporter construct (Fig. 2). To be assured that function of the protein is not affected by the reporter (Prasher *et al.*, 1992), it is wise to design constructs where the tag is inserted in different positions in the coding sequence. To ensure that the construct is functioning like the endogenous protein, the transgene should be assayed for its ability to rescue the mutant phenotype, if a mutant is available, or for the anticipated behavior following ectopic overexpression.

Finally, because of the possibility that the 3′UTR of a gene might be under control of a micro-RNA (miRNA), inclusion of the gene's native 3′UTR may be required for the construct to reflect the expression of endogenous protein. Predictions of miRNA

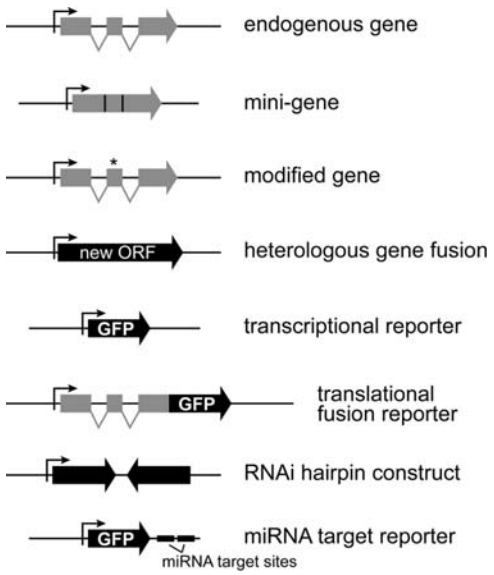

Fig. 2 Schematic showing examples of different types of transgenes (not meant to be exhaustive).

binding sites (Lall *et al.*, 2006) found in WormBase may be informative as to whether or not consideration should be given to possible post-transcriptional regulation in the design of a reporter fusion. Where it is desired to test only the effect of a 3′UTR on gene regulation, sensor transgenes carrying the particular 3′UTR can be tested for responsiveness to miRNA regulation (Wightman *et al.*, 1993). Expression patterns of miRNA genes can be determined with reporter fusions to GFP by using sequences upstream of the mature miRNA as regulatory element (Hayes *et al.*, 2006).

Researchers wishing to know whether the expression of a gene has been studied should first check WormBase (Table I), where expression patterns carried out by gene-specific or genomewide expression studies (Hunt-Newbury *et al.*, 2007) are available. Information on WormBase is often not completely up-to-date and so a literature search should always be performed at the same time. It should be anticipated that a documented expression pattern might not have considered the particular stage, tissue, or condition that is of interest. Hence, the investigator may simply wish to obtain a previously constructed reporter strain, at least for comparison purposes, from either the authors that produced them or from the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota (Table II). Additional expression information may exist in the form of *in situ* hybridization data published by the Kohara laboratory in Japan (Kohara, 2001), which is accessible in the Nematode Expression Pattern Database (Table I).

Table I
Internet links (current as of May, 2011)

Website	Web host	Method/notes
http://www.wormbase.org	WormBase	Contains information about *C. elegans* genes, including sequences
http://wiki.wormbase.org/index.php/Cosmids/YACs	WormBase	Information about obtaining *C. elegans* clones
http://www.wormbook.org/chapters/www_transformationmicroinjection/transformationmicroinjection.html	WormBook – WormMethods	*C. elegans* microinjection. Excellent step-by-step instructions on microinjection and microparticle bombardment procedures
http://www.wormbook.org/chapters/www_reportergenefusions/reportergenefusions.html	WormBook – WormMethods	An excellent description of techniques to generate reporter gene fusions
http://www.wormbook.org/chapters/www_transgenic/transgenic.html	WormBook – WormMethods	Considerations for generation of transgenes that express in the germ line
http://worfdb.dfci.harvard.edu/	ORFeome	Source of *C. elegans* ORFs
http://www.geneservice.co.uk/products/clones/Celegans_Prom.jsp	Promoterome	The library contains 6000 predicted promoters, available from Source Bioscience
http://wormbase.org/db/searches/expr_search	WormBase	Expression pattern search tool. Can be used to identify promoters active in particular cells or tissues
http://nematode.lab.nig.ac.jp/db2/index.php	Nematode Expression Pattern Database (NEXTDB)	Contains *in situ* expression patterns for a large number of genes
http://www.cbs.umn.edu/CGC/	*Caenorhabditis* Genetics Center at University of Minnesota	Source for many of the strains used in transgenesis experiments
http://sites.google.com/site/jorgensenmossci/Home	Jorgensen Lab, Utah	Mos Single Copy Insertion (MosSCI). Detailed protocol on plasmid construction and screening methods
http://www.addgene.org	Addgene	Source for many of the plasmids described here, including for MosSCI
http://www.faculty.ucr.edu/~mmaduro/int.html	Maduro Lab, UC Riverside, CA	Summary of integration techniques using gamma rays, chemical mutagenesis, or UV treatment
http://www.med.yale.edu/mbb/koelle/protocols/protocol_integrating_array.html	Koelle Lab, Yale School of Medicine, New Haven, CT	Step-by-step integration protocol using gamma rays or X-rays
http://www.addgene.org/pgvec1?f=c&cmd=showcol&colid=1	Addgene	Links to documentation for Fire Lab plasmids

C. Rescue of a Chromosomal Mutation

The *C. elegans* system is powerful primarily because of its genetics. For recessive mutations, transgenes carrying the wild-type version of a gene should be able to complement the mutation. For dominant mutations, a transgene carrying the dominant allele should be able to confer a similar phenotype onto an otherwise wild-type strain. Both of these strategies are used for marking transgenes in transformation

experiments. A discussion of markers commonly used for making transgenic lines is included below.

In one variant of complementation of a chromosomal mutation, the transgene is used to rescue an animal with a phenotype resulting from treatment with RNAi. In this case, the transgene is engineered to have resistance to the RNAi effect. When the transgene is combined with tissue-specific or altered promoters, this strategy allows for the assessment of genes that act in multiple tissues or at different stages of development (Green *et al.*, 2008).

Introduction of transgenic sequences is also useful for functional characterization of a gene (Fig. 2). For example, a transgenic construct carrying an altered version of a gene can be assayed to determine if it rescues some or all functions provided by the wild-type gene product (i.e., a sufficiency assay). Introduction of predicted orthologs or paralogs, under the control of a *C. elegans* promoter, can determine conservation of functional domains or gene products. Transgenic constructs containing altered or entirely different regulatory sequences can be used to examine the consequences of ectopic or reduced expression. An altered transgene can be designed to test the function of a particular splice isoform. The transgenes can also be fused to sequences that target them to specific subcellular locations or cause them to be secreted. Several sequences are known that provide subcellular targeting, which includes nuclear localization (SV40 NLS or histone), membrane targeting, secretion, or mitochondrial import (Fire *et al.*, 1990; Portereiko and Mango, 2001; Strome *et al.*, 2001 and Fire Lab Vector information; Addgene). Each of these strategies permits the researcher to manipulate gene activity in order to better characterize the function of a gene of interest.

D. Marking Tissues and Cells for Other Manipulations

The rich variety of existing transgenes allows investigators to mark tissues so that they are more easily followed for live cell imaging, to characterize the effect of environmental or genetic manipulation on development of particular cell types or substructures, or to follow cells in a non-natural context, such as after dissociating embryonic blastomeres. A large number of well-characterized promoters can be searched indirectly by expression in particular tissues, stages, or cells on WormBase. The use of reporters in combinations allows the detection of multiple expression patterns in the same animal, an analysis that is particularly useful for determining lineage-specific expression. Reporters of differing absorption/emission spectra can be used, such as the combination of CFP and YFP, or mCherry with GFP. With mCherry and GFP using standard TRITC and FITC filter sets, the two reporter signals show very little overlap. With CFP and YFP, specific filter sets are used to prevent cross-detection (Miller *et al.*, 1999). Signals in strains expressing all four fluorescent proteins can be discerned because of the behavior of each fluorescent protein in each optimal filter set (Table II). This analysis permits both the deeper understanding of mutant phenotypes and the expression patterns of newly characterized gene products.

Table II
Cross-detection of popular fluorescent reporters in common filter sets

Fluorophore	Appearance in filter set			
	TRITC (Chroma 31002)*	YFP (Chroma 41029)*	GFP (Omega XF100-2)*	CFP (Chroma 31053)*
mCherry	Red	Faint red	Not visible	Not visible
dsRed	Red	Orange	Faint orange	Not visible
YFP	Faint red	Green	Green	Not visible
GFP	Not visible	Green	Green	Green
CFP	Not visible	Not visible	Green	Cyan

* Specifications of the various filters can be found on the manufacturer's websites (http://www.chroma.com; http://www.omegafilters.com).

E. Disruption of Gene Activity: RNA Interference

Extrachromosomal arrays have been used to generate dsRNA *in vivo* that can elicit RNAi. Because of the ability of RNAi to spread among tissues (systemic RNAi), expression of the RNAi construct does not need to occur throughout the entire animal. The constructs can consist of separate sense and antisense RNA transgenes, or constructs expressing a single hairpin (stem-loop) construct (Fig. 2). Expressing hairpin constructs within neurons has been effective for RNAi knockdown of genes that might be more difficult to achieve by feeding-based RNAi (Johnson *et al.*, 2005; Tavernarakis *et al.*, 2000). One difficulty with hairpin constructs is that the DNA constructs are unstable in *E. coli*; this limitation may be overcome by using stem-loops with introns in the loop portion, or the use of *E. coli* strains that are more tolerant to such structures (e.g., SURE2 cells; Stratagene).

F. Mosaic Analysis of Gene Function

Researchers often need to test the requirement of a gene within the context of a subset of its normal expression. This may be done to avoid a requirement for the function of a gene in an earlier developmental stage, or to test if gene function is cell-autonomous. Restricted expression of a gene product can be achieved using a variety of techniques, including creating mosaics through loss of extrachromosomal arrays carrying a gene of interest or by fusion of the gene of interest to a specific promoter. A number of other strategies that promise to permit even more sophisticated analysis of tissue-specific gene expression have also been recently developed (Table III).

The classic approach to making mosaic animals in *C. elegans* is to use extrachromosomal arrays as surrogate chromosomal free duplications, which experience mitotic loss within a single animal at a low frequency (0.1×10^{-3} to 5×10^{-3} loss per cell division) (Hedgecock and Herman, 1995; Lackner *et al.*, 1994; Miller *et al.*, 1996; Yochem and Herman, 2005). Extrachomosomal arrays have an advantage over free duplications because the researcher can determine their composition. To

Table III
Mosaic analysis of gene function

Technique	Applications	Considerations	References
Loss of extrachromosomal arrays	Permits expression of gene in a lineage-specific manner. When introduced into a mutant background, permits analysis of lineage-specific presence or loss of gene activity	Marker may not always correlate with gene activity. Expression levels of gene altered due to silencing, overexpression, or perdurance of gene product. Lineage analysis can be difficult and may not be specific enough to limit expression to a small number of tissues	Hedgecock and Herman (1995); Lackner *et al*. (1994); Miller *et al*. (1996); Yochem and Herman (2005)
Expression of gene under control of tissue-specific promoters	Permits expression of genes in a very specific set of tissues, cells, or developmental stages	Limited by availability of specific, well-characterized promoters. Use of non-native regulatory elements may produce inappropriate levels of gene product. Requires a new construct for each gene of interest	
Addition of long 3′UTR that alters gene product stability	Permits temperature-sensitive regulation of gene expression	Some background gene expression in the off state. Requires work in a nonsense-mediate decay mutant background	
FLP-recombinase gene activation	Sensitive spatial and temporal control of gene expression. Allows for use of endogenous promoters and other regulatory elements in gene of interest. Creates a set of strains that can be used with different constructs	Change in gene expression due to FLP activation is not reversible. Time delay associated with FLP expression and recombination	Davis *et al*. (2008); Voutev and Hubbard (2008)
Controlled expression of heat-shock sensitivity	Sensitive spatial and temporal control of gene expression. Relatively rapid changes in gene expression levels. Creates a set of strains that can be used with different constructs.	Need to work in *hsf-1(lof)* background. Use of non-native regulatory elements may produce inappropriate levels of gene product. Heat-shock response does not allow for sustained gene expression	
Reconstituting gene activity from two components	Sensitive spatial and temporal control of gene expression. Used for cell-specific labeling and killing of cells. Sets of strains can be combined in different ways	Limited to genes or processes that can be reconstituted from two components	Chelur and Chalfie (2007); Zhang *et al*. (2008)

(Continued)

Table III *(Continued)*

Technique	Applications	Considerations	References
Cell-specific delivery of heat shock	Sensitive spatial and temporal control of gene expression using a focused laser microbeam	Requires laser apparatus and ability to identify cells. Care is required to avoid damaging the induced cell(s)	Stringham *et al.* (1992)
Temperature-sensitive *mec-8*-dependent splicing	Permits controlled, temperature-sensitive regulation of gene expression, including RNAi-sensitivity	Perdurance of *mec-8* activity can make precise regulation difficult. Splicing event requires low-doses of MEC-8. Need to work in *mec-8* background	Calixto *et al.* (2010)
Selective depolarization of cells by light stimulation ("optogenetics")	Activation of transgene-driven light-sensitive proteins such as channelrhodopsin-2 (ChR2) (Nagel *et al.*, 2003).	Light activation can be delivered broadly, as only cells expressing ChR2 will become depolarized. Light-sensitive channels that respond to different wavelengths can be used simultaneously	Stirman *et al.* (2011)
Tissue-specific *RNAi* sensitivity	Permits specific loss of gene activity in a subset of cells	RNAi effectiveness can be variable	Qadota *et al.* (2007)

identify which cells/tissues have inherited the array, cells carrying the wild-type copy of a gene could be identified by tagging the gene with GFP, by including a ubiquitous reporter such as *sur-5*::GFP (Yochem and Herman, 2005), by including rescue of *ncl-1*, whose function can be scored cell-autonomously (Hedgecock and Herman, 1995), or by using a nuclear-localized GFP::LacI to mark LacO sequences present in the array (Gonzalez-Serricchio and Sternberg, 2006) (discussed below). By referring back to the *C. elegans* lineage (Sulston *et al.*, 1983), the researcher can conclude which cell(s) lost the array in a particular animal, and, if this loss includes tissues of interest, conclusions can be made about cell-autonomous and cell nonautonomous functions. Finally, arrays can be specifically lost in the maternal germ line, so that progeny animals can be produced that lack both maternal and zygotic contributions of the gene (Hunter and Kenyon, 1996). While this approach has been immensely powerful, using array loss to examine tissue-specific gene expression does have limitations, which include the sometimes-complex lineage analysis required to understand emerging phenotypes.

A second technique for examining tissue-specific expression relies on the use of tissue-specific promoters linked to one's gene of interest (Table III). While this technique has also significantly contributed to our understanding of tissue-specific gene expression, this analysis can be restricted by the limited availability of well-characterized promoters.

A number of strategies for temporally and spatially controlling gene expression have been recently developed (Table IV). In general, these techniques depend on

Table IV
Markers used to identify transgenics

Marker	Plasmid	Notes
rol-6(su1006)	pRF4	Confers a dominant right-handed Roller phenotype to animals (Kramer *et al.*, 1990). Male Rollers do not mate well. Plasmid available from most *C. elegans* laboratories
unc-119 rescue	pDP#MM016B or *Cb-unc-119(+)* in transgene plasmid	Rescues uncoordinated *unc-119* mutants to a wild-type phenotype (Maduro and Pilgrim, 1995; Maduro and Pilgrim, 1996). Mutant strain *unc-119(ed4)* available from the *Caenorhabditis* Genetics Center (CGC). For use with microparticle bombardment *unc-119(+)* is usually included in the transgene plasmid. For MosSCI, the *C. briggsae* gene is inserted into the targeting vector. Plasmid is available from the Maduro lab (University of California, Riverside, CA)
lin-15 rescue	pEKL15	Rescues temperature-sensitive multivulva (Muv) phenotype of *lin-15(n765)* (Clark *et al.*, 1994). The *lin-15(+)* plasmid pEKL15 is available from the Horvitz laboratory (Massachusetts Institute of Technology, Cambridge, MA). Strains harboring *lin-15(n765)* are available from the CGC
pha-1 rescue	pBX or pC1	Rescues larval lethality of *pha-1(ts)* mutants to a wild-type phenotype. Strain is maintained at 15°C, used at 25°C. Mutant strain *pha-1(e2123)* is available from the CGC (Kramer *et al.*, 1990)
dpy-20 rescue	pMH86	Rescues strong Dumpy phenotype of *dpy-20(ts)* mutants to a wild-type phenotype. Mutant strain *dpy-20(e1282ts)* available from the CGC (Clark *et al*., 1995). The plasmid is available from the Han lab (University of Colorado, Boulder, CO)
spe-26 rescue	pJV145	Rescues *spe-26(hc138ts)* (H. Smith and S. Ward, personal communication; Praitis, 2006)
Puromycin resistance	pBCN21-R4R3 or pBCN22-R4R3	Plasmids confer resistance to puromycin (Semple *et al.*, 2010)
G418/neomycin resistance	pdestDD04Neo, pdestRG5271Neo, pdestRG5273Neo	Resistance to G418 (neomycin) (Giordano-Santini *et al.*, 2010)
myo-2::mCherry	pCFJ90	Expresses mCherry in pharynx muscle. Plasmid available from Addgene
elt-2::NLS::GFP::lacZ	pJM66	Expresses GFP in intestinal nuclei (Fig. 3D). Plasmid available from McGhee Lab (University of Calgary, AB)
sur-5::GFP	pTG96	Expresses GFP in all nuclei. Plasmid available from the Han lab (University of Colorado, Boulder, CO)
let-858::GFP	pBK48.1	Expresses GFP in all nuclei. Plasmid available from Kelly Lab (Emory University, Atlanta, GA)
unc-119::GFP	pDP#MMUGF12	Expresses GFP throughout nervous system and in some head muscles (Fig. 3E). GFP, YFP, CFP, and mCherry versions of this reporter are available from the Maduro lab (University of California, Riverside, CA)

controlling gene structure, gene product stability, or gene activity using either tissue-specific promoters or by controlling the presence of inducers or repressors of gene expression. One technique takes advantage of the well-characterized FLP recombinase system to activate a gene of interest in specific tissues or at specific times of development. In this strategy, the regulatory element is separated from the gene of interest by a sequence that is cleaved upon tissue-specific expression of FLP recombinase, thereby activating the gene (Davis *et al.*, 2008; Voutev and Hubbard, 2008). Until recently, the available methods for delivering transgenes, which tend to be higher copy number and contain rearrangements, limited the effectiveness of this technique for temporal or tissue-specific knockdown (as opposed to activation) of gene activity. A second technique that permits control of gene expression depends on reconstituting gene activity from two gene components, each under the control of a specific promoter. When the two elements are combined, as would be expected in a small number of specific cells, gene activity is restored (Chelur and Chalfie, 2007; Zhang *et al.*, 2008). This technique is limited to genes or processes that can be reconstituted from two components. A third technique requires altering the 3′UTR of a gene and taking advantage of temperature sensitivity of nonsense-mediated decay of RNA products to promote gene stability or decay (Drake *et al.*, 2003). However, the effects on gene-expression levels are not always absolute. A fourth technique depends on rescuing a heat-shock deficient *hsf-1(sy441)* mutant in a cell-specific manner by controlled expression of wild-type *hsf-1*, permitting expression of a heat-shock inducible promoter linked to one's gene of interest in only those cells (Bacaj and Shaham, 2007). This technique is limited by the temperature-sensitivity of the process and by the transient nature of the heat-shock response. A fifth method takes advantage of the observation that MEC-8 is required to splice *mec-2* intron 9, thereby regulating the expression of *mec-2* splice variants. By creating a transgene carrying the *mec-2* intron 9 sequence upstream of a gene of interest in a *mec-8(u218ts)* strain, one can regulate expression of the gene using temperature shifts. This technique was used to control expression of the RNAi gene *rde-1* to create a line with temperature-dependent RNAi (Calixto *et al.*, 2010). Potential limitations of this technique include the need to work in a *mec-8* strain, as well as the relative stability of MEC-8 and dose-sensitivity of the splicing event. Despite some limitations, each of these techniques offers researchers valuable tools for selective expression of their gene of interest.

Knockdown of gene function in specific tissues can also be used to examine gene activity. The cell-autonomous requirement for RDE-1 function in RNAi can be exploited by providing wild-type function of *rde-1* in a tissue-specific manner to an *rde-1* mutant strain. Animals then treated with RNAi to a gene of interest will experience knockdown only in cells carrying RDE-1 function (Qadota *et al.*, 2007). While this strategy has been used effectively for some genes, the strength of the RNAi response can be variable.

In summary, the *C. elegans* researcher has a large set of techniques that can be used to understand the role of a gene in a specific cell or developmental process, bypass requirements at specific stages, or examine the consequences of ectopic gene expression. The specific gene studied, the hypothesis being tested, and the

limitations and advantages of each strategy will dictate which technique is best for a given application.

G. Marking Extrachromosomal Arrays to Probe Gene Regulation

The interaction of the *E. coli* LacI protein with *lacO* lactose operator sequences was exploited as a method for marking chromosomes in yeast (Belmont and Straight, 1998) and has been used as a marker for transgenes in *C. elegans* as well (Gonzalez-Serricchio and Sternberg, 2006). Use of the LacI/LacO systems has also been used to label extrachromosomal arrays to study gene regulation (Fig. 3A). In such experiments, a GFP-tagged endogenous transcription factor is expressed in the presence of an extrachromosomal array that carries a promoter that contains its target *cis*-regulatory sites. The factor will interact with the many copies of the target promoter in the array, producing a subnuclear spot. LacI tagged with a different marker can label *lacO* sequences in the same target array, allowing an independent means by which to verify interaction of the GFP-tagged factor with the array (Carmi *et al.*, 1998). Researchers have also used the GFP::LacI/LacO system to demonstrate that transgenes move to different locations in the nucleus depending on whether they are active or inactive in a given cell or tissue (Meister *et al.*, 2010).

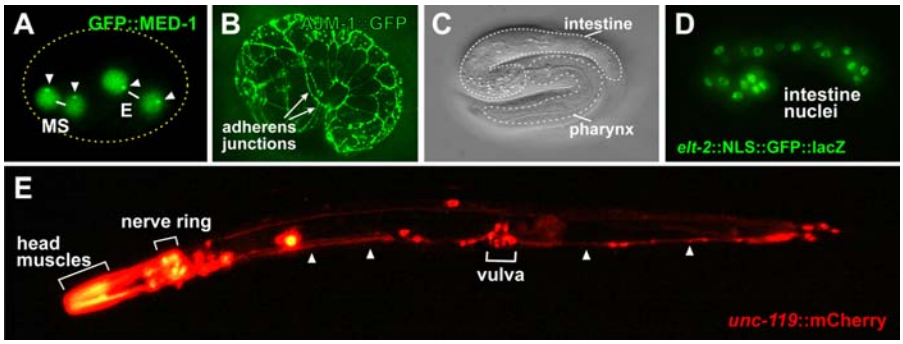

Fig. 3 Examples of types of transgenes and their expression patterns. (A) Expression of a chromosomally-integrated *med-1*::GFP::MED-1 translational reporter in the early embryo, showing nuclear GFP expression in the daughters of the blastomeres MS and E (Maduro *et al.*, 2002). Due to the presence of a separate extrachromosomal array carrying a transcriptional *lacZ* reporter for the MED-1 target gene *end-3*, the GFP::MED-1 localizes to subnuclear spots representing the extrachromosomal array (arrowheads) in each nucleus. (B) Expression of a translational fusion of the adherens junction marker *ajm-1* in mid-embryogenesis. GFP becomes localized to adherens junctions, giving an outline of epidermal cells (Koppen *et al.*, 2001). (C) DIC image of a late embryo, just prior to hatching, with the pharynx and intestine indicated. (D) Expression of an *elt-2*::NLS::YFP::lacZ reporter transgene in the same embryo as in (C) localized to intestinal nuclei (and excluded from nucleoli). (E) A *C. elegans* adult hermaphrodite showing expression of an *unc-119*::mCherry transcriptional reporter throughout the nervous system (including the nerve ring, neurons around the vulva, and the ventral nerve cord indicated by arrowheads) and in head muscles (Maduro and Pilgrim, 1995). The head muscle expression has been overexposed. Anterior is to the left. A *C. elegans* embryo is approximately 50 μm long, while adults are approximately 1mm long. (See color plate.)

III. Construction of Transgenes

An excellent description of the many considerations for construction of plasmid reporters can be found in Boulin *et al.* (2006); Mounsey *et al.* (1999), which we have updated here. There do not appear to be any sequence requirements for the stable inheritance of arrays in *C. elegans* (Mello *et al.*, 1991), as DNA from plasmids or phage, for example, appears to be incorporated into arrays. Hence, standard molecular biology techniques can be used to construct most transgenes. When segments of wild-type DNA are needed, polymerase chain reaction (PCR) can be used to amplify directly from genomic DNA, or larger clones, such as a cosmids or fosmids, can be ordered and used for either direct subcloning or PCR. Information on ordering clones is available on WormBase (Table I). Researchers are cautioned that some larger clones are unstable when propagated in bacteria or yeast, such that a particular isolate of a clone could be missing regions of DNA. When working with these constructs it is always advisable to check that sequences have not been lost.

For simple reporter fusions of zygotically expressed genes, it is usually sufficient to clone a suitable upstream promoter fragment (3–10 kbp is a good start, without taking sequences from the neighboring gene upstream) along with a small part of the coding region. The fragment is cloned into one of the available GFP vectors (generated by the laboratory of Andrew Fire). These vectors supply a useful polylinker, synthetic introns to increase expression, and a 3′UTR from the *unc-54* gene. Variants are available that encode other fluorescent proteins (YFP or CFP), include a nuclear localization signal (NLS), or are a fusion to both GFP and lacZ. Other vectors use a histone H2B coding sequence as a more effective means to localize GFP to nuclei. A number of useful vectors as well as additional documentation from the Fire lab can be obtained from Addgene (http://www.addgene.org). Where an investigator hypothesizes sequence requirements that necessitate a much larger context for the reporter (e.g., tens of kilobasepairs), manipulations can be performed using recombination in yeast or fosmids (Dolphin and Hope, 2006; Tursun *et al.*, 2009; Zhang *et al.*, 2008).

Other applications of transgenes, such as the fusion of a promoter to a different downstream sequence, will require approaches unique to each application (Fig. 2). Additional resources available to the research community can simplify cloning or allow rapid scaling-up of construct production. For example, it is now possible to use the Gateway recombination cloning system to fuse promoters from the "promoterome" library into a suitable reporter. For making novel fusions of promoters to different coding regions, clones from the promoterome can be combined with clones from the ORFeome (Dupuy *et al.*, 2004a; Hope *et al.*, 2004; Reece-Hoyes *et al.*, 2005).

For expression of heterologous coding regions, it may be cost-efficient for an investigator to order an open reading frame to be synthesized *de novo*. Custom gene synthesis can now be achieved for a relatively low cost per base pair. This would also allow engineering for efficient expression in *C. elegans*, such as by the introduction of short introns, or the selection of codons that are optimized for maximal gene

expression (Duret, 2000; Okkema *et al.*, 1993). Hence, some labs may consider that particular manipulations, such as modification of protein coding regions, might be best achieved by direct synthesis, considering the time and/or number of manipulations that would otherwise be necessary.

Expression of genes in the germ line or early embryo can be less straightforward than expression in other tissues or stages of development. The repetitive nature of conventional transgenes results in germ-line silencing (Kelly *et al.*, 1997). Conventional extrachromosomal arrays are compatible with maternal (germ line) expression for only a small subset of GFP reporters (Fire *et al.*, 2006). There are several approaches for achieving expression of maternal transgenes. One is to use conventional arrays, but to coinject genomic DNA that has been digested with a restriction enzyme that leaves blunt ends (Kelly *et al.*, 1997). This approach appears to achieve expression of maternal transgenes because the arrays are made complex and less prone to silencing. In some instances, maternal expression can be achieved by using a promoter and 3′UTR that seem to be compatible with expression from a multicopy array, such as from *glh-2* (Bessereau *et al.*, 2001). More reliable approaches for germ-line or maternal expression use microparticle bombardment or MosSCI (Fig. 1B), both of which deliver fewer copies of the transgene, which makes them less prone to silencing. Both of these techniques require special consideration for the plasmids that carry the transgenes, as described below.

IV. Obtaining Transgenic Animals

A. Considerations for Marking of Transgenics

It is usual practice to mark the presence of a transgene by a convenient marker that can be scored visually in larvae or adults, to facilitate identification following transgene delivery, and when transformants are obtained, during crosses or screening for integrants (Figs. 1A,B, Table IV). Transgenes that confer a readily detectable change in phenotype from nontransgenic animals (e.g., rescue of a visible mutation or very bright GFP reporter), may not need a coinjection marker unless a positive control for the injection process is desired. During the process that gives rise to conventional transgene arrays, recombination among the injected plasmids (if present at high enough relative concentrations) will almost always ensure that multiple, separate plasmids become incorporated into the same array. For microparticle bombardment, the marker is often but not always included in the same plasmid as the transgene, because the low copy number of the resulting insertions makes it less likely that both will become integrated. For Mos-directed chromosomal insertion, the marker and transgene must both be included in between the flanking homology segments.

Simple transgenes in a wild-type background can be marked with the plasmid pRF4, which encodes the *su1006* allele of the *rol-6* gene, also called *rol-6D* (Fig. 1A) (Mello *et al.*, 1991). pRF4 induces an obvious right-handed Roller (Rol) phenotype.

Unfortunately, the effect greatly reduces the mating efficiency of males, which can make crosses more difficult. Some chromosomal integrants of *rol-6D*-marked transgenes show a much weaker Rol phenotype as heterozygotes, or they can be combined with mutations in some genes (e.g., *dpy-11*), which can suppress the Rol effect.

Where a wild-type phenotype is desired from the transgenic animals, it is convenient to start with a strain carrying a recessive mutation and use rescue of the mutation as the transgene marker (Fig. 1B). Markers commonly used are rescue of *pha-1*, *dpy-20*, and *unc-119*. Loss of *pha-1* is lethal, but the allele used is temperature-sensitive (ts), so that animals are propagated at 15°C and selected for transgenics at 25°C (Granato *et al.*, 1994). Loss of *dpy-20* results in a viable dumpy (Dpy) phenotype, but Dpy adults are more difficult to inject, so a ts allele is used (Clark *et al.*, 1995). Until such transgenes are integrated, maintenance requires propagation at 25°C, which may make downstream genetics more challenging (e.g., if a transgene were to be crossed into another ts mutant background). *unc-119* mutants do not form dauer larvae, an alternative larval stage that allows worms to survive prolonged starvation. As a result, non-Unc-119 transgenics can be identified from large populations since they are viable after starvation. For MosSCI or microparticle bombardment, in which a very small fraction of animals becomes transgenic, rescue of *unc-119* has been the most frequently used marker, although a number of other markers have been used successfully (Praitis, 2006). Use of the more compact *C. briggsae* homolog of *unc-119* is convenient as it facilitates cloning of the transgene and marker into the same plasmid. Inclusion of the *unc-119* marker into transgenic constructs has also been made simpler by a recent modification of recombineering techniques (Ferguson and Fisher, 2009; Zhang *et al.*, 2008).

As an alternative to using rescue of a mutation, transgenes can be marked by the presence of a GFP reporter to *myo-2* (Okkema *et al.*, 1993), *elt-2* (Fig. 3D) (Fukushige *et al.*, 1998), *sur-5*, or *let-858* (Kelly *et al.*, 1997; Yochem *et al.*, 1998). Access to a dissecting microscope equipped with a fluorescent lamp and appropriate filters or a fluorescent worm sorter are necessary for identifying and maintaining lines carrying extrachromosomal arrays. Other transgene markers include antisense-*unc-22*, which imposes a twitching paralysis, and selection for resistance to antibiotics (Fire *et al.*, 1991; Giordano-Santini *et al.*, 2010; Semple *et al.*, 2010).

As a final consideration, expression of one gene on a single array may be precluded by the presence of the second gene. In such cases, it may be desirable to obtain separate transgene reporters, and combine the two strains together. If this is done, the researcher may wish to consider different strategies for marking the presence of either transgene. For example, if both are *rol-6D* marked transgenes, it may be difficult to identify double-transgenics or to even mate them together. In such cases, rescue of *unc-119* and *rol-6D* could be used to make separate transgenes, and then the two strains can be combined by crossing rescued *unc-119* transgenics to the *rol-6D* strain that is homozygous for *unc-119*. The double transgenics will be Rol non-Unc.

B. Delivery Methods

The *C. elegans* germ line is the target organ for microinjection. It contains a syncytium of germ-line nuclei sharing a common cytoplasm (Klass *et al.*, 1976). Researchers have two choices for delivery of transgenes to *C. elegans*, microinjection or microparticle bombardment (Fig. 1C).

Microinjection is typically the first technique tried, as it requires only a small number of animals, and F_1 animals are scored within a few days after injection. An inverted microscope setup with differential interference contrast (DIC) or similar optics, a needle puller, and a micromanipulator are necessary. Pressurized nitrogen, delivered through a regulator with a foot pedal controller, is usually used to force injection mixtures through the needle. Alternatively, other lower-cost arrangements are possible. Laboratories performing *Drosophila* microinjection may have similar setups that can be used. A detailed protocol for *C. elegans* transformation using microinjection can be found online from the WormMethods section of WormBook and from other published sources (Evans, 2006; Kadandale *et al.*, 2009; Mello and Fire, 1995). For laboratories that desire low copy number transgenes, for example, to avoid toxicity or to achieve maternal expression, injection can be modified by the inclusion of digested genomic yeast or nematode DNA (Kelly *et al.*, 1997). Alternatively, bombardment or MosSCI, both of which yield low copy integrants, can be used. When stable lines are required, extrachromosomal transgenic arrays can be integrated using chemical mutagens or radiation, as described below.

Microparticle bombardment requires more time initially, as a large number of starting *unc-119* mutant animals are required, and there is usually a 10–14-day post-treatment wait time before active screening for transformants begins. However, the chief advantages of the technique are that both integrants and extrachromosomal arrays are obtained in the same procedure, the technique relies on a selection that yields only the most stable lines, and it requires little technical expertise. Access to a Biolistic PDS-1000 Helium Microparticle Bombardment machine or other delivery device and several consumables are needed for this procedure. Laboratories may find access to such a machine if there is a nearby facility that performs plant cell transformations. Detailed descriptions of the microparticle bombardment procedure are available in WormBook or in other published sources (Evans, 2006; Green *et al.*, 2008; Jackstadt *et al.*, 1999; Praitis *et al.*, 2001; Rieckher *et al.*, 2009; Wilm *et al.*, 1999).

The delivery of transgenes for directed chromosomal insertion using Mos transposition (Fig. 1C) is really just a special case of direct microinjection into *unc-119* mutant animals, as the desired transgenics do not require infinite passage of the extrachromosomal transgene array. The injected plasmids serve primarily as the chromosomal repair source (the "targeting plasmid") and to activate Mos transposition in the germ line. To distinguish *bona fide* chromosomal insertions from transmission of an extrachromosomal array, several reporter plasmids are coinjected simultaenously. In one version of the approach, chromosomal insertions are rescued for the *unc-119* phenotype but fail to express the other coinjected transformation

markers, including a plasmid encoding the temperature-sensitive, dominant negative selection marker *twk-18(cn110)*. The plasmids are microinjected simultaneously in a manner similar to that for conventional arrays, but the wait time following microinjection is about 10–14 days. Some 25–50 animals are needed for injection, and the equipment is identical to that needed for normal extrachromosomal arrays (Frokjaer-Jensen *et al.*, 2008). Hence, laboratories that are already established for regular microinjection will find it easier to work with Mos-directed insertions if their goal is to obtain low copy number arrays.

Researchers wishing to try both microparticle bombardment and MosSCI may consider constructing their transgene into a targeting vector for MosSCI, as the resultant plasmid can then be used directly for bombardment, MosSCI or a conventional multicopy array transgene, as all can be delivered to *unc-119* mutants. It is worth noting that as MosSCI insertions are targeted to predetermined locations, researchers may wish to consider which location (and corresponding targeting vector) they will use if there is a later need to combine transgenes into one strain. There are currently two locations, on chromosomes II and IV (Frokjaer-Jensen *et al.*, 2008), though it is anticipated that additional targeting loci will become available over time.

C. Integration of Extrachromosomal Arrays

Transgenes carried on extrachromosomal arrays can be integrated into a chromosomal location, which eliminates mitotic and meiotic loss of the array (Evans, 2006). Spontaneous integration of extrachromosomal arrays has been observed by many investigators, which may be more likely to be seen in large populations propagated for many generations, especially if there is a selective advantage to the integrants. Otherwise, spontaneous integration is rare enough that it is not convenient to expect it to occur for any given transgene. Hence, most investigators use chemical mutagens or ionizing radiation (gamma rays or ultraviolet light) to induce integration of an array into a chromosome. This is usually done by mutagenizing a small starting population of animals, establishing several hundred single F_1 animals, and testing F_2 progeny individually for 100% transmission of the transgene to subsequent generations (Evans, 2006). Coinjection of oligonucleotides can also stimulate integration of arrays (Mello *et al.*, 1991), and integration is observed if oocyte nuclei are directly injected (Fire, 1986). However, neither of these approaches appears to be in wide use. Once integrated, it is usually no longer necessary to follow a transgene by the coinjection marker. This may simplify subsequent genetic manipulations and permit combining multiple transgenes into a single strain.

For all integrated transgenic lines, strains should be backcrossed several times to eliminate background changes to the genome introduced by the integration treatment. It is also important to examine phenotypes and expression patterns in several integrated lines to be assured that results are not dependent on the site of integration or any linked background mutations.

D. Transgenics in Other Nematode Species

With the recent availability of genome sequences for other nematodes, researchers may wish to perform gene manipulations in these species. The closely related *C. briggsae* is the most frequent choice for comparative work, perhaps because it is hermaphroditic like *C. elegans* (most others are male–female), and because its genome sequence is of very high quality (Stein *et al.*, 2003). Like *C. elegans*, *C. briggsae* can be made transgenic by microinjection with the use of *rol-6D* or rescue of mutants. Mutants in *unc-119* have been made available that permit the use of microparticle bombardment (Zhao *et al.*, 2010). There are Mos insertions that have been made in *C. briggsae* but the MosSCI approach is still being developed (Marie Delattre, personal communication). In the more distant nematode *Pristionchus pacificus*, also a hermaphroditic species, transgenics can be made, though with some difficulty (Schlager *et al.*, 2009), using an adaptation of the protocol for using complex arrays in *C. elegans* (Kelly *et al.*, 1997). Routine transgenesis in male–female nematode species has not been developed, although in principle coinjection of a dominant marker that does not affect male mating, such as a GFP reporter, could be used to mark transgenics. The *rol-6D* phenotype compromises male mating, which would make maintenance of homozygous transgenic integrants more difficult. As well, the basis for identification of transgenics in bombardment and MosSCI – *unc-119* rescue – would be impossible in male–female species because *unc-119* blocks male mating. Microparticle bombardment may be the best possibility for transgenesis in other species, if a system can be devised to identify rare transgenics. One promising breakthrough, a transformation strategy that depends on conferring drug resistance, will likely make it simpler to generate transgenics in a large number of nematode species (Giordano-Santini *et al.*, 2010; Semple *et al.*, 2010).

V. Perspectives: What Lies on the Horizon?

While recent research has added to an already rich suite of applications for transgenesis in *C. elegans*, there are some technologies, used in other systems, which are still being developed or refined in worms. One essential technique for studying gene function is a simple, reproducible method for knocking out, tagging, or otherwise manipulating a gene at its endogenous locus. Several recently developed methods for creating lines carrying homologous integrations, using negative/positive selection after microparticle bombardment or MosSCI, promise to make homologous, targeted modifications the standard in *C. elegans* (Frokjaer-Jensen *et al.*, 2008; Vazquez-Manrique *et al.*, 2010).

A second essential technique that would permit more sophisticated analysis of gene function is one that promotes or prevents gene expression in a precise spatial or temporal pattern, similar to the *Drosophila* GAL4 system. In principle, several recently developed techniques described in the mosaic analysis section of this chapter could achieve this aim (Table III). Particularly promising are those techniques that create strains that confer specific expression or inhibition of any

appropriately constructed transgene or RNAi construct. Once a toolbox of these strains has been created, it could be used by anyone in the community who requires a specific expression pattern for their gene of interest. Another exciting possibility is that the FLP recombinase system, which has been used to induce gene expression (Davis *et al.*, 2008; Voutev and Hubbard, 2008), could be used for tissue-specific elimination of gene expression. This seems a likely future development, given that single-gene insertions and homologous recombination techniques have been developed (Frokjaer-Jensen *et al.*, 2008; Vazquez-Manrique *et al.*, 2010).

A variation on mosaic analysis that would be useful for researchers is a technique that permits tissue- or spatially restricted knockdown of gene activity for use in a genetic screen. This technique is crucial for any researcher studying a process that requires genes essential at earlier developmental time points or for other processes. Emerging techniques that restrict RNAi sensitivity to a small set of cells or to specific developmental stages could, in theory, permit these types of genomewide screens (Calixto *et al.*, 2010; Qadota *et al.*, 2007).

Another technology emerging in other systems that may have applications in *C. elegans* is the use of zinc finger nucleases. These are heterodimers consisting of engineered C_2H_2 zinc finger arrays expressed as fusions to the nuclease domain of the restriction enzyme *Fok*I (Kim *et al.*, 2010). These enzymes are capable of single site-specific cleavage of DNA, which in theory can result, as with transposon excision, in imprecise repair of the break (i.e., generating a mutation) or incorporation of sequences from a transgene repair template. At least one such enzyme has been found to function somatically in *C. elegans* (Carroll *et al.*, 2008; Morton *et al.*, 2006), raising the hope that if germ-line expression can be achieved, it may be possible to cause germ-line site-specific chromosome modification.

VI. Summary

The creation of transgenic strains is one of the most important tools for analysis of gene function. Two different delivery methods for *C. elegans* transgenesis, microinjection and microparticle bombardment, have been developed. From these basic methods, a plethora of techniques have emerged that permit analysis of gene expression and function in a range of key cellular and developmental processes. The future holds promise for even greater precision and sophistication in experimentally manipulating gene expression in *C. elegans*.

References

Bacaj, T., and Shaham, S. (2007). Temporal control of cell-specific transgene expression in *Caenorhabditis elegans*. *Genetics* **176**, 2651–2655.

Bazopoulou, D., and Tavernarakis, N. (2009). The NemaGENETAG initiative: Large scale transposon insertion gene-tagging in *Caenorhabditis elegans*. *Genetica* **137**, 39–46.

Belmont, A., and Straight, A. (1998). *In vivo* visualization of chromosomes using lac operator–repressor binding. *Trends Cell Biol.* **8**, 121–124.

Berezikov, E., *et al*. (2004). Homologous gene targeting in *Caenorhabditis elegans* by biolistic transformation. *Nucleic Acids Res.* **32**, e40.

Bessereau, J. L., *et al*. (2001). Mobilization of a *Drosophila* transposon in the *Caenorhabditis elegans* germ line. *Nature* **413**, 70–74.

Boulin, T., and Bessereau, J. (2007). Mos1-mediated insertional mutagenesis in *Caenorhabditis elegans*. *Nat. Protoc.* **2**, 1276–1287.

Boulin, T., *et al*. (2006). Reporter Gene Fusions. *In* (The *C. elegans* Research Community, ed.). WormBook. doi/10.1895/wormbook.1.108.1.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

Calixto, A., *et al*. (2010). Conditional gene expression and RNAi using MEC-8-dependent splicing in *C. elegans*. *Nat. Methods* .

Carmi, I., *et al*. (1998). The nuclear hormone receptor SEX-1 is an X-chromosome signal that determines nematode sex. *Nature* **396**, 168–173.

Carroll, D., *et al*. (2008). Gene targeting in *Drosophila* and *Caenorhabditis elegans* with zinc-finger nucleases. *Methods Mol. Biol.* **435**, 63–77.

C. elegans Genome Sequencing Consortium(1998). Genome sequence of the nematode C. elegans: a platform for investigating biology. *Science* **282**, 2012–2028.

Chalfie, M., *et al*. (1994). Green fluorescent protein as a marker for gene expression. *Science* **263**, 802–805.

Chelur, D. S., and Chalfie, M. (2007). Targeted cell killing by reconstituted caspases. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 2283–2288.

Clark, D. V., *et al*. (1995). Molecular cloning and characterization of the *dpy-20* gene of *Caenorhabditis elegans*. *Molec. Gen. Genet.* **247**, 367–378.

Clark, SG., *et al*. (1994). The *Caenorhabditis elegans* locus lin-15, a negative regulator of a tyrosine kinase signaling pathway, encodes two different proteins. *Genetics* **137**, 987–997.

Davis, M., *et al*. (2008). Gene activation using FLP recombinase in *C. elegans*. *PLoS Genet.* **4**, e1000028.

Dolphin, C., and Hope, I. (2006). *Caenorhabditis elegans* reporter fusion genes generated by seamless modification of large genomic DNA clones. *Nucleic Acids Res.* **34**, e72.

Drake, J., *et al*. (2003). Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1-42) in a transgenic *Caenorhabditis elegans* model. *Neurobiol. Aging* **24**, 415–420.

Dupuy, D., *et al*. (2004 a). A first version of the *Caenorhabditis elegans* promoterome. *Genome Res.* **14**, 2169–2175.

Dupuy, D., *et al*. (2004 b). A first version of the *Caenorhabditis elegans* promoterome. *Genome Res.* **14**, 2169–2175.

Duret, L. (2000). tRNA gene number and codon usage in the *C. elegans* genome are co-adapted for optimal translation of highly expressed genes. *Trends Genet.* **16**, 287–289.

Duverger, Y., *et al*. (2007). A semi-automated high-throughput approach to the generation of transposon insertion mutants in the nematode *Caenorhabditis elegans*. *Nucleic Acids Res.* **35**, e11.

Elemento, O., and Tavazoie, S. (2005). Fast and systematic genome-wide discovery of conserved regulatory elements using a non-alignment based approach. *Genome Biol.* **6**, R18.

Evans, T. C. (2006) Transformation and Microinjection. *In* (The *C. elegans* Research Community, ed.). Wormbook, Vol. doi/10.1895/wormbook.1.108.1.

Ferguson, A., and Fisher, A. (2009). Retrofitting ampicillin resistant vectors by recombination for use in generating *C. elegans* transgenic animals by bombardment. *Plasmid* **62**, 140–145.

Fire, A. (1986). Integrative transformation of *Caenorhabditis elegans*. *EMBO J.* **5**, 2673–2680.

Fire, A., *et al*. (1991). Production of antisense RNA leads to effective and specific inhibition of gene expression in *C. elegans* muscle. *Development* **113**, 503–514.

Fire, A., *et al*. (2006). Unusual DNA structures associated with germline genetic activity in *Caenorhabditis elegans*. *Genetics* **173**, 1259–1273.

Fire, A., *et al*. (1990). A modular set of lacZ fusion vectors for studying gene expression in *Caenorhabditis elegans*. *Gene* **93**, 189–198.

Fire, A., and Waterston, R. H. (1989). Proper expression of myosin genes in transgenic nematodes. *EMBO J.* **8**, 3419–3428.

Fire, A., *et al*. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811.

Frokjaer-Jensen, C., *et al*. (2008). Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat. Genet.* **40**, 1375–1383.

Fukushige, T., *et al*. (1998). The GATA-factor elt-2 is essential for formation of the *Caenorhabditis elegans* intestine. *Dev. Biol.* **198**, 286–302.

Giordano-Santini, R., *et al*. (2010). An antibiotic selection marker for nematode transgenesis. *Nat. Methods* **7**, 721–723.

Gonzalez-Serricchio, A., and Sternberg, P. (2006). Visualization of *C. elegans* transgenic arrays by GFP. *BMC Genet.* **7**, 36.

Granato, M., *et al*. (1994). *pha-1*, a selectable marker for gene-transfer in *C. elegans*. *Nucl. Acids Res.* **22**, 1762–1763.

Green, R., *et al*. (2008). Expression and imaging of fluorescent proteins in the *C. elegans* gonad and early embryo. *Methods Cell Biol.* **85**, 179–218.

Hayes, G. D., *et al*. (2006). The mir-84 and let-7 paralogous microRNA genes of *Caenorhabditis elegans* direct the cessation of molting via the conserved nuclear hormone receptors NHR-23 and NHR-25. *Development* **133**, 4631–4641.

Hedgecock, E. M., and Herman, R. K. (1995). The ncl-1 gene and genetic mosaics of *Caenorhabditis elegans*. *Genetics* **141**, 989–1006.

Hope, I. (1999). *C. elegans: A Practical Approach.* Oxford University Press, Oxford, U.K.

Hope, I., *et al*. (2004). Feasibility of genome-scale construction of promoter::reporter gene fusions for expression in *Caenorhabditis elegans* using a multisite gateway recombination system. *Genome Res.* **14**, 2070–2075.

Hunt-Newbury, R., *et al*. (2007). High-throughput *in vivo* analysis of gene expression in *Caenorhabditis elegans*. *PLoS Biol.* **5**, e237.

Hunter, C., and Kenyon, C. (1996). Spatial and temporal controls target pal-1 blastomere-specification activity to a single blastomere lineage in *C. elegans* embryos. *Cell* **87**, 217–226.

Jackstadt, P., *et al*. (1999). Transformation of nematodes via ballistic DNA transfer. *Mol. Biochem. Parasitol.* **103**, 261–266.

Johnson, N., *et al*. (2005). Heritable and inducible gene knockdown in *C. elegans* using Wormgate and the ORFeome. *Gene* **359**, 26–34.

Kadandale, P., *et al*. (2009). Germline transformation of *Caenorhabditis elegans* by injection. *Methods Mol. Biol.* **518**, 123–133.

Kelly, W. G., *et al*. (1997). Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. *Genetics* **146**, 227–238.

Kim, J. S., *et al*. (2010). Genome editing with modularly assembled zinc-finger nucleases. *Nat. Methods* **7**, 91 author reply 91-2.

Klass, M., *et al*. (1976). Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. *Dev. Biol.* **52**, 1–18.

Kohara, Y. (2001). [Systematic analysis of gene expression of the *C. elegans* genome]. *Tanpakushitsu Kakusan Koso* **46**, 2425–2431.

Koppen, M., *et al*. (2001). Cooperative regulation of AJM-1 controls junctional integrity in *Caenorhabditis elegans* epithelia. *Nat. Cell Biol.* **3**, 983–991.

Kramer, J. M., *et al*. (1990). The *Caenorhabditis elegans* rol-6 gene, which interacts with the sqt-1 collage gene to determine organismal morphology, encodes a collagen. *Molec. Cell Biol.* **10**, 2081–2089.

Kuntz, S., *et al*. (2008). Multigenome DNA sequence conservation identifies Hox *cis*-regulatory elements. *Genome Res.* **18**, 1955–1968.

Lackner, M. R., *et al*. (1994). A MAP kinase homolog, mpk-1, is involved in ras-mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev.* **8**, 160–173.

Lall, S., *et al*. (2006). A genome-wide map of conserved microRNA targets in *C. elegans*. *Curr. Biol.* **16**, 460–471.

MacMorris, M., *et al*. (1994). Analysis of the VPE sequences in the *Caenorhabditis elegansvit-2* promotor with extrachromosomal tandem array-containing transgenic strains. *Mol. Cell Biol.* **14**, 484–491.

Maduro, M., and Pilgrim, D. (1995). Identification and cloning of unc-119, a gene expressed in the *Caenorhabditis elegans* nervous system. *Genetics* **141**, 977–988.

Maduro, M., and Pilgrim, D. (1996). Conservation of function and expression of unc-119 from two *Caenorhabditis* species despite divergence of non-coding DNA. *Gene* **183**, 77–85.

Maduro, M. F., *et al*. (2002). Dynamics of a developmental switch: Recursive intracellular and intranuclear redistribution of *Caenorhabditis elegans* POP-1 parallels Wnt-inhibited transcriptional repression. *Dev. Biol.* **248**, 128–142.

Meister, P., *et al*. (2010). The spatial dynamics of tissue-specific promoters during *C. elegans* development. *Genes Dev.* **24**, 766–782.

Mello, C., and Fire, A. (1995). DNA transformation. *Methods Cell Biol.* **48**, 451–482.

Mello, C. C., *et al*. (1991). Efficient gene transfer in *C. elegans*: Extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959–3970.

Miller, D. M., *et al*. (1999). Two-color GFP expression system for *C. elegans*. *Biotechniques* **26**, 914–918 920-1.

Miller, L. M., *et al*. (1996). Mosaic analysis using a ncl-1 (+) extrachromosomal array reveals that lin-31 acts in the Pn.p cells during *Caenorhabditis elegans* vulval development. *Genetics* **143**, 1181–1191.

Morton, J., *et al*. (2006). Induction and repair of zinc-finger nuclease-targeted double-strand breaks in *Caenorhabditis elegans* somatic cells. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 16370–16375.

Mounsey, A., *et al*. (1999). Gene expression patterns. *In* "*C. elegans*: A Practical Approach," (and I. A. Hope, ed.), pp. 181–199. Oxford University Press, Oxford.

Murray, J. I., *et al*. (2008). Automated analysis of embryonic gene expression with cellular resolution in *C. elegans*. *Nat. Methods* **5**, 703–709.

Nagel, G., *et al*. (2003). Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 13940–13945.

Okkema, P., *et al*. (1993). Sequence requirements for myosin gene expression and regulation in *Caenorhabditis elegans*. *Genetics* **135**, 385–404.

Plasterk, R. H., and Groenen, J. T. (1992). Targeted alterations of the *Caenorhabditis elegans* genome by transgene instructed DNA double strand break repair following Tc1 excision. *EMBO J.* **11**, 287–290.

Portereiko, M. F., and Mango, S. E. (2001). Early morphogenesis of the *Caenorhabditis elegans* pharynx. *Dev. Biol.* **233**, 482–494.

Praitis, V. (2006). Creation of transgenic lines using microparticle bombardment methods. *Methods Mol. Biol.* **351**, 93–107.

Praitis, V., *et al*. (2001). Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. *Genetics* **157**, 1217–1226.

Prasher, D. C., *et al*. (1992). Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* **111**, 229–233.

Qadota, H., *et al*. (2007). Establishment of a tissue-specific RNAi system in *C. elegans*. *Gene* **400**, 166–173.

Reece-Hoyes, J., *et al*. (2005). A compendium of *Caenorhabditis elegans* regulatory transcription factors: A resource for mapping transcription regulatory networks. *Genome Biol.* **6**, R110.

Rieckher, M., *et al*. (2009). Transgenesis in *Caenorhabditis elegans*. *Methods Mol. Biol.* **561**, 21–39.

Ringrose, L. (2009). Transgenesis in *Drosophila melanogaster*. *Methods Mol. Biol.* **561**, 3–19.

Robert, V., *et al*. (2009). Mos1 transposition as a tool to engineer the *Caenorhabditis elegans* genome by homologous recombination. *Methods* **49**, 263–269.

Sanford, J. (1989). Biolistic plant transformation. *Physiologia Plantarum* **79**, 206–209.

Schlager, B., *et al*. (2009). Molecular cloning of a dominant roller mutant and establishment of DNA-mediated transformation in the nematode *Pristionchus pacificus*. *Genesis* **47**, 300–304.

Semple, J. I., *et al*. (2010). Rapid selection of transgenic *C. elegans* using antibiotic resistance. *Nat. Methods* **7**, 725–727.

Shaner, N. C., *et al*. (2004). Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat. Biotech.* **22**, 1567–1572.

Stein, L. D., *et al*. (2003). The genome sequence of *Caenorhabditis briggsae*: A Platform for Comparative Genomics. *PLoS Biol.* **1**, E45.

Stinchcomb, D. T., *et al*. (1985). Extrachromosomal DNA Transformation of *Caenorhabditis elegans*. *Molec. Cell Biol.* **5**, 3484–3496.

Stirman, JN., *et al*. (2011). Real-time multimodal optical control of neurons and muscles in freely behaving *Caenorhabditis elegans*. *Nat. Methods* **8**, 153–158.

Stringham, E. G., *et al*. (1992). Temporal and spatial expression patterns of the small heat shock (hsp16) genes in transgenic *Caenorhabditis elegans*. *Mol. Biol. Cell* **3**, 221–233.

Strome, S., *et al*. (2001). Spindle dynamics and the role of gamma-tubulin in early *Caenorhabditis elegans* embryos. *Mol. Biol. Cell* **12**, 1751–1764.

Sulston, J. E., *et al*. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64–119.

Tavernarakis, N., *et al*. (2000). Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. *Nat. Genet.* **24**, 180–183.

Tursun, B., *et al*. (2009). A toolkit and robust pipeline for the generation of fosmid-based reporter genes in *C. elegans*. *PLoS One* **4**, e4625.

Vazquez-Manrique, R., *et al*. (2010). Improved gene targeting in *C. elegans* using counter-selection and Flp-mediated marker excision. *Genomics* **95**, 37–46.

Voutev, R., and Hubbard, E. (2008). A "FLP-Out" system for controlled gene expression in *Caenorhabditis elegans*. *Genetics* **180**, 103–119.

Wightman, B., *et al*. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* **75**, 855–862.

Williams, D., *et al*. (2005). Characterization of Mos1-mediated mutagenesis in *Caenorhabditis elegans*: A method for the rapid identification of mutated genes. *Genetics* **169**, 1779–1785.

Wilm, T., *et al*. (1999). Ballistic transformation of *Caenorhabditis elegans*. *Gene* **229**, 31–35.

Yochem, J., *et al*. (1998). A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. *Genetics* **149**, 1323–1334.

Yochem, J., and Herman, R. K. (2005). Genetic Mosaics. *In* (T. C. e. R. Community, ed.). WormBook, Vol. doi/10.1895/wormbook.1.58.1.

Zhang, Y., *et al*. (2008). A simplified, robust, and streamlined procedure for the production of *C. elegans* transgenes via recombineering. *BMC Dev. Biol.* **8**, 119.

Zhao, Z., *et al*. (2010). New tools for investigating the comparative biology of *Caenorhabditis briggsae* and *Caenorhabditis elegans*. *Genetics* 853–863.

Ziemienowicz, A. (2010). Plant transgenesis. *Methods Mol. Biol.* **631**, 253–268.

Zorio, D. A., *et al*. (1994). Operons as a common form of chromosomal organization in *C. elegans*. *Nature* **372**, 270–272.