Workshop review: Engineered nucleases for genome editing in nematodes

John Calarco[1], Hui Chiu[2], Daniel J. Dickinson[3], Ari E. Friedland[4], Junho Lee[5], Te-Wen Lo[6], Hillel Schwartz[2], Yonatan Tzur[4] and Jordan D. Ward[7]

[1]FAS Center for Systems Biology, Harvard University, Cambridge MA, [2]Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena CA, [3]Department of Biology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill NC, [4]Department of Genetics, Harvard Medical School, Boston MA, [5]Department of Biophysics and Chemical Biology, Seoul National University, Seoul 151-742, Korea, [6]Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California, Berkeley CA, [7]Department of Cellular and Molecular Pharmacology, University of California, San Francisco CA

Correspondence to: Hillel Schwartz (hillels@caltech.edu), Jordan D. Ward (jordan.ward@ucsf.edu)

Introduction

The recent development of genome modification technologies such as TAL effector nucleases (TALENs) and the CRISPR-Cas9 system has allowed unprecedented modification of eukaryotic genomes. TALENs are a sequence-specific nuclease comprised of TALE DNA binding domains fused to the FokI nuclease. CRISPR-Cas9 consists of the Cas9 nuclease plus two small RNAs: one that base-pairs with a 19 bp target (crRNA) and another that activates Cas9 (trcRNA). This system can be simplified by making a synthetic guide RNA (sgRNA), a hybrid of the two small RNAs. Both systems are used to make DNA double-strand breaks at desired genomic locations. At the recent International *C. elegans* meeting, we presented in a workshop about harnessing these technologies to edit the nematode genome. We have summarized the workshop and describe the reagents and protocols we anticipate distributing to the community. The presentations covered a broad range of successful approaches:

DNA-based (CRISPRs)

A.E.F. (Church lab), J.C., and Y.T. (Colaiàcovo lab) presented their system for targeted mutagenesis, which involved injecting a cocktail of three vectors: a worm codon optimized Cas9 driven by the *eft-3* promoter, an sgRNA driven by a *U6* promoter, and an mCherry marker driven by the *myo-3* promoter (Friedland et al., 2013). Targeting four different genes with this system, they recovered mutant progeny with random inserts and deletions at the expected loci. Progeny of these F_1 animals were screened and also carried these mutant alleles, indicating that the targeted disruptions are heritable. Reagents are available on Addgene at http://www.addgene.org/crispr/calarco/.

By co-injecting an engineered homologous recombination template and a single Cas9+sgRNA expression plasmid, D.J.D. (Goldstein lab) and J.D.W. (Yamamoto lab) inserted *gfp* into endogenous genes, resulting in GFP "knock-in" fusion proteins expressed under the control of all native regulatory elements. They also made multiple targeted point mutations in endogenous genes. The *unc-119(+)* marker used to select for recombinants can be excised with Cre recombinase, allowing complicated genome edits to be made with minimal "scarring." Knock-in strains took less than 1 month to produce (about 2 days total hands-on time) and cost about $200 (mainly the cost of PCR primers). Plasmids will be distributed via Addgene after acceptance of the manuscript.

Protein-based (CRISPRs)

J.L. reported work from Jihyun Lee (his lab) and S.W. Cho (J.S. Kim lab); they generated gene-

specific heritable mutations by germline injection of Cas9 protein complexed with sgRNA. X-linked genes *dpy-3* and *unc-1* were selected for targeting to facilitate identifying mutations through their visible phenotypes in homozygotes and hemizygotes. Indels at target sites were successfully confirmed in F_1 animals by T7E1 assay and sequencing in both cases. Surprisingly, visible F_1 mutants were often observed, and one Dpy mutant turned out to be a *trans*-heterozygote of two independent mutations in *dpy-3*, demonstrating the high efficiency of the method.

RNA-based (CRISPRs and TALENs)

H.C. and H.S. (Sternberg lab) injected *in vitro*-synthesized RNAs into the *C. elegans* germline: a capped and polyadenylated mRNA for humanized Cas9 and an sgRNA. F_2 progeny were inspected for phenotypic homozygous mutants. Mutants were recovered at varying frequencies, up to one allele for every five P_0s. A majority of mutations were large deletions (>1 kbp). Analysis of high-throughput sequencing of two closely related but independent *dpy-11* mutants did not identify off-target changes to the genome, suggesting CRISPR mutagenesis was highly specific for targeted gene disruption.

T.W.L. (Meyer lab) reported on highly effective strategies using TALENs and CRISPR-Cas9 nucleases to create heritable, precise insertion, deletion, or substitution mutations at specific DNA sequences at targeted endogenous loci. This was achieved by germline injection of nuclease mRNAs and single-strand DNA templates. They created nucleotide changes both close to and far from double-strand breaks to gain and lose genetic function, to tag proteins made from an endogenous gene, and to excise entire loci through targeted FLP-FRT recombination. These methodologies were effective across nematode species divergent by 300 million years: hermaphroditic and gonochoristic species within *Caenorhabditis* (*elegans* and species 9) and *P. pacificus*. Thus, genome-editing tools now exist to transform non-model nematode species into genetically tractable model organisms.

Perspectives

The adoption of these modification technologies promises to transform nematode genetics. Going forward, the rules of CRISPR targeting must be better elucidated, the kinetics of insertion/deletion and homologous recombination events can be optimized, and high-throughput screening strategies must be developed. The workshop highlighted the diversity of techniques successfully developed for nematode genome modification, with the best technique depending on the desired experimental outcome.

Reference

Friedland AE, Tzur YB, Esvelt KM, Colaiácovo MP, Church GM, and Calarco JA. (2013). Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. Nat. Methods 10, 741-743.