Editorial

Genome Editing of a CArG Element in the Mouse Genome Establishes its Role in Gene Expression

Kiran Musunuru

In the February issue, Han et al present the use of a novel clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated 9 (Cas9) method to inactivate the calponin-1 gene involved in smooth muscle contraction, by introducing a knockin mutation to cleanly disrupt a CC(A/T)6GG (CArG) element in the first intron of the gene. Previous work by the group had suggested a critical role of the CArG element in calponin-1 gene expression in both humans and mice. Here, the authors injected the components of CRISPR-Cas9—an mRNA for the Cas9 endonuclease, a guide RNA containing the 20-nucleotide sequence matching the sequence of the CArG element, and a 135-nucleotide single-strand DNA oligonucleotide overlapping the CArG element and containing the desired knockin mutation—into the cytoplasm of fertilized mouse eggs (Figure). This resulted in stable incorporation of the CArG knockin mutation into the genome in some of the eggs. After implantation of the resulting blastocysts into surrogate mothers, the injected eggs yielded founder mice in 3 weeks. Remarkably, 3 of 18 founder mice (17%) carried the desired knockin mutation on ≥1 chromosome, with 1 founder having the mutation on both chromosomes. The founder mice were successfully bred, with germline transmission of the knockin mutation. With calponin-1 being a smooth muscle cell–restricted gene, the authors examined smooth muscle tissues from wild-type, heterozygous, and homozygous mice and found almost complete suppression of calponin-1 gene expression in the homozygous mice. This simple yet elegant experiment, performed within a time frame of a few months, unequivocally establishes the critical role of the CArG element in calponin-1 gene expression in smooth muscle tissues.

See accompanying article on page 312 in the February 2015 issue

To fully appreciate the game-changing nature of CRISPR-Cas9, consider a similar study performed in the pregenome-editing era. Khromov et al engineered a knockin mouse telokin locus. Fourth, embryonic stem cells were injected into blastocysts and implanted into surrogate mothers to yield chimeric mice. Fifth, the chimeric mice were bred to obtain mice that had inherited the mutant allele through the germline. Sixth, as part of the breeding, male mice expressing Cre recombinase in the germline were used to remove the antibiotic resistance cassette. Finally, the floxed alleles were bred to homozygosity to yield the final mice for study. A conservative estimate of the amount of time required to carry out all these steps is ≥1 year and probably ≥2 years; yet, despite all this effort, the end result was a mutant allele in which the 30-nucleotide CARG-bearing fragment was replaced with a 34-nucleotide loxP sequence, effective but crude. In contrast, Han et al were able to carry out their study in just a few months while creating a more subtle mutant allele in which several nucleotides were substituted to impair the CArG element, with no need for antibiotic resistance, the Cre-loxP system, and so on.

The tremendous use of CRISPR-Cas9 in generating knockout and knockin mice was initially demonstrated by Rudolf Jaenisch’s group and has since been validated with its ability to correct pathogenic mutations in mouse embryos and to generate knockout and knockin mutations in a wide variety of model organisms, most impressively in monkeys. The work by Han et al represents the first case in which CRISPR-Cas9 has been used to manipulate smooth muscle gene expression by editing a key noncoding regulatory element and can easily be extended to rapidly and efficiently interrogate the function of any number of noncoding regulatory elements throughout the mouse genome. This successful demonstration of the method should have a long-time impact not only in the field of smooth muscle biology but also in many other fields of biomedical research.

Disclosures

None.

References


**KEY WORDS:** gene expression ■ gene knockout ■ smooth muscle ■ transgenic mice
Genome Editing of a CArG Element in the Mouse Genome Establishes its Role in Gene Expression
Kiran Musunuru

Arterioscler Thromb Vasc Biol. 2015;35:496-497
doi: 10.1161/ATVBAHA.115.305175

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/35/3/496

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/