PROFILE

Profile of Dana Carroll

Jennifer Viegas, *Science Writer*

University of Utah biochemist Dana Carroll was among the first scientists to develop reagents for genome editing. These tools can make site-specific double-strand DNA breaks to stimulate desired recombination and repair. The technology that Carroll spearheaded, zinc-finger nucleases (ZFNs), laid the groundwork for other genome-editing platforms, such as transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR-Cas9). In his Inaugural Article (1), Carroll, who was elected to the National Academy of Sciences in 2017, explores a limitation of CRISPR-Cas9. The results could inform DNA target selection for genome editing in agricultural, medical, and other research applications.

Generations of Scientists

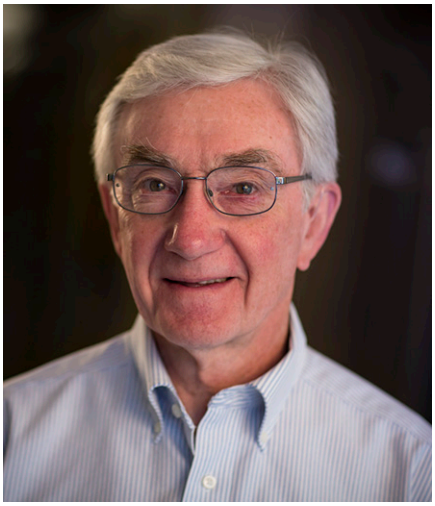
Carroll was born into a family of scientists. His grandfather, William Ernest Carroll, was a student of animal nutrition at the University of Illinois and contributed to the then-emerging field of animal science. A member of the Utah Agricultural College, W. E. Carroll conducted research on animal husbandry. Several of his influential papers and books on the subject, such as *Swine Production* (2), remain in circulation.

Carroll's father, William Robert Carroll, conducted research on protein chemistry at the National Institutes of Health for more than two decades. "Many of our family friends were other NIH scientists and their families, but there was little discussion in our house or in social gatherings about science per se," Carroll says. "I only really learned about my father's research interests and those of his colleagues when I was in graduate school. If my upbringing had an influence on my career choices, it was indirect."

From Transcriptional Regulation to Genetic Recombination

As a youth, Carroll was interested in mathematics and science. Undaunted by what he describes as a "crushingly boring" high school chemistry class, Carroll chose to major in chemistry at Swarthmore College in Pennsylvania, where he earned a bachelor's degree in 1965. Later, physical chemistry became the focus of his doctoral work at the University of California, Berkeley. His thesis advisor, Ignacio Tinoco, Jr., gave him a sense of independence and an appreciation for rigorous research as they analyzed nucleic acid structure.

When Carroll earned his doctorate in 1970, he envisioned becoming a molecular or cell biologist. His first postdoctoral fellowship, with a focus on transcriptional regulation, was with John Paul at the Beatson Institute for Cancer Research in Glasgow, Scotland. During a second postdoctoral stint at the Carnegie Institution of Washington's embryology department in Baltimore, Maryland, Carroll characterized the 5S ribosomal DNA in the model organism *Xenopus laevis* (3, 4) with Donald Brown, who purified these repeated genes. "Don Brown worked at the bench, had a laser-like focus on the projects at hand, and displayed a nice balance of strong support and high expectations," Carroll says. Another early role model was cell biologist Joseph Gall. Both Gall and Brown sparked Carroll's interest in genetic recombination.



Dana Carroll. Image courtesy of University of Utah Health.

Mechanism of Homologous Recombination

In 1975 Carroll accepted the position of assistant professor in the University of Utah's Department of Microbiology. In 1995 he moved to the Department of Biochemistry as co-chair, then served as solo chair from 1998 to 2009. He was named a distinguished professor in 2015. When Carroll established his laboratory at the university, he continued to study *X. laevis*, which led to questions about the fate of DNA injected into the organism's oocytes.

An answer came to light in 1991, when Carroll and a colleague found evidence confirming a nonconservative mechanism of homologous recombination that depends on double-strand breaks in the DNA (5). The dependence of recombination on DNA breaks started Carroll thinking about ways to introduce targeted breaks at specific chromosomal targets.

This is a Profile of a member of the National Academy of Sciences to accompany the member's Inaugural Article on page 9351.
Published online September 10, 2018.

Development of ZFN Technology

Five years later, biochemist Srinivasan Chandrasegaran and his colleagues at the Johns Hopkins University reported the engineering of ZFNs based on the modular structure of the enzyme FokI (6). The researchers demonstrated that ZFNs can cleave DNA in a sequence-specific manner. Carroll contacted Chandrasegaran and suggested a collaboration. They subsequently coauthored studies that defined the requirements for specific DNA target cleavage using ZFNs (7) and demonstrated that these enzymes can find and cleave targets in a eukaryotic cell, thereby stimulating homologous recombination (8).

Because ZFNs not only cut DNA but also possess a separate DNA-binding domain that imparts sequence specificity, Carroll reasoned that they could be engineered to achieve particular genetic outcomes. His proof-of-principle experiment involved using ZFNs to generate a targeted color mutation—brown to yellow—in a whole organism, the fruit fly *Drosophila melanogaster* (9). "In this species, the phenotype reflects the genotype so directly," Carroll says.

He recalls the moment when, looking through a microscope, Carroll and his team first saw the desired patches of yellow on the insect's cuticles and bristles, signifying the experiment's success: "Coauthor Kent Golic rarely shows emotion, but when he observed the yellow patches, his eyes widened, as he said, 'If I were you, I'd be pretty excited.'" Carroll and his colleagues later successfully applied ZFN-mediated mutagenesis to the plant *Arabidopsis thaliana* (10) and the nematode *Caenorhabditis elegans* (11).

Homologous Genome Editing

Using ZFNs again in *Drosophila*, Carroll and his team generated DNA cleavage to stimulate gene targeting when a homologous donor DNA was provided (12). Homologous genome editing with programmable nucleases in a whole organism marked a major milestone in genome editing. Before long, Carroll and his team demonstrated high-frequency germline gene targeting in *Drosophila* (13). He says, "We simplified the delivery of ZFNs by direct embryo injection of mRNAs, and we reported that the recovery of homologous repair products was enhanced by inactivation of [the enzyme] DNA ligase IV."

The injection procedure proved to be more rapid than earlier approaches and made possible the generation and recovery of targeted DNA alterations at any genome locus within two generations of the organism. For these contributions, Carroll received the Novitski Prize from the Genetics Society of America (2012) and the Herbert Sober Lectureship from the American Society of Biochemistry and Molecular Biology (2014).

Putting TALENs to the Test

Carroll remains interested in the cellular processes on which genome editing depends. He investigated homology requirements and DNA conversion tracts in *Drosophila* (14). The findings have enabled better experimental designs for gene targeting in this and other organisms. They were also applied to a comparison of ZFN technology with the TALEN genome-editing platform, which consists of a second-generation class of gene-targeting enzymes (15).

TALENs are based on proteins known as effectors that are expressed by plant bacterial pathogens. They include transcription activator-like effector DNA-binding domains. While ZFNs recognize any specific sequence of nucleotide triplets, each TALEN domain recognizes a single nucleotide using a simple one-module-to-one-base pair recognition code. After comparing their utility with that of ZFNs in manipulating targeted *Drosophila* genes, Carroll and his team concluded that TALENs were easy to design for new targets and induced desired mutations at a high rate.

Evidence for a CRISPR-Cas9 Limitation

During a sabbatical from 2014 to 2015, Carroll focused on CRISPR-Cas9 research at the University of California, Berkeley Innovative Genomics Institute. Unlike ZFNs and TALENs, this technology relies on Watson–Crick base pairing and not on protein-DNA recognition. A guide RNA (gRNA) directs a Cas endonuclease enzyme to cleave the target sequence. The gRNAs can be designed quickly at low cost, explaining why the CRISPR-Cas9 editing reagent is now more popular than ZFNs and TALENs.

In his Inaugural Article (1), Carroll investigated why the efficiency of CRISPR-Cas9 genome editing varies widely at different targets and in different cells. Observing DNA cleavage of specific, unique targets in real time, Carroll and his team determined that chromatin structure influences the technology's effectiveness. The presence of nucleosomes, in particular, strongly inhibits cleavage by CRISPR-Cas9. No such inhibition is observed for ZFNs, which sever nucleotide linkages equally well at nucleosome-occupied and nucleosome-depleted targets.

The findings have implications for the choice of genome-editing targets and platforms in various applications. Carroll says, "There are situations where researchers are working with cells that are not dividing or that otherwise have more static nucleosome structures. There may also be strict requirements on where the cut must be made."

In Vivo Genome Editing

Genome-editing research is elucidating the basic biology underlying many diseases, thereby identifying possible therapeutic targets. Genetic diseases of blood cells, such as sickle cell disease, are prime candidates for such research. Together with researchers from the Innovative Genomics Institute, Carroll used CRISPR-Cas9 to modify hematopoietic stem and progenitor cells (16). Ongoing research is directed at achieving levels of gene correction to confer potential clinical benefits.

The biotechnology company Sangamo Therapeutics, Inc., based in Richmond, California, licensed Carroll's ZFN gene-editing platform in 2004. Related preclinical trials for treatments for two forms of

hemophilia and two lysosomal storage diseases, including Hunter syndrome (MPS II), are now underway. In 2017 Sangamo Therapeutics announced its foray into in vivo human genome editing for MPSII. The male patient, deficient in a necessary enzyme, received a corrective gene via viral delivery along with ZFNs to his liver cells. The hope is that the patient's liver will produce a stable supply of the enzyme over time. Sangamo Therapeutics is planning to conduct similar trials with different dosage levels administered at multiple medical research sites.

Advocate for Ethical Research

Over the years, Carroll has engaged in international discussion of ethical issues surrounding the medical and agricultural applications of genome editing. "Who will decide what products or treatments are developed, and who will decide who gets them?" Carroll wrote in a *Yale Journal of Biology and Medicine* article published in 2017 (17).

The genetic engineering revolution that Carroll helped launch is now moving quickly, raising valid concerns over safety and the current reality that market forces, rather than humanitarian considerations, often drive key decisions. "Are we comfortable with this or do we need governmental participation at the national and international levels to change the situation?" Carroll asks. "Count me as an advocate for the latter."

1 Yarrington RM, Verma S, Schwartz S, Trautman JK, Carroll D (2018) Nucleosomes inhibit target cleavage by CRISPR-Cas9 in vivo. *Proc Natl Acad Sci USA* 115:9351–9358.
2 Carroll WE (1950) *Swine Production* (McGraw Hill, New York).
3 Carroll D, Brown DD (1976) Repeating units of *Xenopus laevis* oocyte-type 5S DNA are heterogeneous in length. *Cell* 7:467–475.
4 Carroll D, Brown DD (1976) Adjacent repeating units of *Xenopus laevis* 5S DNA can be heterogeneous in length. *Cell* 7:477–486.
5 Maryon E, Carroll D (1991) Characterization of recombination intermediates from DNA injected into *Xenopus laevis* oocytes: Evidence for a nonconservative mechanism of homologous recombination. *Mol Cell Biol* 11:3278–3287.
6 Kim YG, Cha J, Chandrasegaran S (1996) Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain. *Proc Natl Acad Sci USA* 93:1156–1160.
7 Smith J, et al. (2000) Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains. *Nucleic Acids Res* 28:3361–3369.
8 Bibikova M, et al. (2001) Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. *Mol Cell Biol* 21:289–297.
9 Bibikova M, Golic M, Golic KG, Carroll D (2002) Targeted chromosomal cleavage and mutagenesis in *Drosophila* using zinc-finger nucleases. *Genetics* 161:1169–1175.
10 Lloyd A, Plaisier CL, Carroll D, Drews GN (2005) Targeted mutagenesis using zinc-finger nucleases in *Arabidopsis*. *Proc Natl Acad Sci USA* 102:2232–2237.
11 Morton J, Davis MW, Jorgensen EM, Carroll D (2006) Induction and repair of zinc-finger nuclease-targeted double-strand breaks in *Caenorhabditis elegans* somatic cells. *Proc Natl Acad Sci USA* 103:16370–16375.
12 Bibikova M, Beumer K, Trautman JK, Carroll D (2003) Enhancing gene targeting with designed zinc finger nucleases. *Science* 300:764.
13 Beumer KJ, et al. (2008) Efficient gene targeting in *Drosophila* by direct embryo injection with zinc-finger nucleases. *Proc Natl Acad Sci USA* 105:19821–19826.
14 Beumer KJ, Trautman JK, Mukherjee K, Carroll D (2013) Donor DNA utilization during gene targeting with zinc-finger nucleases. *G3 (Bethesda)* 3:657–664.
15 Beumer KJ, et al. (2013) Comparing zinc finger nucleases and transcription activator-like effector nucleases for gene targeting in *Drosophila*. *G3 (Bethesda)* 3:1717–1725.
16 DeWitt MA, et al. (2016) Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. *Sci Transl Med* 8:360ra134.
17 Carroll D (2017) Genome editing: Past, present, and future. *Yale J Biol Med* 90:653–659.