The 2012 Novitski Prize
Dana Carroll

THE Edward Novitski Prize is named in honor of *Drosophila* geneticist Edward Novitski (1928–2006) and is awarded to recognize the "creativity and intellectual ingenuity" that goes into solving a difficult genetic problem. This year's Novitski Prize recognizes Dana Carroll for his insight into the potential use of zinc-finger nucleases (ZFNs) for genome modification and for his work demonstrating the broad applicability of this technique. ZFNs can be used to create targeted mutations in a broad range of organisms, allowing efficient "reverse" genetics in those organisms whose genomes are otherwise very difficult to manipulate. The basic technology can be used to correct defective genes as well, opening the door for the potential use of ZFNs in gene therapy.

Dana's career path has not been that of a typical Novitski Prize winner. He was formally trained as a chemist rather than as a geneticist. He majored in chemistry as an undergraduate at Swarthmore College and subsequently received a Ph.D. in biophysical chemistry at the University of California at Berkeley, where he worked with Ignacio Tinoco, Jr., on nucleic acid structure. Dana's first postdoctoral experience was with John Paul at the Beatson Institute for Cancer Research in Scotland, where he was introduced to the more the biological side of science. He then moved to the Carnegie Institution of Washington in Baltimore for further postdoctoral work in the lab of Don Brown, and it was there that Dana initiated studies on 5S ribosomal DNA in *Xenopus laevis*. Although these studies continued when Dana set up his own lab at the University of Utah in 1975, his focus shifted to the fate of DNA injected into *Xenopus* oocytes in the mid-1980s. He subsequently made important contributions to the recombination field, elucidating basic mechanisms of homogolous and nonhomologous/illegitimate recombination using the *Xenopus* system (Carroll 1998). Minus one sabbatical, Dana has remained at Utah his entire career, which has included a 24-year stint as chair of the Department of Biochemistry.

doi: 10.1534/genetics.112.138925

With the development of recombinant DNA technologies and methods to introduce DNA into cells in the 1970s, it became feasible to modify the genetic material of model microorganisms. These developments were, in large part, responsible for the rapid emergence of *Saccharomyces cerevisiae* as the premier eukaryotic genetic system. What set yeast apart from other experimental systems was the robust nature of homologous relative to nonhomologous recombination, which allowed efficient targeting of plasmid DNA to the cognate genome location. This is in stark contrast to most other eukaryotes, where nonhomologous recombination events predominate over homology-based events, and exogenous DNA integrates randomly and unpredictably. It was subsequently discovered that linearizing the exogenous DNA dramatically increased the gene-targeting efficiency in yeast and hence that double-strand breaks (DSBs) were efficient initiators of recombination (Orr-Weaver *et al.* 1981). Finally, the use of mega-endonucleases with large recognition sites (primarily the HO and I-SceI nucleases) was pioneered in yeast as a method to target a DSB to a single genomic position in a large population of cells (Haber 1995). In the presence of a homologous repair template, the DSB was accurately repaired by copying sequence from the donor molecule.

The major roadblock to gene targeting in higher eukaryotes has been devising ways to identify and/or enrich the desired homologous recombination event among a sea of nonhomologous events. Although the meganuclease approach for creating a recombination-initiating DSB works in mammalian cells, one first has to engineer a recognition site for the enzyme into the desired target sequence—not a trivial task. The basic intellectual framework for targeted genome manipulation was in place, however, and the challenge was to generalize the technique for introducing the required DSB. Dana had the key insight that solved what seemed an insurmountable problem: Instead of modifying the target so that it can be recognized by the meganuclease, why not take the reverse approach and modify the enzyme so that it recognizes the desired target? Dana was aware that such "designer" enzymes—ZFNs—were being developed by Chandrasegaran and colleagues (Kim *et al.* 1996) and so pursued their use as potential gene-targeting agents.

ZFNs are based on the modular structure of the restriction enzyme *Fok*I, which has a nuclease domain that cuts DNA and a separate DNA-binding domain that imparts sequence specificity. Because of the modular structure, enzymes with novel sequence specificity can be created by fusion of the nuclease domain to a heterologous DNA-binding domain. Significantly, Chandrasegaran had demonstrated that the DNA-binding zinc fingers of transcription factors could be used to generate ZFNs. By stringing together different zinc fingers, each of which recognizes a 3-bp sequence, Dana reasoned that it should be possible to design a custom ZFN that would cleave at a single preselected site within a complex genome. His hunch proved to be correct, with the proof-of-principle experiment being to make targeted genetic changes to the Drosophila *yellow* gene (Bibikova *et al.* 2002, 2003). The general technique has been pushed forward by Dana and has now been extended to over 15 different organisms and cell types, with an efficiency in the 10% range (Carroll 2011). Replacements can be achieved by providing donor DNA at the time at which the ZFN is induced; in the absence of a repair template, the break is joined in an error-prone manner to yield a gene knockout. Building on the success of ZFNs, a second-generation class of gene-targeting enzymes has emerged that uses DNA-binding modules based on transcription-activator-like (TAL) effectors of plant bacterial pathogens (Bogdanove and Voytas 2011). Although the TAL-effector nucleases, or TALENs, have technical advantage over the original ZFNs, ZFNs provided the intellectual framework for their development.

The Novitski Award acknowledges Dana for his insight into how designer enzymes can be created and effectively used to generate a single DSB within a complex genome. Dana has provided the genetics community with an invaluable tool that extends the genome modification techniques available in model microorganisms to virtually any organism.

Literature Cited

Bibikova, M., M. Golic, K. G. Golic, and D. Carroll, 2002 Targeted chromosomal cleavage and mutagenesis in Drosophila using zinc-finger nucleases. Genetics 161: 1169–1175.

Bibikova, M., K. Beumer, J. K. Trautman, and D. Carroll, 2003 Enhancing gene targeting with designed zinc finger nucleases. Science 300: 764.

Bogdanove, A. J., and D. F. Voytas, 2011 TAL effectors: customizable proteins for DNA targeting. Science 333: 1843–1846.

Carroll, D., 1998 Modes of DNA repair in Xenopus oocytes, eggs and extracts, pp. 597–616 in *DNA Damage and Repair: Biochemistry, Genetics and Cell Biology,* edited by Hoekstra, M. F., and J. A. Nickoloff. Humana Press, Clifton, NJ; Totowa, NY.

Carroll, D., 2011 Genome engineering with zinc-finger nucleases. Genetics 188: 773–782.

Haber, J. E., 1995 *In vivo* biochemistry: physical monitoring of recombination induced by site-specific endonucleases. Bioessays 17: 609–620.

Kim, Y. G., J. Cha, and S. Chandrasegaran, 1996 Hybrid restriction enzymes: zinc finger fusions to *Fok* I cleavage domain. Proc. Natl. Acad. Sci. USA 93: 1156–1160.

Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA 78: 6354–6358.

Sue Jinks-Robertson