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A revolution coming to a classic model organism

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Abstract

Classic gene targeting and gene replacement can now be achieved in zebrafish after cleaving the genome with engineered nucleases in the presence of donor DNA. This simple-to-implement method enables new classes of biological study in this important model organism.

Since George Streisinger introduced the zebrafish as a model organism that could yield special insights into the genetic and cellular basis of vertebrate biology¹, work with zebrafish has helped provide a unifying molecular model of tissue origins in the vertebrate embryo, brought profound insights to our understanding of the evolution of vertebrate genomes, provided a genetically tractable model for studying the basis of tissue and organ regeneration, generated new models for the study of human disease and provided a new platform for the discovery of pharmaceuticals. Nevertheless, this model system has more undeveloped potential to revolutionize the study of cell biological processes in vertebrates. If we could harness the methods of gene targeting used so imaginatively and productively in the mouse to alter zebrafish gene composition and manipulate gene expression, then we would have a dramatically new ability to study the behaviors of cells, the regulation of cell signaling events or the behaviors of proteins within cells in the native context of a developing animal. In an article in this issue of *Nature Methods*, Zu *et al.*² describe a major breakthrough in the realization of this potential.

The method introduced by Zu *et al.*² affords the potential to manipulate the genome of the zebrafish in all the ways currently available in the mouse. At minimum, it appears that any several-kilobase-pair segment of the zebrafish genome may be replaced with gene sequences of interest—a procedure termed ‘gene targeting’. The new method is thus an advance beyond the recent breakthrough that established an approach for generating very small sequence changes in the genome³. Certainly the new method will make it easy to rapidly create zebrafish models that recapitulate biochemical pathway defects that contribute to disease states. This alone will be an extraordinary practical advance for the discovery of new medicines, as the zebrafish embryo, which can be readily distributed and assayed in multiwell plates, has proven ripe for drug screens to identify compounds that augment or inhibit physiologically relevant signaling pathways⁴. Similarly, simple methods for gene targeting will lead to tests of the presumed regulatory roles of small sequence motifs thought to act, for instance, as enhancers or as targets of microRNAs.

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However, the most transformative applications are likely to be those that exploit the distinguishing features that were recognized by Streisinger as making the zebrafish so powerful for the study of vertebrate biology. First, the life cycle of the fish is well-suited for both forward and reverse genetic analyses of gene function. Second, it has a rapidly developing, nearly transparent embryo whose entire developmental progression from the fertilized egg onward can be assayed with single-cell resolution *in situ* as the changes occur. Third, the fecundity and ease of propagation in the laboratory mean thousands of near-synchronously developing embryos can be collected for biochemical and molecular genetic analyses.

As a result, gene targeting in the zebrafish will likely revolutionize the study of cell biological and gene regulation processes. The precise insertion of small protein-coding sequences will allow us to generate endogenous proteins fused to fluorescent or other peptide tags expressed at physiological levels and locations. The optical accessibility of the embryo will allow us to discover how developmental changes, morphogenesis or signaling events affect the dynamic intracellular behavior of such tagged proteins. Similar type of genome engineering will allow study of the activities of developing neural circuits in the intact embryo. Finally, the biochemical analysis of tagged endogenous proteins in the zebrafish will help push the study of gene regulation beyond its current realm, one that is often restricted to static populations of tissue culture cells examined under nonphysiological conditions. Investigations in zebrafish embryos should allow dynamic studies of transcription-factor binding and chromatin structure during cell differentiation. Understanding how the genome is regulated during normal or aberrant differentiation will provide insight into changes that contribute to cancer.

The essence of the method hinges on taking advantage of the host's endogenous response to induced chromosome breaks⁵. The integrity of chromosome structure is important to preserve: cells have extensive molecular machinery for detecting and repairing DNA breaks. In the absence of additional DNA, the nonhomologous end-joining pathway rejoins broken chromosome arms even at the expense of occasional alterations in the DNA sequence. The pathway can be used to induce mutations in specific genes of the zebrafish: RNA encoding an engineered nuclease that is directed to cleave a unique site in the genome is injected directly into a zebrafish egg, and the nuclease is synthesized as the embryo grows. In an environment of recurrent exposure to the nuclease, repair-mediated sequence changes accrue, and mutations can be induced in the gene targeted by the nuclease (Fig. 1).

In the work of Zu *et al.*², targeted breaks induced by a synthetic nuclease are repaired via a distinct mechanism using the homologous recombination pathway. In the presence of DNA molecules with homology to the site of the lesion, homologous recombination machinery will execute an exchange of DNA sequence information, incorporating into the host chromosome sequences that span the break. In this way new gene sequences can be permanently introduced into the zebrafish genome. Gene modifications, such as the repair of a zebrafish mutation, can be accomplished simply by the simultaneous injection of a synthetic nuclease targeting a mutant locus along with donor wild-type gene sequences. The experiments of Zu *et al.*² do not indicate yet how extensively the genome may be altered, but

they do indicate that it will not be difficult to replace an endogenous gene with sequences encoding a foreign protein, such as a fluorescent reporter.

The approach to gene targeting in the zebrafish is wholly different from that used in the mouse, where homologous donor DNA molecules are introduced into embryonic stem cells, and those that have experienced a very rare recombination event are selected in tissue culture, amplified and then introduced into host embryos where they may contribute to the germ line⁶. In the absence of established zebrafish stem cell lines, a very efficient method was needed to produce altered genomes in the context of a living embryo, which harbors relatively few cells that will ultimately contribute to the germ line.

Haber, Jasin and their colleagues initially demonstrated *in vitro* that induction of a targeted double-strand break in a host chromosome could stimulate homologous recombination at that locus by orders of magnitude^{7,8}. Carroll and colleagues tailored this insight to accomplish gene targeting in *Drosophila*, using a synthetic ‘zinc-finger nuclease’ (ZFN) comprising a DNA-binding domain composed of zinc-finger motifs fused to the nuclease domain of the FokI enzyme to initiate the targeted breaks⁹. However, ZFNs could not be easily programmed to target any sequence. The key breakthrough that set the stage for gene targeting in the zebrafish was the recent development of synthetic transcription activator–like effector nucleases, or TALENs, that can induce a DNA break at virtually any desired spot in the genome^{10,11}. TALENs are highly efficient in the zebrafish; in many cases well over half of the copies of a targeted gene present in a growing embryo can be cleaved. Moreover, because the TALEN architecture commonly used is a bit more flexible than that of the ZFNs, TALENs often can recleave a targeted locus that may have been repaired and slightly modified by the nonhomologous end-joining pathway. Thus, it is likely that in practice TALENs generate more target locus DSBs over time than do ZFNs, and thus are particularly well-suited for generating the lesions needed to stimulate homologous recombination.

Although the experiments reported by Zu *et al.*² represent early days in the application of this new technology, and the parameters that might improve its efficiency are still unexplored, two important outcomes have been established. First, precise gene targeting can be accomplished in zebrafish. The products of recombination appear to be perfect, with endogenous genome sequences faithfully replaced with donor ones. Second, even with donor molecules that have little identity in common with the targeted locus (1-kilobase-pair homologous sequence flanking each side of a foreign donor sequence), homologous recombination events can be recovered at frequencies that are of practical use. The gene-targeting method used here in zebrafish should be applicable in any cell type, contingent only on successful delivery of a synthetic nuclease together with donor DNA molecules. As the method matures, it will surely become widely implemented beyond zebrafish, bringing about a revolution in our ability to alter the genomes of many different kinds of organisms.

References

1. Streisinger G, Walker C, Dower N, Knauber D, Singer F. Nature. 1981; 291:293–296. [PubMed: 7248006]
2. Zu Y, et al. Nat Methods. 2013; 10:329–331. [PubMed: 23435258]

3. Bedell VM, et al. *Nature*. 2012; 491:114–118. [PubMed: 23000899]
4. Peterson RT, et al. *Nat Biotechnol*. 2004; 22:595–599. [PubMed: 15097998]
5. Carroll D. *Genetics*. 2011; 188:773–782. [PubMed: 21828278]
6. Capecchi MR. *Nat Rev Genet*. 2005; 6:507–512. [PubMed: 15931173]
7. Jasin M. *Trends Genet*. 1996; 12:224–228. [PubMed: 8928227]
8. Paques F, Haber JE. *Microbiol Mol Biol Rev*. 1999; 63:349–404. [PubMed: 10357855]
9. Bibikova M, Beumer K, Trautman JK, Carroll D. *Science*. 2003; 300:764. [PubMed: 12730594]
10. Bogdanove AJ, Voytas DF. *Science*. 2011; 333:1843–1846. [PubMed: 21960622]
11. Mussolino C, Cathomen T. *Curr Opin Biotechnol*. 2012; 23:644–650. [PubMed: 22342754]

Figure 1.

Engineering the zebrafish genome with TALENs. The schematics show repair of a TALEN-induced double-stranded (ds)DNA break via homologous recombination (HR)-mediated or nonhomologous end joining (NHEJ)-mediated mechanisms. The micrographs illustrate that mutations can be repaired in the presence of donor DNA (left) and induced in its absence (right) in an endogenous zebrafish pigmentation gene. The TALEN recognizes the *golden* gene, which determines eye and body color.