Knockout Rats via Embryo Microinjection of Zinc-Finger Nucleases

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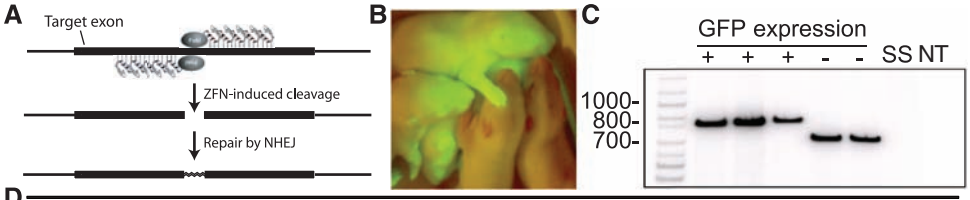
The laboratory rat is a well-established model for the genetic dissection of human disease-related traits (*1*) despite the fact that targeted modification of its genome is largely intractable. We investigated the application of engineered zinc-finger nucleases [ZFNs (*2*)] for the elimination of specific rat gene functions and generation of knockout rats. ZFNs induce site-specific, double-strand DNA breaks that can be repaired by the error-prone nonhomologous end-joining DNA repair pathway to result in a targeted mutation (Fig. 1A). In the fruit fly and zebrafish, direct embryo injection of ZFN-encoding mRNA has been used to generate heritable knockout mutations at specific loci (*2*).

The design and validation of three sets of ZFN reagents that target the green fluorescent protein (GFP) gene and two endogenous rat genes, *Immunoglobulin M* (*IgM*) and *Rab38*, were performed as described (*3*) and are detailed in (*4*). To take advantage of the potential for greater specificity of action afforded by longer (and therefore rarer) targets, we used five- and six-finger ZFNs.

We delivered these ZFNs to 36 hemizygous GFP-transgenic (*5*) inbred SS (Dahl S; GFP ZFNs), 91 inbred FHH (Fawn-hooded hypertensive; Rab38 ZFNs), and 2793 outbred SD (Sprague Dawley; IgM ZFNs) embryos by pronuclear or intracytoplasmic injection of ZFN-encoding DNA or mRNA at different concentrations (table S1). Screening 295 founder animals yielded 35 (12%) that harbored targeted mutations.

Full knockout of the GFP transgene was achieved because mutant animals lacked both GFP expression and wild-type GFP sequence (Fig. 1, B and C). Thirty-two IgM mutants and the single Rab38 mutant carried 25 to 100% disrupted target chromosomes (fig. S1). Sequence analysis of 18 founders revealed deletion alleles ranging from 3 to 187 base pairs; of note, one animal carried biallelic mutations in *IgM* (table S1). Furthermore, ZFN-mediated gene disruption demonstrated high fidelity for each target sequence because no ZFN-induced mutations were detected in target gene–disrupted animals at any of 20 predicted ZFN off-target sites (figs. S2 and S3). After breeding to wild-type animals, one out of one GFP and three out of four *IgM* mutations were transmitted through the germline, one of which was subsequently bred to homozygosity (table S1 and fig. S4).

The high percentage of disrupted chromosomes demonstrates that ZFNs are active in early rat embryos from three strains, leading to both mono- and biallelic gene disruption. Although we observed no cleavage at predicted off-target sites, such events could be segregated away from the desired mutation by backcrossing to the parental strain. ZFN-driven gene disruption and germline transmission can be accomplished in 4 months' time, and ZFNs can be engineered against a broad range of sequences (*6*, *7*); this strategy adds a valuable tool to an increasingly powerful rat genetic toolbox, opening up a range of new experiments and models of human disease.



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Strain	Target/ Construct	Route/ Dose (ng/ul)	Injected/ Transferred (%)	Founders (%)*	Mutants (%)†	Bred/ F1 inheritance
SS	GFP/mRNA	PNI/1.5	36/25 (69)	5 (20)	2 (40)	1/1
SD	IgM/Plasmid	PNI/10	609/493 (81)	54 (11)	6 (11)	4/3
SD	IgM/Plasmid	PNI/2	605/468 (77)	82 (18)	8 (10)	ND
SD	IgM/Plasmid	PNI/0.4	511/423 (83)	62 (15)	4 (6)	ND
SD	IgM/mRNA	PNI/10	186/104 (56)	14 (13)	4 (29)	ND
SD	IgM/mRNA	PNI/2	230/142 (62)	21 (15)	4 (19)	ND
SD	IgM/mRNA	PNI/0.4	183/118 (64)	19 (16)	1 (5)	ND
SD	IgM/mRNA	IC/10	272/197 (72)	4 (2)	3 (75)	ND
SD	IgM/mRNA	IC/2	197/134 (68)	17 (13)	2 (12)	ND
FHH	Rab38/mRNA	IC/2	91/83 (91)	17 (20)	1 (6)	ND

PNI - pronuclear injection; IC - intracytoplasmic; * - of transferred; † - of liveborn founders; ND - not determined

Fig. 1. ZFN-mediated gene disruption in rat embryos. (**A**) ZFNs containing five or six fingers were designed to target coding sequences of interest (gray lines) for site-specific cleavage. (**B**) Two of five pups born after microinjection of GFP-targeted ZFNs were devoid of GFP expression. (**C**) Polymerase chain reaction using GFP-specific primers revealed truncated but no wild-type sequence in each of the GFP negative pups compared with positive littermates. SS indicates Dahl S control DNA; NT indicates no template. (**D**) Table of injection data revealing successful mutagenesis of the three gene targets after multiple delivery methods and doses in three rat strains.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5939/433/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 and S2
References

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