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**COMPETING FINANCIAL INTERESTS**

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology

**Jens Nielsen**1 & **Jay D Keasling**2–4

1Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden. 2Joint Bioenergy Institute, Emeryville, California, USA. 3Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California, USA. 4Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA.
e-mail: jdkeasling@lbl.gov


**Correspondence**

**To the Editor:**

The recent description of highly active transcription activator-like effector nucleases (TALENs) prompted us to explore their utility for genome engineering in the laboratory rat. The rat is a valuable experimental animal because of its suitability for modeling human disease and toxicology. Zinc-finger nuclease (ZFN) technology and the isolation of rat embryonic stem cells have enabled targeted modifications of the rat genome2–5. Recently, *Xanthomonas*-derived transcription activator-like (TAL) effector proteins have elicited much interest because of their apparently simple rules for sequence-specific DNA recognition6,7. Several investigators have fused the FokI nuclease domain to TAL effector proteins to create TALENs1,8–12. However, only optimal truncation of the TAL effector protein allowed high-frequency gene disruption of endogenous loci and targeted DNA integration1,13,14. Here we use TALENs to disrupt the rat *IgM* locus, creating heritable mutations that eliminate *IgM* function. Our results establish the use of TALEN technology for in vivo gene knockout in mammals.

We designed and assembled TALENs to exon 2 of rat *IgM* and tested their ability to alter the *IgM* locus in rat S16 cells (Fig. 1a and Supplementary Sequences). The TALEN pair modified ~21% of chromosomes when delivered as DNA and up to 13% when delivered as mRNA (Fig. 1b). We injected titrations of these nucleic acids into one-cell rat embryos and assayed the resulting pups for alteration of the *IgM* locus (Fig. 1c). Over all doses, 7/74 (9.5%) of rat one-cell embryos injected with DNA and 51/88 (58%) of rat embryos injected with mRNA were modified at *IgM* (Table 1). Of the *IgM*-mutated mRNA-injected rats, 13/51 (25%) were biallelically modified in this single step, several containing frame-shifting mutations predicted to eliminate *IgM* function on both alleles (Supplementary Table 1). *IgM* mutation frequency was a function of TALEN dose, with the highest percentage of modified rats (75%) derived from injection of 10 ng/µl and 4 ng/µl mRNA (Table 1). The frequency of biallelically modified rats was also dose-dependent: 8/15 (53%) of rats injected with 10 ng/µl mRNA, 5/27 (19%) at 4 ng/µl, and none at 0.8 ng/µl. No biallelically modified rats were obtained from DNA injections in this study, consistent with the lower overall frequency of *IgM* modification in this founder population.

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Table 1 Disruption of the rat IgM locus via TALEN cleavage

<table>
<thead>
<tr>
<th>Injection/route</th>
<th>Dose (ng/µl)</th>
<th>Injected</th>
<th>Transferred</th>
<th>Newborns</th>
<th>Founders</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA/PNI</td>
<td>10</td>
<td>166</td>
<td>98 (59%)</td>
<td>13 (13%)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>DNA/PNI</td>
<td>2</td>
<td>236</td>
<td>150 (63%)</td>
<td>53 (35%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>DNA/PNI</td>
<td>0.4</td>
<td>84</td>
<td>48 (57%)</td>
<td>8 (17%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>mRNA/IC</td>
<td>10</td>
<td>200</td>
<td>146 (73%)</td>
<td>20 (14%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>mRNA/IC</td>
<td>4</td>
<td>187</td>
<td>127(68%)</td>
<td>36 (28%)</td>
<td>27 (75%)</td>
</tr>
<tr>
<td>mRNA/IC</td>
<td>0.8</td>
<td>86</td>
<td>73 (85%)</td>
<td>32 (44%)</td>
<td>9 (28%)</td>
</tr>
<tr>
<td>Plasmid 1 DNA/PNI</td>
<td>2</td>
<td>402</td>
<td>235 (58%)</td>
<td>47 (20%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Plasmid 2 DNA/PNI</td>
<td>2</td>
<td>353</td>
<td>256 (72%)</td>
<td>51 (20%)</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

TALENs were injected into the pronucleus as DNA or into the cytoplasm as mRNA at three concentrations each. Survival statistics from injection of two unrelated plasmid DNAs are shown for comparison. The percentages shown in the right three columns were derived using the number in each column as the numerator and the number in the column to the left as the denominator, times 100%. PNI, pronuclear injection; IC, intracytoplasmic.

Our optimally truncated TALENs cleave well when the TALEN dimer has a 12–23 bp intrarip spacing;1 the IgM TALENs described here have a 17-bp spacer. We recovered no modified alleles with a small gain of nucleotides and many with deletions larger than typically seen with ZFNs. The potential of the IgM TALENs to recluse alleles with small insertions and deletions in the spacer region likely accounts in part for the observed spectrum of alleles. Additionally, two specific alleles, Δ6 and Δ12, were unexpectedly commonly recovered (the specific instances of which are both present in rat 17.5). Both of these alleles were likely formed by microhomology-mediated reclosure of the double-strand break using TCCT and CT, respectively.

Whereas ZFNs have been used to disrupt >25 rat genes,15 this is the first report of rat gene knockout using TALENs. This relative lack of experience with TALEN technology prevents a definitive comparison of ZFNs and TALENs with respect to rat gene disruption. However, the prior use of ZFNs to disrupt the rat IgM locus permits a limited comparison of TALENs and ZFNs designed to target IgM. The overall frequency of mutated animals was identical (9%) when the nucleases were injected as DNA and as mRNA—and behaved comparably to our previously described work with ZFNs.

Together, our results suggest that TALE nucleases, when optimally designed and constructed1,13,14, are compatible with in vivo gene disruption in rats. TALENs therefore represent a validated tool for the targeted genetic modification of this important experimental animal.

Note: Supplementary information is available on the Nature Biotechnology website.

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Laurent Tesson1, Claire Usal1, Séverine Ménoret1, Elo Leung2, Brett J Niles2, Séverine Remy1, Yolanda Santiago3, Anna I Vincent2, Xiangdong Meng3, Lei Zhang4, Philip D Gregory2, Ignacio Anegon5 & Gregory J Cost2

1INSERM UMR 643, Nantes, France; Platform Transgenic Rats IBISA-CNRS; CHU Nantes, Nantes; Université de Nantes; Sangamo Biosciences, Richmond, California, USA. e-mail: gcost@sangamo.com or ignacio.anegon@univ-nantes.fr