microPublication BIOLOGY

3/27/2019 - *Open Access*

An isoform-specific allele of the *sax-7* locus

Dylan Rahe[1], Ines Carrera[2,3], Filip Cosmanescu[2] and Oliver Hobert[1,2§]

[1]Department of Biological Sciences, Columbia University, New York, NY, USA, Howard Hughes Medical Institute

[2]Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY, USA

[3]Current Address: School of Chemistry, University of the Republic, Montevideo, Uruguay

[§]To whom correspondence should be addressed: or38@columbia.edu

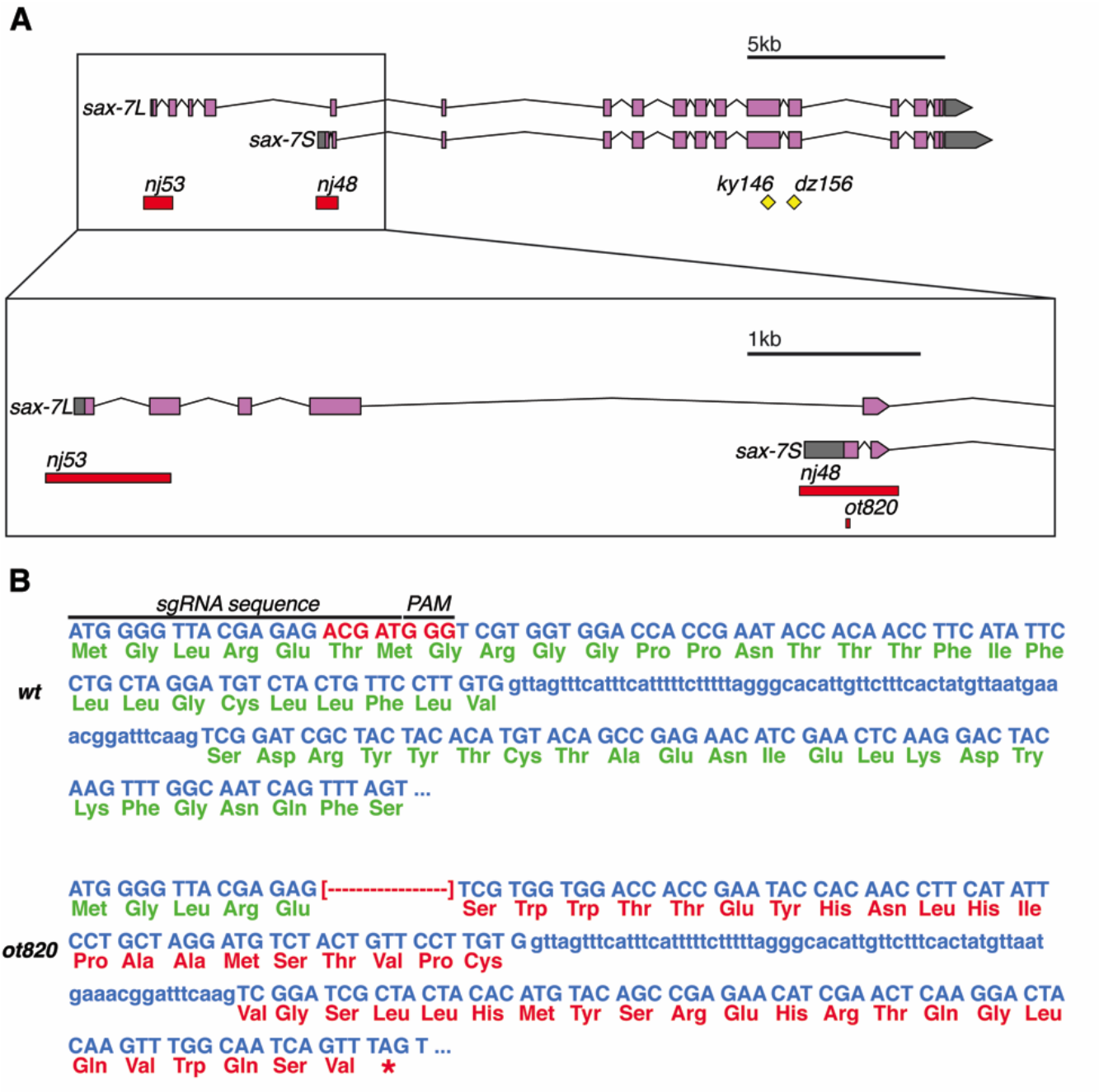

Figure 1 : *ot820* is a novel isoform-specific allele of the *sax-7* locus. A. *sax-7* locus encodes two isoforms of an L1CAM homolog. Existing null alleles affect either the long isoform (*nj53*) or both isoforms (*nj48*, *ky146*, and *dz156*). *ot820* is a short deletion mutant that affects only the short isoform. **B.** sgRNA targets the beginning of the first exon of *sax-7S*, and *ot820* is a 8bp deletion 15bp from the start codon.

3/27/2019 - *Open Access*

Description

The immunoglobulin superfamily member *sax-7* produces a long and short isoform that appear to have distinct functions (Chen *et al.* 2001; Wang *et al.* 2005; Sasakura *et al.* 2005; Pocock *et al.* 2008). An isoform-specific allele for the long isoform, *sax-7L*, was previously reported (Sasakura *et al.* 2005), but an isoform-specific allele for the short isoform, *sax-7S*, has been lacking (Fig. 1A). We used CRISPR/Cas9 to generate such an allele, using the *unc-22* co-CRISPR method (Kim *et al.* 2014). The *ot820* allele was isolated using sgRNA targeted to the first exon of *sax-7S* (sgRNA sequece: 5' – TGGGGTTACGAGAGACGAT – 3'). Twitching progeny were screened by PCR and Sanger sequencing, and an 8bp deletion 15bp from the start codon was isolated (screening primers: 5' – GGTGCTTCTCTGGTGGTAGC – 3' and 5' – TGTTGGCAAACAAAATACACG – 3', Fig. 1B). While the *sax-7L* isoform is predicted to be entirely unaffected by this allele, the resultant frameshift is predicted to generate a 49 amino acid protein in which all but the first five amino acids of *sax-7S* are aberrant and has no predicted signal sequence, before terminating in a premature stop in the second exon (Fig. 1B). This allele therefore likely represents a null for the *sax-7S* isoform. After we generated this allele, a recent paper reported an additional *sax-7S*-specific allele, with somewhat similar, but not identical sequence properties (Chen et al., 2019). Consistent with previous evidence of distinct functions of *sax-7* isoforms, these authors showed differing axon fasciculation defects between these two alleles, which is further distinct from a total null allele. These new *sax-7S* alleles should help to reveal new insights into the role of L1CAM/SAX-7 isoforms in the nervous system.

Reagents

OH13830 *sax-7(ot820) IV; oyIs14 V*. Will be available at CGC.

References

Chen, L., Ong, B., & Bennett, V. LAD-1, the Caenorhabditis elegans L1CAM homologue, participates in embryonic and gonadal morphogenesis and is a substrate for fibroblast growth factor receptor pathway-dependent phosphotyrosine-based signaling. J Cell Biol. 2001;154(4):841-55. PMID: 11502758.

Chen CH, Hsu HW, Chang YH, Pan CL. Adhesive L1CAM-Robo Signaling Aligns Growth Cone F-Actin Dynamics to Promote Axon-Dendrite Fasciculation in C. elegans. Dev Cell. 2019 Jan 28;48(2):215-228. PMID: 30555000.

Kim, H., Ishidate, T., Ghanta, K. S., Seth, M., Conte, D., Shirayama, M., & Mello, C. C. A co-CRISPR strategy for efficient genome editing in Caenorhabditis elegans. Genetics. 2014;197(4):1069-80. PMID: 24879462.

Pocock R., Bénard C.Y., Shapiro L., Hobert O. Functional dissection of the C. elegans cell adhesion molecule SAX-7, a homologue of human L1. Mol Cell Neurosci. 2008 Jan;37(1):56-68. PMID: 17933550.

Sasakura, H., Inada, H., Kuhara, A., Fusaoka, E., Takemoto, D., Takeuchi, K., & Mori, I. Maintenance of neuronal positions in organized ganglia by SAX-7, a Caenorhabditis elegans homologue of L1. EMBO J. 2005;24(7):1477-88. PMID: 15775964.

Wang X., Kweon J., Larson S., & Chen L. A role for the C. elegans L1CAM homologue lad-1/sax-7 in maintaining tissue attachment. Dev Biol. 2005 Aug 15;284(2):273-91. PMID: 16023097.

Funding: This work was supported by the Howard Hughes Medical Institute.

Reviewed By: Anonymous

History: Received March 4, 2019 **Accepted** March 22, 2019 **Published** March 27, 2019

Citation: Rahe, D; Carrera, I; Cosmanescu, F; Hobert, O (2019). An isoform-specific allele of the *sax-7* locus. microPublication Biology. https://doi.org/10.17912/micropub.biology.000092