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04/19/2019 – *Open Access*

New alleles of the *lin-22*/Hairy bHLH transcription factor

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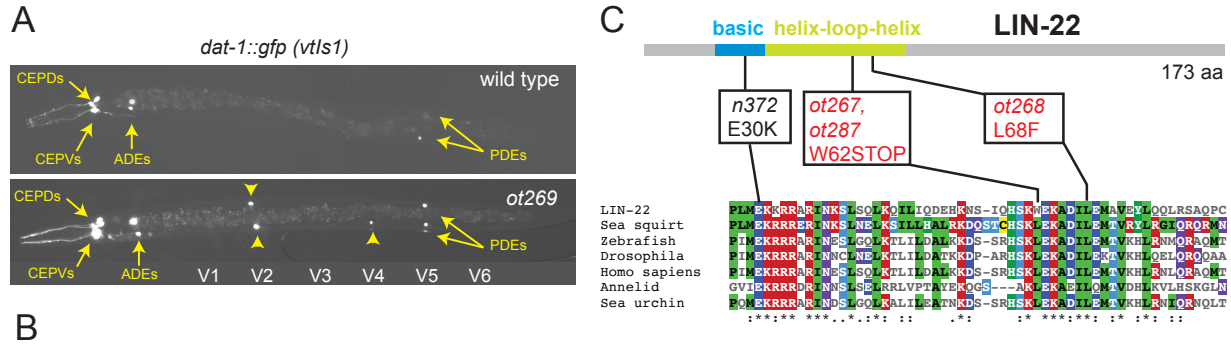

B

Genotype	% animals with defects in head dopamine neurons	% animals with ectopic PDE neurons in V neuroblast lineage as assessed by ectopic *dat-1::gfp (vtIs1)* expression							Sequence change in or around *lin-22* locus (underlined and bold)
		V1	V2	V3	V4	V5	V6	*n*	
vtIs1	0	0	0	0	0	100	0	120	
ot267; vtIs1	0	6	61	50	66	100	0	108	G to A in ATTCCAAATG**G**GAAAAAGCT
ot268; vtIs1	0	28	95	84	89	100	0	82	C to T in TGATATT**C**TCGAAATGGCTGT
ot269; vtIs1	0	0	13	1	19	100	0	96	C to T at -4940 from the ATG in TTTTAT**C**TTGATTTACGTGT
ot287; vtIs1	0	16	68	68	64	100	0	50	G to A in ATTCCAAATG**G**GAAAAAGCT
n372; vtIs1	0	17	78	84	87	100	0	40	

Figure 1. Alleles of *lin-22*. (A) *lin-22* mutant alleles display an ectopic expression of *dat-1::gfp (vtIs1;* Nass et al., 2002). One representative example is shown. (B) Quantification of *lin-22* mutant defects and sequence changes. (C) Sequence change in protein coding sequences. Sequences of Hairy homologs from different animal phyla are shown.

Description

We screened for mutants that affect expression of dopaminergic neuron identity, using a transcriptional reporter for expression of the dopamine transporter *dat-1*. We previously published and characterized a number of mutants that affect *dat-1* expression in different neuron types (Doitsidou et al., 2008). Four alleles that we did not publish in our original screening paper are described here. While wild-type animals only display a single *dat-1::gfp(+)* neuron pair in the midbody region, the PDE neuron pair from the postdeirid lineage, all 4 mutant alleles display ectopic *dat-1::gfp* expression along the anterior/posterior axis of the animal (Fig.1A,B). Postdeirid lineage duplication defects were previously described in animals lacking the bHLH transcription factor *lin-22/Hairy* (Wrischnik and Kenyon, 1997). We find that the canonical *lin-22* allele, *n372*, indeed displays *dat-1::gfp* expression defects similar to those observed in our mutants (Fig.1B). We sequenced the *lin-22* locus in all of our four, independently isolated alleles. Two of them are premature stop codons, one is a missense mutation affecting a conserved leucine residue and all display a similar penetrance of defects (Fig.1B,C). The fourth and weakest allele, *ot269*, displayed no sequence alteration in the *lin-22* coding sequence or in exon/intron boundaries. *ot269* failed to complement *ot267, ot268, ot287* and the canonical *lin-22* allele *n372*. Furthermore, the *ot269* phenotype was rescued by injection of the fosmid WRM0627dG07, which contains *lin-22* and one additional complete gene. We found that *ot269* harbors a single nucleotide change in the upstream intergenic region of *lin-22*, almost 5kb away from the start of the gene (sequence change shown in Fig.1B). Subsequent work has shown that this mutation affects a binding site for a GATA transcription factor (Katsanos et al. 2017).

Reagents

OH4265 *lin-22(ot267);vtIs1*
OH4270 *lin-22(ot268);vtIs1*
OH4271 *lin-22(ot269);vtIs1*
OH4320 *lin-22(ot287);vtIs1*

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Strains are available at the CGC.

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Acknowledgements. We thank Chi Chen for injections, Andrea J. Pretorian and Albert Lee for help with screening, Alexander Boyanov and Berta Vidal for help with sequencing.

Funding This work was funded was funded by the US National Institutes of Health (R01NS050266), the Howard Hughes Medical Institute and a European Molecular Biology Organization long-term fellowship to M.D.

Author Contributions: Conceptualization: M.D.; Data Curation: M.D.; Formal Analysis: M.D.; Investigation: M.D.; Methodology: M.D.; Project administration: M.D., O.H.; Visualization: M.D.; Validation: M.D.; Writing – original draft: O.H; Writing – review and editing: M.D., O.H.; Funding Acquisition: M.D., O.H.; Supervision: O.H.

Reviewed by Jintao Luo

Received 04/16/2019. **Accepted** 04/19/2019. **Published Online** 04/19/2019.

Citation: Doitsidou, M., & Hobert, O. New alleles of the lin-22/Hairy bHLH transcription factor. microPublication Biology. https://doi.org/10.17912/MICROPUB.BIOLOGY.000111.