nature metabolism

Article

https://doi.org/10.1038/s42255-023-00765-3

Alkaline taste sensation through the alkaliphile chloride channel in *Drosophila*

In the format provided by the authors and unedited

Supplementary Information

I. Materials

Antibodies

Primary antibodies: Rabbit anti-Alka polyclonal antibody (generated in this study); rabbit anti-GFP antibody (polyclonal) (Thermo Fisher, catalog number A-11122, RRID:AB_221569); mouse anti-GFP antibody (monoclonal 3E6) (Thermo Fisher, catalog number A11120, RRID:AB_221568); mouse anti-Myc antibody (monoclonal 9E10) (Cell Center Services, University of Pennsylvania, catalog number 3207); mouse anti-nc82 antibody (monoclonal) (Developmental Studies Hybridoma Bank (DSHB), RRID:AB_528108); and mouse anti-mCherry antibody (monoclonal) (DSHB, catalog number DSHB-mCherry-3A11, RRID:AB_2617430).

Secondary antibodies: Goat anti-rabbit AlexaFluor 488 (Jackson Immuno Research, catalog number 111-545-003, RRID:AB_2338046); goat anti-rabbit 594 (Jackson Immuno Research, catalog number 111-585-003, RRID:AB_2338059); goat anti-mouse AlexaFluor 488 (Jackson Immuno Research, catalog number 115-545-003, RRID:AB_2338840); and donkey anti-mouse AlexaFluor 594 (Jackson Immuno Research, catalog number 715-585-150, RRID:AB_2340854).

II. Methods

1. Generating the *alka*^{1} null mutant

To generate an *alka* deletion allele, we took advantage of the CRISPR/Cas9 gene-editing system. We identified candidate guide RNA (gRNA) sequences using the flyCRISPR Target Finder tool (https://flycrispr.org/target-finder/) and selected two gRNA target sites (gRNA1 and gRNA2), which are predicted to have no off-target activity. These two gRNAs are approximately 1 kb apart, spanning exons 6-9 of the *alka* gene. We ligated their corresponding oligos separately into the *pU6-BbsI-chiRNA* plasmid (AddGene, RRID: Addgene_45946). The sequences of these gRNAs are as follows:

gRNA1:
Sense: 5' CTTCGTTCGACGAGACTTCGGGAT 3'
Antisense: 5' AAACATCCCGAAGTCTCGTCGAAC 3'

gRNA2:
Sense: 5' CTTCGATGAAGTCGTCCTAGGATT 3'
Antisense: 5' AAACAATCCTAGGACGACTTCATC 3'

The gRNA1 and gRNA2 constructs were co-injected into embryos of the *nos-cas9 attp2* line (Blooming Drosophila Stock Center, RRID:BDSC_78782) (Rainbow Transgenic Flies, Camarillo, CA). We crossed the injected flies with w^{1118} flies and then screened their offspring for deletion mutants by performing genomic polymerase chain reaction (PCR) using the following primers:

Forward: 5’ CGCGCTTGAACCTAGACGTA 3’
Reverse: 5’ GCCAGTGACGCTAGGTCTTT 3’

We successfully obtained an *alka*^{1} mutant with 1,124 base-pairs (bp) removed (location in the fly genome: 2R:10,828,634 to 10,829,758) from the *alka* gene (**Extended Data Figs. 2a-c**). In this *alka*^{1} mutant, the DNA sequences encoding a portion of the N-terminus, all four transmembrane segments, and the entire C-terminus of the Alka protein are ablated. Thus, we assert that the *alka*^{1} allele is nonfunctional. To eradicate any potential off-target mutations, we outcrossed the *alka*^{1} mutant to wild type for five generations. We used the outcrossed *alka*^{1} mutant flies for all experiments presented in this study.

2. Generating the *alka-Gal4* line

To generate a promoter *Gal4* line for *alka*, we cloned the *alka* promoter sequence, the span roughly 3 kb upstream from the ATG site, using the following primers:

Forward: 5’ ATACATACTAGAATTCGCACATGTGTTTCTCTGTATTTACAAAAG 3’
Reverse: 5’ TTTGCTTACGGGATCCAAACTGAATGGAGCGGAACTGAG 3’

We then ligated the promoter fragment into the *pCaSpeR-gal4* vector using the In-Fusion® HD Cloning Kit (Takara, catalog number 639649). Next, the *pCaSpeR-alka-gal4* construct was used to transform *w*^{1118} fly embryos. Transgenic adults were selected based on red-eye color and then outcrossed to *w*^{1118} flies for five generations, creating the *alka-gal4* line.

3. Generating *PCS2+MT-Myc-alka* and *PCS2+MT-Myc-alka*^{P276A}

To amplify the Alka protein-coding sequences from an *alka* cDNA construct (Drosophila Genomic Research Center, UFO04392), we use the following primers:

Forward: 5’ AGAGGACTTGAATTCAATGCTCGACAAATTCAACACAAA 3’
Reverse: 5’ GTTCTAGAGGCTCGAGCTAGGACGACTTCATCAGCAGTA 3’

The resulting amplicon was subcloned into the EcoR1 and XhoI restriction sites of the *PCS2+MT-Myc* expression vector. We then verified the final construct with DNA sequencing.

To make the *alka*^{P276A} construct, we performed site mutagenesis using the Agilent QuikChange site-directed mutagenesis kit (Agilent Technologies, catalog number 200523-5). The *alka* cDNA was mutated so that cytosine (C) 826 was changed to guanine (G), inducing a proline-to-alanine substitution at the amino acid residue 276, with the following primers:

Forward: 5’ GGACTGGACGCCATTGCGGGCGC 3’
Reverse: 3’ CCTGACCTGCGGTAACGCCCCGCG 5’
The C-to-G mutation is denoted by the red nucleotide within the primer sequence.

We confirmed the C-to-G mutation by DNA sequencing and subcloned the mutant *alka*^{P276A} gene into a new *PCS2+MT-Myc* vector.

4. Generating *UAS-alka* and *UAS-alka*^{P276A} lines

We cloned the *alka* and *alka*^{P276A} cDNA fragments from the *pCS2+MT-alka* and *pCS2+MT-alka*^{P276A} constructs, respectively, using the following primers:

Forward: 5' GTCCGGACTCAGATCTATGCTCGACAAATTCAACACAAA 3'
Reverse: 5' TAGATCCGGTGGATCCCTAGGACGACTTCATCAGCAGTA 3'

We subsequently ligated *alka* or *alka*^{P276A} into the BglII and BamH1 sites of the pUASp vector. The resultant *UAS-alka* or *UAS-alka*^{P276A} construct was transformed into *w*^{1118} embryos to make transgenic flies (BestGene, Chino Hills, CA). After outcrossing the *UAS-alka* or *UAS-alka*^{P276A} flies to *w*^{1118} flies for five generations, we eventually established the *UAS-alka* or *UAS-alka*^{P276A} lines.

5. Making Alka antibodies

We selected a peptide (AA 371-420) from the Alka protein region due to its little homology to any other fly proteins. We amplified the corresponding cDNA sequence of this peptide using the following primers:

Forward: 5' GGGGCCCCTGGGATCCTCGCGGAAGGCCACTCAGAC 3'
Reverse: 5' GATGCGGCCGCTCGAGTTACTCTCGCCACATGATCTTCTCCACGCC 3'

The amplified fragment was ligated into the PGEX-6P-1 vector (AddGene, catalog number 27-4597-01) between the BamH1 and Xho1 restriction sites. After verifying the construct with DNA sequencing, we transformed it into BL21 *Escherichia coli* (New England BioLabs, catalog number C2530H) and induced protein expression with isopropyl β-d-1-thiogalactopyranoside (IPTG) (LabScientific, catalog number I-555). The Alka-GST fusion protein was purified from BL21 cell lysate on GST purification affinity columns (Cytiva, catalog number 28401745). Next, we removed the Alka peptide from the bound GST tag with PreScission protease (Cytiva, catalog number 27084301). The purified Alka proteins were injected into a rabbit to generate polyclonal antibodies (Pacific Immunology, Ramona, CA).

III. Extended Data Movies

Extended Data Movie 1. The *alka-Gal4* control fly showing persistent sucrose (500 mM) feeding in the presence and absence of an intense red-light stimulus (2000 Lux).

Extended Data Movie 2. The *alka-Gal4,UAS-CsChrimson*;*Orco-Gal80* fly exhibiting normal feeding of sucrose (500 mM) in the absence of red light but a cessation of feeding in response to a moderate red-light stimulus (1200 Lux).