On the nature of alleles ectopically inducing the small heat shock protein reporter.

Wadim Kapulkin and Chris Link

Institute for Behavioral Genetics, University of Colorado-Boulder

The article describes the mutant screen for alleles ectopically activating the small heat shock protein reporter variant. Provided the described experiments were conducted in 2000-2002 and it has been many years since I implemented those experiments, I thought I will provide the abbreviated description as I think the results could be of more general interests.

The story starts, with the construction of a small heat shock protein GFP reporter. I constructed the translational reporter gene by inserting, in frame, the entire promoter region followed by the entire coding sequence of the hsp-16.2 (Y46H3A.3) gene including its single intron, into the Fire lab vector pPD95.77 (containing GFP gene followed by a 3'UTR). The resulting construct was functional, as judged by the appearance of the heat stress-inducible sHSP:GFP foci. sHSP:GFP foci (presumably representing intracellular small heat shock protein-containing granules) emerged upon heat stress in most cells and tissues, visible earliest in head neurons starting from 2hrs after exposure to elevated temperature. The particular reason, for choosing the hsp-16.2, was that it has been shown the particular small heat shock protein directly binds to transgenic beta-amyloid aggregates (1). Induced sHSP:GFP foci were demonstrated in muscles in backgrounds expressing beta-amyloid (1, 2). In general, the above observations were consistent with the expected expression pattern of small heat shock protein reporter (perhaps except for somewhat unexpected the granular or vacuolar appearance of the subcellular sHSP:GFP foci).

Having established few independent transgenic lines transmitting the above sHSP:GFP reporter , by injecting the plasmid construct into the WT N2 background (where in the absence of heat stress the background expression of sHSP:GFP is almost negligible), I have exposed one of the reporter lines to the mutagen ethyl methanesulphonate (EMS). Among the progeny of 30 mutagen exposed parental hermaphrodites, a fraction of animals expressed easily detectable sHSP:GFP foci in the absence of the heat stress. The expression patterns observed in the progeny of the mutagenized hermaphrodites were discrete and clearly different from the ubiquitous pattern observed upon the exposure to heat stress. Typically, the expression patterns observed in the progeny of mutagenized worms were restricted to few individual cells (i.e. unidentified cells in the head) or organs / tissues (i.e. enterocytes) (Figure 1.).
In mutants, the sHSP:GFP foci were observed constitutively in populations otherwise not exposed to external heat shock response triggers. Collectively, the observed expression patterns of the sHSP:GFP observed in the progeny of the EMS-mutagenized hermaphrodites, were interpreted as resulting from newly induced alleles capable of ectopic induction of the stress sensitive, transgenic reporter gene. We propose to term this allele class an ‘ectopic activators of heat shock protein’ (*eah* alleles).

Considering the possible nature of the alleles which acquired the ability of the ectopic sHSP activation, the following allele categories could be proposed:
1. Mutations acquired in the transgenic small HSP promoter sequence.
2. Gain-of-function mutations in hsf-1: heat shock factor alleles capable of activating the reporter and presumably endogenous small heat shock loci.
3. 'Second-site mutations' where mutated allele acquires the ability to trigger the heat shock protein response.

In theory, the alleles could be assigned to the above categories by the complementation tests with hsf-1 alleles and crosses with different small heat shock protein reporter lines (to exclude for the mutations acquired into the reporter transgene). Given the established role of heat shock proteins in cellular proteostasis, the last category of alleles appears of exceptional interests. The last category of *eah* alleles

presumably represents the genetic events when the 'second site mutations', causes the background alleles to increase the cellular demands for chaperoning. In the simplest scenario, that class of alleles, would represent the mutations affecting the ability of the newly translated polypeptide to fold into the proper 3-dimiensional structure (either directly or indirectly, however, more elaborate scenarios could be drawn i.e. involving the compensatory changes (3.)).

The *eah* alleles are of possible interests in the aging field and also in the area of stress-response and pathogenesis. Depending on the nature of the particular alleles, mutated lines could have an effect on the lifespan of the organism. Provided sHSP driven reporter is regarded as a predictor of the longevity in *C. elegans* (4.), depending on the tissue/cell type where the ectopic activation of the small HSP is observed as well as the nature of the particular allele, either positive or negative impact on the lifespan could be expected.

Given the small heat shock promoter based constructs are commonly used to drive transgenic expression (i.e. heritable hairpin RNAi transgenes, viral RNA replicons, Mos1 transposon constructs and more recently the inducible Cas9 expression) we think some of the above alleles might be also interesting outside the aging and pathogenesis field, primarily as a mean to launch the transgenic expression of constructs of interests in cell and tissue-specific manner.

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2. Link CD, Taft A, Kapulkin WJ, Duke K, Kim S, Fei Q, Wood DE, Sahagan BG.
Gene expression analysis in a transgenic Caenorhabditis elegans Alzheimer's disease model.
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3. Kapulkin WJ, Hiester BG, Link CD. Compensatory regulation among ER chaperones in C. elegans.
FEBS Lett. 2005 Jun 6;579(14):3063-8.

4. Mendenhall, A. R., Tedesco, P. M., Taylor, L. D., Lowe, A., Cypser, J. R., & Johnson, T. E. (2012).
Expression of a Single-Copy hsp-16.2 Reporter Predicts Life span. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 67(7), 726–733.

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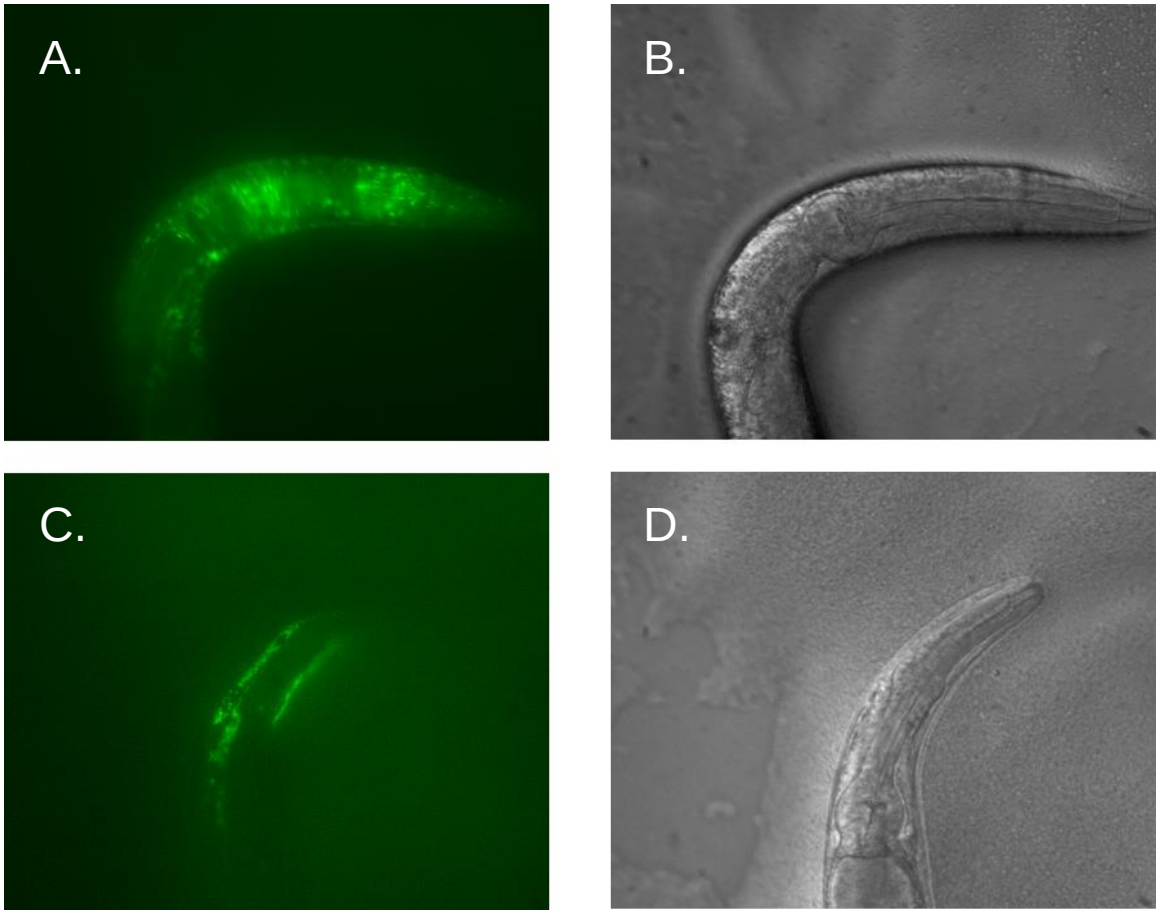
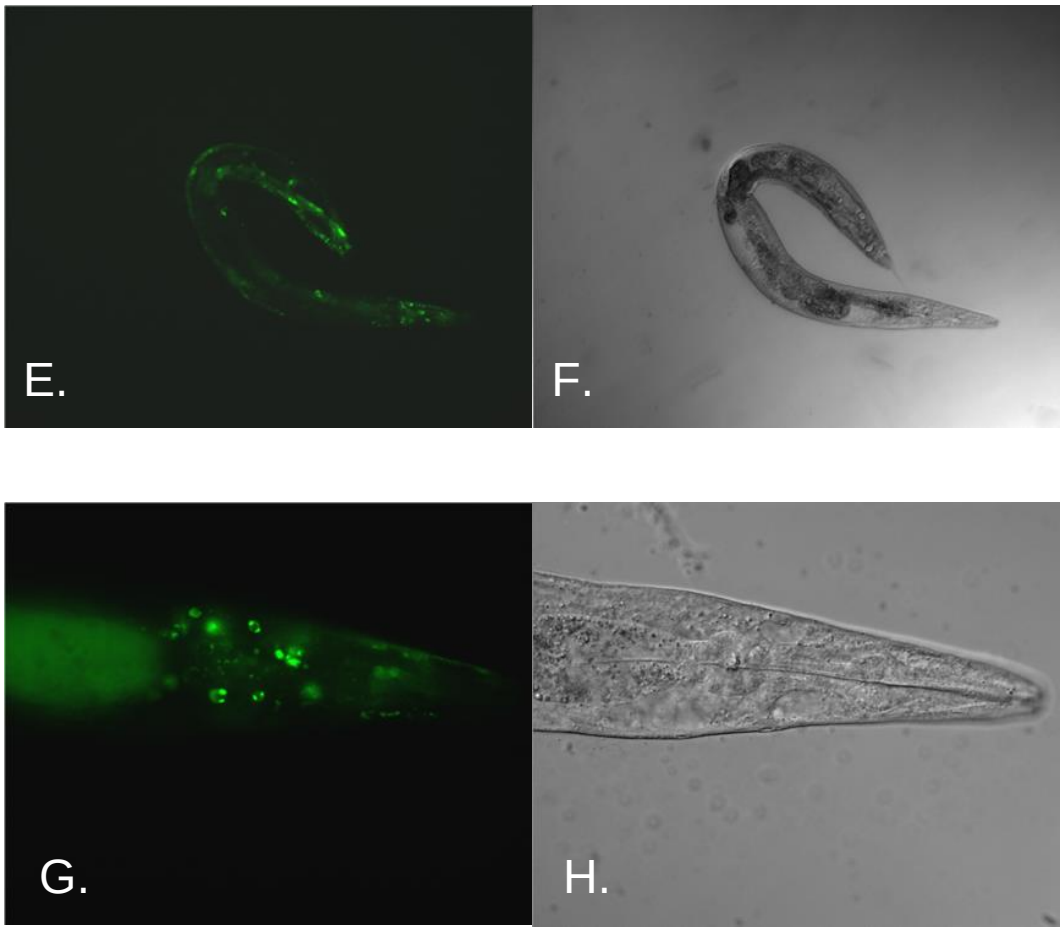
Figure 1.

Figure description:

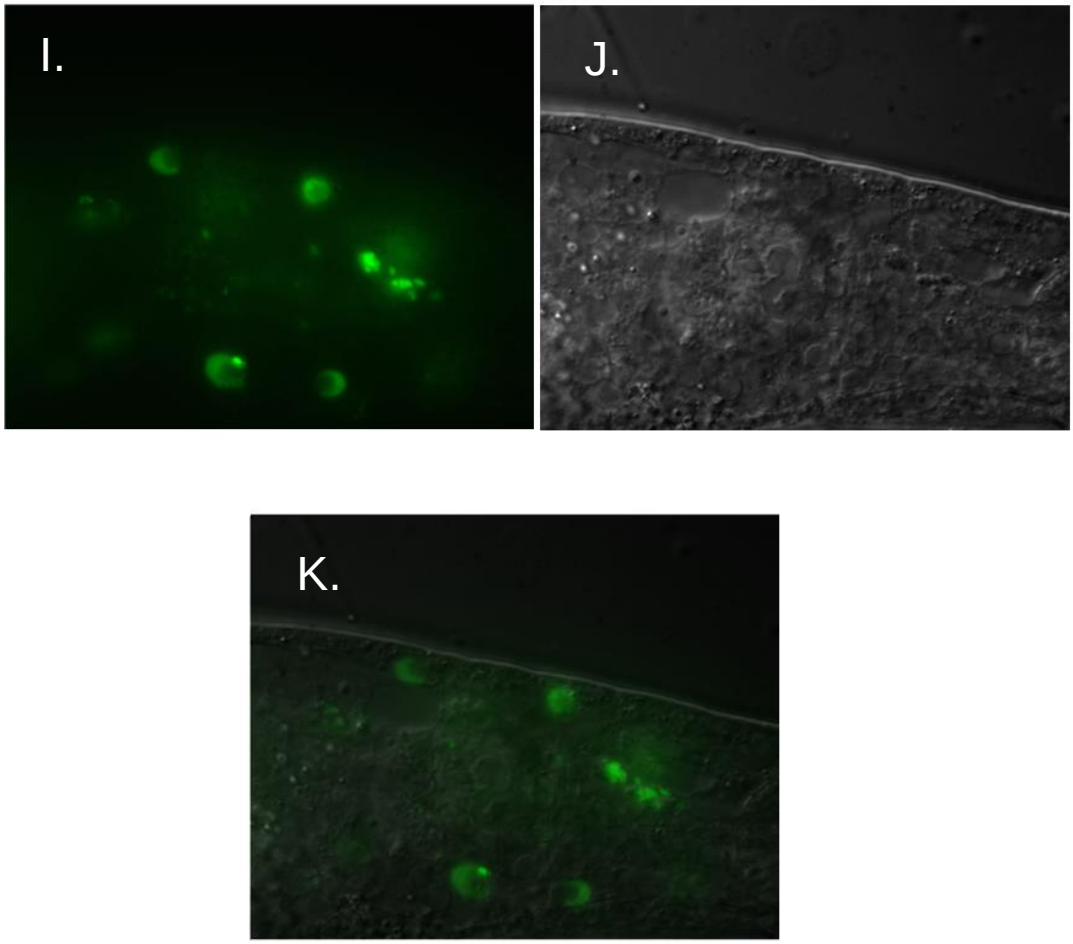
(A. and B.) The beta-amyloid binding, small heat shock protein (hsp-16.2) sHSP:GFP reporter fusion is induced by the heat stress in WT N2(Bristol) background. Expression of the sHSP:GFP foci emerge upon 1-2hrs of non-lethal exposure to the temperature above the physiological range (2hrs at 37ºC). Expression pattern of the reporter transgene indicates the presence of the distinct foci of the hybrid sHSP:GFP protein, distributed across most of the cells and tissues. Upon induction, fluorescent sHSP:GFP foci are first observed in neurons of an animal. (C. and D.) Body wall muscle specific expression pattern of the beta-amyloid binding sHSP:GFP hybrid protein in the double transgenic line. sHSP:GFP reporter is encoded by the extrachromosomal transgenic array maintained in the chromosomally integrated beta-amyloid expressing background *dvIs2* (CL2006). Constitutive expression of sHSP:GFP foci is observed in body-wall muscles under the physiological range of temperatures tolerated by *C. elegans*. Plate images focused on the head region of the representative animal is shown in both cases. Both transgenes expressing the sHSP:GFP are propagated as extrachromosomal arrays and both lines do express rol-6(d) used as transformation marker. Image to the left is GFP, right is DIC.



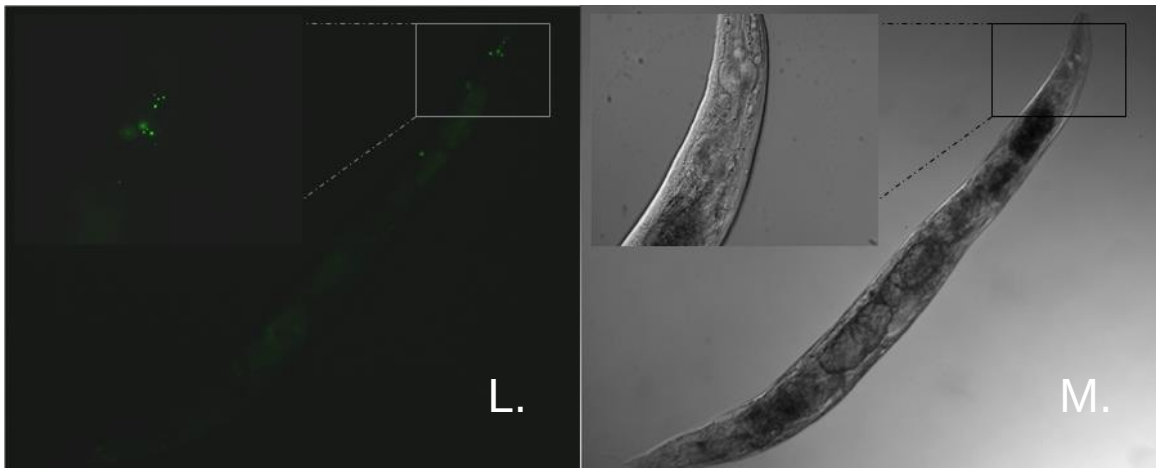
(E-J) The *eah-1* allele. Low-magnification of the mounted whole body of an animal, demonstrating the prominent sHSP:GFP foci (E and F, top panel). Higher magnification image of the sHSP:GFP foci in the head region (G. and H. middle panel). High magnification of the lateral part of the head, focused on the localized group of sHSP:GFP foci (I. and J., bottom panel). (K.) High-magnification GFP and DIC images (I. and J.) digitally merged to impose fluorescent sHSP:GFP foci onto cellular structures in the lateral head. Ring-like structures highlighted by the ectopic fluorescent sHSP:GFP foci are apparent.



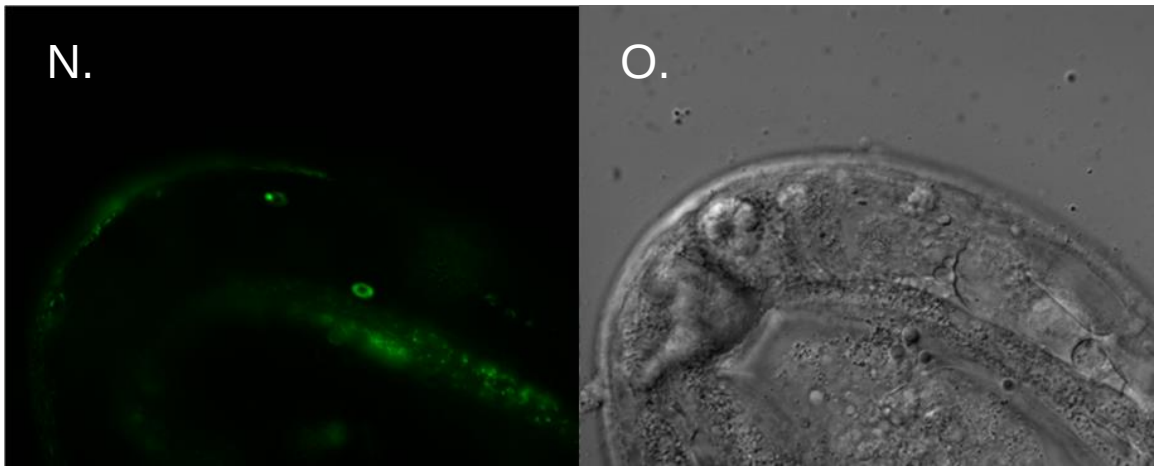
The *eah-1* (cont.) High magnification of the lateral part of the head, focused on the localized group of sHSP:GFP foci (I. and J., bottom panel). (K.) High-magnification GFP and DIC images (I. and J.) digitally merged to impose fluorescent sHSP:GFP foci onto cellular structures in the lateral head. Ring-like structures highlighted by the ectopic fluorescent sHSP:GFP foci are apparent.



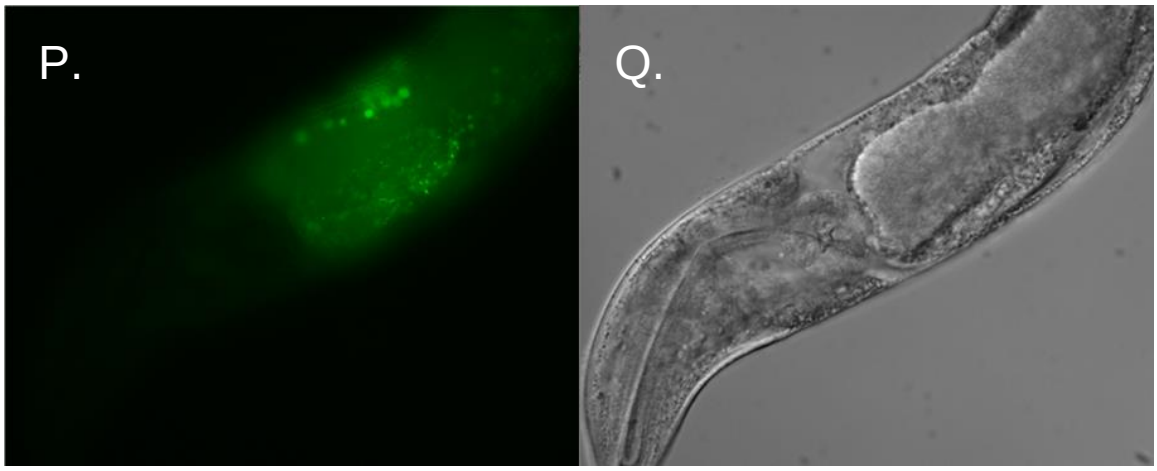
(L. and M.) The *eah-2* allele. Unidentified structures labeled by sHSP:GFP foci (most prominent in the head region). Low-magnification GFP and DIC images of an entire animal body. Inserted boxed area corresponds to a higher-magnification image focused on the brightly fluorescent structures in the head region (left) corresponding DIC (right).



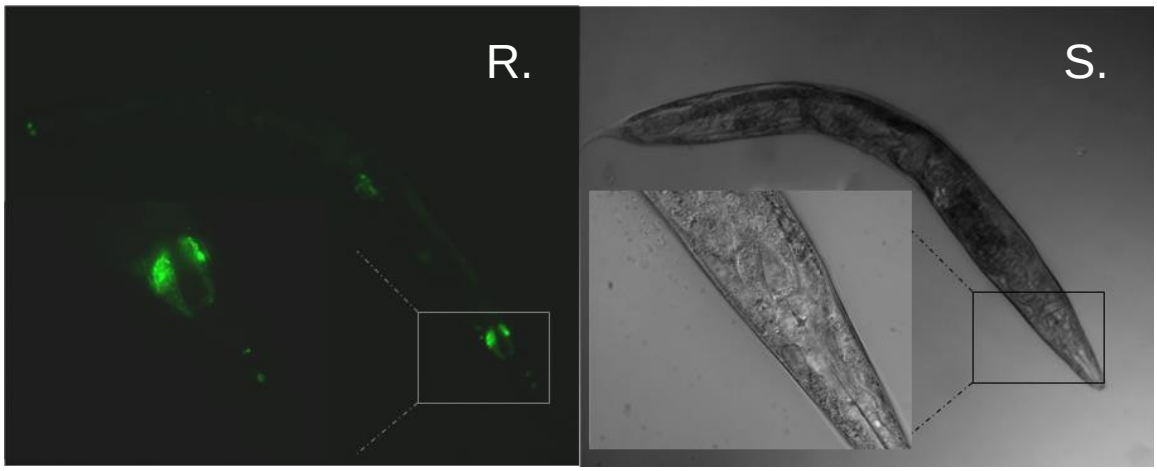
(N. and O.) The *eah-3* allele. Unidentified structures and cells in the body wall.



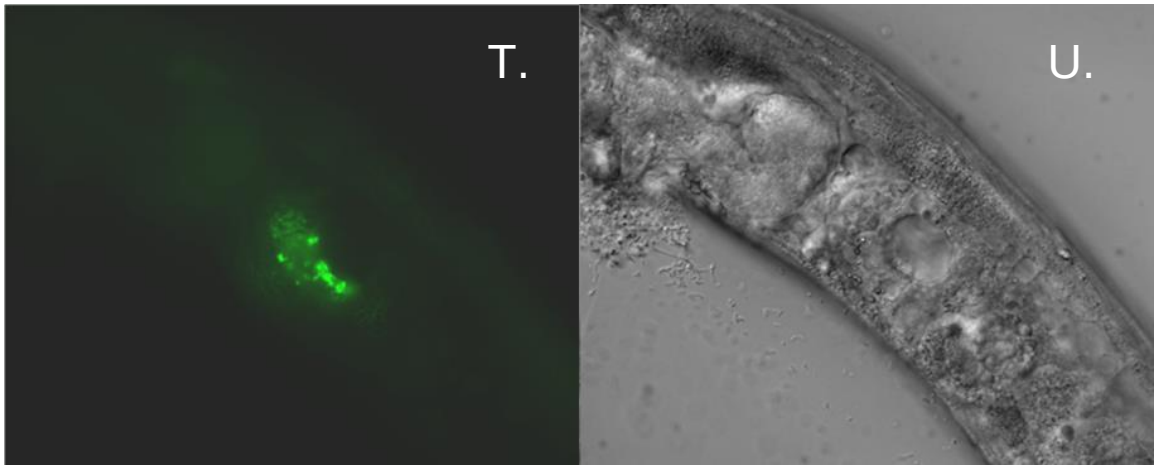
(P. and Q.) The *eah-4* allele. An enteric pattern of the sHSP:GFP foci induction (anterior intestine). The original image is shown (the GFP signal overlaps with enteric granules auto-fluorescence).



(R. and S.) The *eah-5* allele. Unidentified structures labeled by sHSP:GFP foci (most prominent in the head region). Low-magnification GFP and DIC images of an entire animal body. Inserted boxed area corresponds to a higher-magnification image focused on the brightly fluorescent structures in the head region (left) corresponding DIC (right).



(T. and U.) The *eah-6* allele. Unidentified structure in the mid-body.



(W-Z) The *eah-7* allele. Unidentified structures. Low-magnification of the mounted whole body of an animal, demonstrating the sHSP:GFP foci (W., top panel). Higher magnification images of the sHSP:GFP foci in the head region (X. and Y., middle and bottom panel). (W, X, Y) Fluorescent GFP images (left), (Z1-3) corresponding DICs images (right).

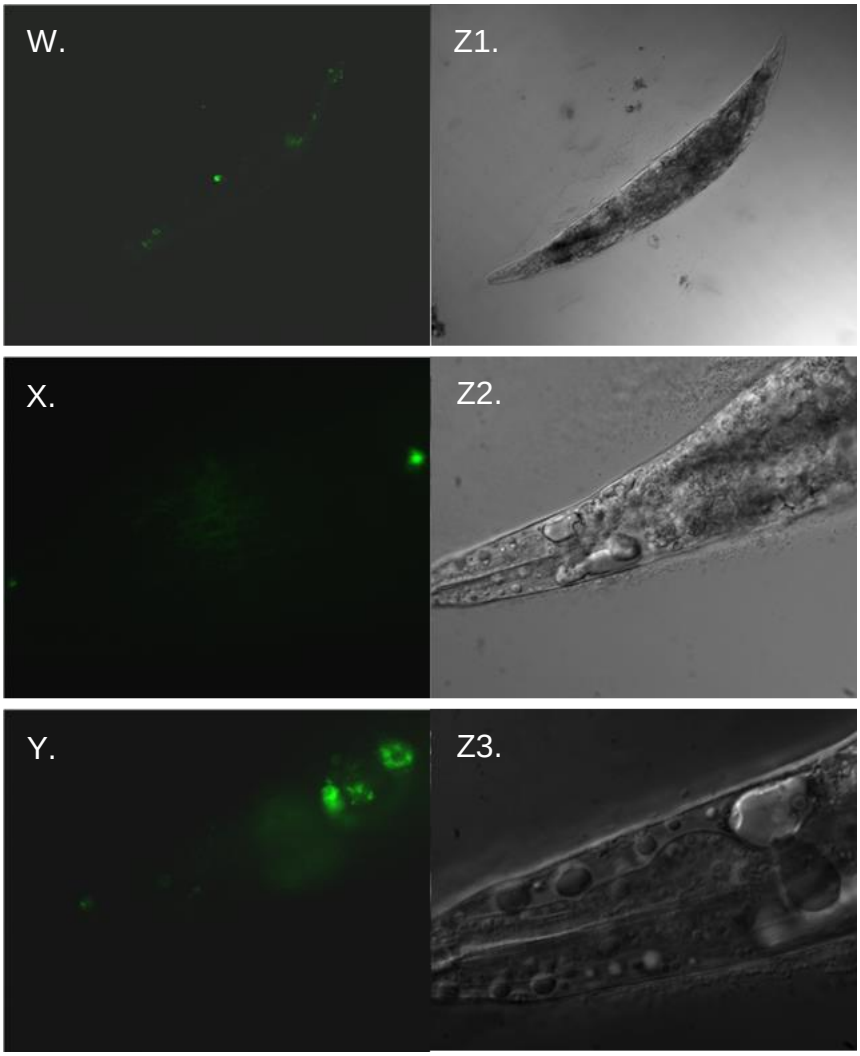


Figure legend: Collage of the individual eah images (single TIFF file with labels).