The Drosophila genome encodes a single TNF homolog, known as Eiger (Egra-like cell death trigger, Egr) (Igaki et al. 2002; Moreno et al. 2002; Narasimamurthy et al.). Egr is expressed in many different tissues and plays various roles in cellular processes such as the immune response, energy homeostasis, and JNK-dependent cell death. Since its identification, numerous studies on cell death and immunity have utilized the egr1 and egr3 alleles, which were generated by imprecise excisions of the Regg1GS9830 element and resulted in deletions of the first coding exon of the egr gene (Igaki et al. 2002). Both egr1 and egr3 strains are homozygous viable and considered severe loss-of-function alleles.

Dead cells in Drosophila are commonly removed from tissues by phagocytic engulfment by plasmatocytes, the most abundant of the circulating hemocytes in the larva (Abrams et al. 1999; Franc et al. 1999; Shklyar et al. 2013). Plasmatocytes carry cell surface receptors for the recognition and rapid engulfment of bacteria, dead cells and cellular debris, such as Eater (Kocks et al. 2005; Chung and Kocks 2011), NimrodC1 (Kurucz et al. 2007; Honti et al. 2013) and Draper (Manaka et al. 2004). A frequently used marker for plasmatocytes in Drosophila is positivity for NimC1, a transmembrane protein characterized by the presence of a special type of EGF repeat known as the NIM repeat, located immediately proximal to a conserved CCxGY motif (Somogyi et al. 2008). The NimC1 gene is part of a cluster of four NimC (NimC1-4) genes in the midst of several other related Nimrod genes at 34E on chromosome 2. Nimrod proteins contain 2–16 NIM repeats as well as additional conserved residues at their amino termini. The Nimrod proteins, together with Eater and Draper, form a conserved superfamily of 12 proteins in vertebrate and mammalian genomes (Melcarne et al. 2019a). Loss of any Nimrod protein diminishes the capacity of hemocytes to fight microbes. For example, RNAi against NimC1 has implicated it in bacterial phagocytosis (Kurucz et al. 2007), while complete loss of NimC1
demonstrated cooperation between NimC1 and Eater in the recognition and phagocytosis of bacteria (Melcarne et al. 2019b). In addition, Egr has been reported to have a role in regulating phagocytosis of certain bacteria (Schneider et al., 2007). Here we report that egr1 and egr3, two commonly used egr alleles, and their parental strain Regg1G0865, carry additional mutations in the NimC1 phagocytosis receptor gene.

**MATERIALS AND METHODS**

**Drosophila genetics and husbandry**

Flies were raised at 25°C on standard cornmeal-molasses food supplemented with fresh dry yeast. The following strains were used: egr1, egr3 and egrRegg1 (Igaki et al. 2002), egr3 (gift of H. Kanda), egr1AG and egr1AG (generated in this work), Hm43-2DRed56/CyO (gift of K. Brückner; Makhijani/Cyo (gift of H. Struhl), act > y+STOP > Gal4 and tub > MycSTOP > Gal4 (de la Cova et al. 2004), Oregon R (Bloomington Drosophila Stock Center).

**Cell death assays**

Eggs from appropriate crosses were collected on yeasted grape plates for 2-4 hr and allowed to develop at 25°C in a humid chamber for 24 hr. Prior to collecting the eggs, two 30 min pre-collections were carried out to allow females to void any developing eggs. At hatching, larvae were transferred to food vials supplemented with fresh yeast paste at a density of less than 50 larvae/vial to prevent crowding. To generate Myc-expressing clones, act > y+STOP > Gal4 was used to generate act > Gal4 clones that expressed UAS-GFP and UAS-Myc in WT and in egr1 mutants. act > Gal4 clones were induced in larvae with a HS in a 37°C water bath for 6 min. Clones were allowed to grow in the wing discs for 24 or 48 hr, as described (de la Cova et al. 2004; Meyer et al. 2014; Alpar et al. 2018). The wing discs were then dissected from larvae at the indicated times after clone induction (ACI). A detailed protocol is available upon request.

**Larval dissection and imaging**

Wing imaginal discs were dissected from third instar larvae as indicated above, and fixed in 4% paraformaldehyde in phosphate-buffered saline (PB-PBS) for 20 min at room temperature, and washed 3-5 times for 20 min each with 0.01% Tween-20 in PBS (PBTw). Larval carcasses were stained with Rabbit anti-Cleaved Caspase-3 (Cas-3) at 1:100 (Cell Signaling Technology, cat. # 9661). Secondary Alexa Fluor 555 Goat anti-Rabbit IgG antibodies (1:600) were purchased from Molecular Probes (cat.# A-21429). Lymph glands were stained with plasmatocyte-specific P1 antibodies (Mouse anti-NimC; 1:100) (Kurucz et al. 2007) and subsequently, Alexa Fluor 555 (Invitrogen) secondary antibodies. Hoechst 33258 (Sigma) was used to stain DNA. Wing discs and lymph glands were mounted in VectaShield Antifade (Vector Laboratories, Cat# H-1000) on glass slides. Images were acquired with a Zeiss Axiohot microscope with Apotome and processed using ImageJ and Adobe Photoshop.

Figure 1 Dying cells transiently accumulate in egr3 mutants. A. Clones in A and B were examined 24 hr after clone induction (ACI). Clones of loser cells in a wildtype (WT) wing disc, expressing GFP (cyan) and cleaved caspase 3 (Cas-3); Cas-3 channel is shown in A’. Larval genotype: yw hsflp/w; tub > myc, y+STOP > Gal4, UAS-GFP/. Genotype of the WT loser clones: yw hsflp/w; tub > Gal4, UAS-GFP/+ . B. Loser clones, expressing GFP and Cas-3, in a egr3 mutant wing disc; Cas-3 channel is shown in B’. Larval genotype: yw hsflp/w; egr3/egr3; tub > myc, y+STOP > Gal4, UAS-GFP/+ , act > Gal4, UAS-GFP/+ . C. Quantification Cas-3-positive, GFP-positive (loser) cells per disc at 24 hr ACI from A-B, and from the same genotypes (WT or egr3) at 48 hr ACI. Numbers of wing discs examined for each genotype is shown in the bars. P values were determined using 2-tailed t-tests with unequal variance. D. Image of a Myc-expressing clone, marked by expression of GFP (cyan), in a wildtype wing disc. Cas-3 positive cells are shown in red. D’ shows Cas-3 as a single channel. Larval genotype: yw hsflp/w; act > CD2STOP > Gal4, expressing “loser” cells in WT and in egr3 mutants. FLR recombinase, under heat shock (HS) control, was activated by HS of larvae in a 37°C water bath for 10 min, at 48 hr after egg laying (AEL). Post-HS, larvae were allowed to develop at 25°C for 24 or 48 hr. To generate Myc-expressing clones, act > y+STOP > Gal4 was used to generate act > Gal4 clones that expressed UAS-GFP and UAS-Myc in WT and in egr1 mutants. act > Gal4 clones were induced in larvae with a HS in a 37°C water bath for 6 min. Clones were allowed to grow in the wing discs for 24 or 48 hr, as described (de la Cova et al. 2004; Meyer et al. 2014; Alpar et al. 2018). The wing discs were then dissected from larvae at the indicated times after clone induction (ACI). A detailed protocol is available upon request.
Hemocyte immunohistochemistry and image processing

Hemocytes were collected from 10-20 experimental larvae, by bleeding from a small tear in the posterior cuticle into a 10-fold volume of PBS. Cells were then transferred to a coverslip and allowed to settle for 30 min at room temperature in a humidity chamber. All subsequent steps were performed directly on the coverslip. Cells were fixed in 4% PFA in PBS for 7 min at room temperature, washed 3 times in PBS, permeablized for 5 min in 0.1% Triton in PBS (PBT), blocked for 5 min in 10% normal goat serum (NGS) in PBT and then incubated with primary antibody in 10% NGS in PBT either overnight at 4°C or for 30 min at room temperature. Washes were carried out in PBT and secondary incubation was performed for 30 min at room temperature in 10% NGS in PBT. Cells were then washed 2 times in PBT, followed by 2 washes in PBS. A final 5 min incubation with DAPI in PBS was performed. Coverslips were mounted in Slowfade Gold Antifade (Molecular Probes, cat # S36937). Primary antibodies used were plasmatocyte-specific Mouse anti-P1 (1:100) (Kurucz et al. 2007). Secondary antibodies were Alexa Fluor 555 (1:500, Invitrogen). Images were collected on a Zeiss Axio Imager M1 and were processed using ImageJ and Adobe Photoshop.

Sequencing of egr mutant strains

Genomic DNA was isolated from homozygous adult female flies. 100 ng of DNA was amplified using the primer sets as described in Honti et al. 2013. The 355bp deletion, 5bp insertion and 6bp micro-deletion were found in the fragment amplified by P11189fw (CGCAGGAGCTACGATAATC) and P11189rev (AAGGAATGTGGACCACATA). The fragments were cloned into a pCR4-TOP-O-TA vector for sequencing using the common sequencing primers M13. Primer sequences are listed in Supplementary Table 3.

Outcrossing and genotyping of egr alleles

egr1 and egr3 alleles (hereafter egr) were treated identically. egr mutant virgin females were crossed to OregonR (OreR) males and the resulting F1 heterozygous egr/+ virgin females were backcrossed to OreR males. Ten F2 males (either egr/+ or +/) were singly crossed to virgin OreR females, killed and used for single-fly PCR (Gloor et al. 1993) to identify males carrying the egr alleles, but no longer carrying the 355bp deletion in NimC1. A single egr-positive, NimC1-negative line for each allele was carried forward by crossing F3 virgin females back to OreR males. This process was repeated four times, after which egr alleles were re-isolated by crossing to +; Sco/CyO actin-GFP, a chromosome II marker/balancer strain similarly crossed into an OreR background. These alleles were named egr1AG and egr3AG. Genotyping of egr1 and egr3 used primers flanking the reported deletion in each strain: Egr_F1 (CCAGAGCCCACTGTATCACC) and Egr_R3 (TCACCTCCTTTTGAACTCG) amplify a ~1500bp and ~2000bp fragment in egr1 and egr3, respectively. Genotyping of NimC1 used primers flanking the 355bp deletion and 5bp insertion between nucleotides 1582 to 1937 (Honti genome annotation). Nimrod_del_F1 (CCGGGCTACGTAATGAGAAA) and Nimrod_del_R1 (CAATTTGAGTGCGGAACCTC) amplify a 656bp fragment. Genotyping of NimC1 used primers flanking the 355bp deletion and 5bp insertion between nucleotides 1582 to 1937 (Honti genome annotation). Nimrod_del_F1 (CCGGGCTACGTAATGAGAAA) and Nimrod_del_R1 (CAATTTGAGTGCGGAACCTC) amplify a 656bp fragment.
fragment in WT animals and a ∼300bp fragment in animals bearing the NimC1 deletion. Primer sequences are listed in Supplementary Table 3.

**Statistical analysis**

T-tests were carried out using two tails of unequal variance.

**Data availability**

Strains and plasmids are available upon request. Supplementary Table 1 lists genotypes and sources of all Drosophila strains and sources and identifiers of other reagents that were used. Supplementary Table 2 lists the genotypes used in Figures 1, 2 and 3. Supplementary Table 3 contains sequences for the primers used in the study. All of this information is also included in the Reagent Table.

**RESULTS**

In the course of studying the role of Egr in cell competition, where apoptosis is induced in so-called “loser” cells, we found that dying cells appeared to accumulate in wing discs from egr3 mutant larvae (Figure 1A-E). That cell death was still induced in egr3 mutant loser cells suggested that Egr is not required for the cells to die under the two conditions we examined. However, because dead cells are typically cleared within 2-4 hr from wild-type wing imaginal disc epithelia (Milan et al. 1997), the accumulation of Cas-3 positive loser cells that we observed in egr3 mutants suggested that loss of egr might impair corpse clearance. This prompted us to examine plasmatocytes in the lymph glands, the major larval hematopoietic organ, from WT and egr3 mutant larvae. We immunostained the lymph glands from both genotypes with anti-NimC1 antibodies, a mixture of P1a and P1b antibodies that specifically recognizes the phagocytic plasmatocytes of the larva (Kurucz et al. 2007). As a control, we also examined larvae that carried Hml∆-DsRed, consisting of a hemocyte-specific enhancer/promoter from the Hemolectin gene fused to red fluorescent protein (DsRed) that identifies larval hemocytes (Makhijani et al. 2011) (Figure 2A). NimC1 is expressed at high levels on the plasma membrane of numerous cells in the primary lymph gland lobes from WT controls, and anti-NimC1 staining overlapped with many Hml∆-DsRed positive cells (Figure 2A, B). Strikingly, however, no NimC1 positive cells were evident in lymph glands from egr3 larvae (Figure 2C). As the egr3 and egr1 alleles were derived from the same parental strain (Igaki et al. 2002), we also tested lymph glands from egr1 larvae, and again found no detectable NimC1 expression (data not shown). To examine circulating plasmatocytes, we isolated hemocytes from larval hemolymph. Although NimC1 was readily observed in circulating hemocytes from OregonR (OreR) controls (Figure 2D), we detected no NimC1-positive hemocytes in the hemolymph from egr3/egr1 transheterozygous larvae, or from egr31 larvae (Figure 2E-F). egr31 is a precise excision of the ReggGS9830 p. element present in the parental strain. Thus all of the egr alleles derived from the ReggGS983 strain lacked circulating and lymph gland resident plasmatocytes that expressed NimC1.

Honti and colleagues reported that several Drosophila strains that were negative for NimC1 staining carried mutations in the NimC1 gene, which they postulated were scars of mobile element mobilization (Honti et al. 2013). Genomic sequencing of these P1-negative strains identified two independent micro-deletions in the NimC1 gene, including a 6 bp deletion between nucleotides 2264 to 2270 (Honti et al. 2013). Another deletion of 355 bp was found between nucleotides 1582 to 1937, accompanied by a 5 bp insertion. Together, Honti et al. 2013 found that the 355 bp deletion and the 5 bp insertion generated a frameshift mutation in both the NimC1 RA and RB transcripts, resulting in new sequences and a premature stop codon (Honti et al. 2013). The alterations were predicted to give rise to a truncated NimC1 protein that

---

**Figure 3 Summary scheme of mutations in the NimC1 locus in various egr mutants.** A. Schematic representation of Chromosome 2, where the NimC1 locus is located on the left arm (2L) and the egr gene on the right arm (2R). B. Representation of the NimC1 locus from yw122 flies, used as a WT strain. This sequence, and that from the outcrossed egr3AG strain, is identical to the reference genome (D. melanogaster version r5.23). PCR genotyping suggests that the OreR WT strain is also wild-type at the NimC1 locus. Numbering as in https://flybase.org/decoratedfasta/FBgn0259896. C. Representation of the NimC1 locus in the egr31 precise excision allele, the parental allele egrRegg1, and the egr1 and egr3 daughter strains. Two deletions, of 355 bp and 6bp, and an insertion of 5 bp, were found in egr1 and egr2. PCR genotyping in egr31 and the parental strain, egrRegg1 indicates that they also carry the 355 bp deletion (E); although not notated here, it is likely that they also carry the 5 bp insertion and 6 bp deletion. D. Representation of the NimC1 locus from Honti et al., 2013. Note that the locus numbering is slightly different than in B-D, presumably due to an earlier genome annotation. The mutations are identical to those found in egr1 and egr2 and similar to egr31 and the parental line, egrRegg1 (C). E. Gel electrophoresis of the results of PCR genotyping of NimC1 in the egr mutants indicated at top, using primers flanking the 355 bp deletion and 5 bp insertion between nucleotides 1582 to 1937 (Honti et al. genome annotation). Primer sequences are listed in Supplementary Table 3 and included in the Reagent Table.
lacks the intracellular and transmembrane domains and four extracellular NIM repeats, which would account for its absence on the plasma membrane of hemocytes (Honti et al. 2013).

To determine whether the egr<sup>l</sup> and egr<sup>r</sup> mutants carried mutations at the NimC1 locus, we carried out genomic sequencing of a 1254 bp region that encompasses most of the NimC1 open reading frame (Figure 3). Our data shows that both egr<sup>l</sup> and egr<sup>r</sup> contain identical microdeletions and insertions within the NimC1 gene, consistent with their common parental origin. Each mutant strain has the same 355 bp deletion, 5 bp micro-insertion, and 6 bp micro-deletion described by Honti et al. 2013 at residues 2264-2270 (Figure 3C). In addition, using primers flanking the larger, 355 bp deletion in PCR reactions, we found that both the Regg<sup>1G595630</sup> and egr<sup>31</sup> strains carried similar lesions (Figure 3E). Since these egr<sup>r</sup> mutants were both NimC1 negative (Figure 2E, F), they very likely also carry the premature stop codon generated by the 355 bp deletion and 5 bp insertion. Altogether, these results suggest that these NimC1 polymorphisms were present in the parental strain (Figure 3C).

To restore the wild-type NimC1 locus to the egr<sup>r</sup> mutants, we outcrossed both egr<sup>l</sup> and egr<sup>r</sup> to the OreR wild-type strain and isolated recombinants with the WT NimC1 locus and either the egr<sup>r</sup> or egr<sup>r</sup> mutation (see Methods). We then sequenced the NimC1 locus in these outcrossed egr alleles (hereafter called egr<sup>LAG</sup> and egr<sup>LAG</sup>) to verify that the recombination removed the mutant sequences. Both the egr<sup>LAG</sup> and egr<sup>LAG</sup> strains lacked the deletions and micro-insertions that characterized the original egr<sup>r</sup> and egr<sup>r</sup> alleles (Figure 3B, E). Consistent with the loss of the deletions, hemocytes from the egr<sup>LAG</sup> and egr<sup>LAG</sup> mutants regained NimC1 positivity (Figure 2G and data not shown).

**DISCUSSION**

Our sequencing data confirm that egr<sup>r</sup> and egr<sup>r</sup> mutants also carry mutations at the NimC1 locus, similar to those found previously in other Drosophila strains (Honti et al. 2013). Since the large deletion and micro-insertion in exon 3 of NimC1 also exist in the original parental line for the egr<sup>r</sup> and egr<sup>r</sup> alleles, egr<sup>LAG</sup>, and also in egr<sup>31</sup>, a precise excision of the Regg<sup>1G595630</sup> P-element, it is highly likely that the NimC1 mutations in each of these egr alleles are derived from the parental strain. These NimC1 mutations are recessive (Honti et al. 2013), and we speculate that their presence on each egr mutant chromosome in our experiments might explain the transient accumulation of dying cells; perhaps they also account for the infection susceptibility found previously in egr<sup>r</sup> mutants (Schneider et al. 2007). Consistent with our sequencing results, the genetic backgrounds of the egr<sup>r</sup> and egr<sup>r</sup> alleles were previously noticed to harbor anomalies that led to egr-independent susceptibility to infection by Gram-positive bacteria (Narasimamurthy et al. 2009). Complete deletion of NimC1 has been reported to prevent phagocytosis of latex beads or yeast zymosan particles by plasmatocytes (Melcarne et al. 2019b), but whether and how phagocytosis of dying cells may be impaired by the NimC1 mutations we found here remains to be determined. NIM repeats are thought to mediate protein-protein interactions and clustering of receptors is proposed to be key in phagocytic removal of apoptotic cells (Shklyar et al. 2013). The truncated mutant NimC1 proteins are aberrantly secreted into the hemolymph, as predicted (Honti et al. 2013), they could interfere with critical NIM interactions. As the egr<sup>l</sup> and egr<sup>r</sup> alleles have been used in numerous studies of immunity and cell death, it may be worthwhile to re-evaluate some of the phenotypes obtained with these alleles.

**ACKNOWLEDGMENTS**

We are grateful to Christina Cary for technical assistance, and members of the Johnston lab for advice. We thank Masayuki Miura, Tatsushi Igaki, Hiroshi Kanda and Iswar Hariharan for providing fly stocks, and István Andó for the gift of anti-NimC1 antibodies. We are indebted to the Bloomington Drosophila Stock Center (BDSC) (NIH P40OD018537) and Flybase (Attrill et al. 2015) for their services. Funding for this work was provided by the NIH R01GM078464 and NCI R01CA192838 (to LAJ) and the NSF (Graduate Research Fellowship to ARG). The egr<sup>LAG</sup> and egr<sup>LAG</sup> alleles, both WT for NimC1, were isolated by ARG in the lab of Iswar Hariharan and will be deposited at the BDSC for general use.

**LITERATURE CITED**

Abrams, J. M., K. White, L. I. Fessler, and H. Steller, 1993 Programmed cell death during Drosophila embryogenesis. Development 117: 29–43.

Alpar, L., C. Bergantinos and L. A. Johnston, 2018 Spatially Restricted Regulation of Spatzle/Toll Signaling during Cell Competition. Dev Cell 46: 706–719. https://doi.org/10.1016/j.devcel.2018.08.001

Attrill, H., K. Falls, J. L. Goodman, G. H. Millburn, G. Antonazzo et al., 2016 FlyBase: establishing a Gene Group resource for Drosophila melanogaster. Nucleic Acids Res. 44: D786–D792. https://doi.org/10.1093/nar/gkv1046

Chung, Y. S., and C. Kocks, 2011 Recognition of pathogenic microbes by the Drosophila phagocytic pattern recognition receptor Eater. J. Biol. Chem. 286: 26524–26532. https://doi.org/10.1074/jbc.M111.240007
de la Cova, C., M. Abril, P. Bellosta, P. Gallant, and L. A. Johnston, 2004 Drosophila myc regulates organ size by inducing cell competition. Cell 117: 107–116. https://doi.org/10.1016/S0092-8674(04)00214-4

Franc, N. C., P. Heitzler, R. A. Ezekowitz, and K. White, 1999 Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. Science 284: 1991–1994. https://doi.org/10.1126/science.284.5422.1991

Gloor, G. B., C. R. Preston, D. M. Johnson-Schlitz, N. A. Nassif, R. W. Phillis et al., 1993 Type I repressors of P element mobility. Genes 135: 81–95.

Honti, V., G. Cinge, G. Csordas, E. Kurucz, J. Zsamboki et al., 2013 Variation of NimC1 expression in Drosophila stocks and transgenic flies. Fly (Austin) 7: 263–266. https://doi.org/10.4161/fly.25654

Igaki, T., H. Kanda, Y. Yamamoto-Goto, H. Kanuka, E. Kuranaga et al., 2002 Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway. EMBO J. 21: 3009–3018. https://doi.org/10.1093/emboj/cdf306

Kocks, C., J. H. Cho, N. Nehme, J. Ulvila, A. M. Pearson et al., 2005 Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell 123: 335–346. https://doi.org/10.1016/j.cell.2005.08.034

Kurucz, E., R. Markus, J. Zsamboki, K. Fokli-Medzhirdzky, Z. Darula et al., 2007 NimroD, a putative phagocytosis receptor with EGF repeats in Drosophila plasmatocytes. Curr. Biol. 17: 649–654. https://doi.org/10.1016/j.cub.2007.02.041

Makhijani, K., B. Alexander, T. Tanaka, E. Rulifson, and K. Bruckner, 2011 The peripheral nervous system supports blood cell homing and survival in the Drosophila larva. Development 138: 5379–5391. https://doi.org/10.1242/dev.067322

Manaka, J., T. Kuraishi, A. Shiratsuchi, Y. Nakai, H. Higashida et al., 2004 Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by Drosophila hemocytes/macrophages. J. Biol. Chem. 279: 48466–48476. https://doi.org/10.1074/jbc.M408597200

Melcarne, C., B. Lemaitre, and E. Kurant, 2019a Phagocytosis in Drosophila: From molecules and cellular machinery to physiology. Insect Biochem. Mol. Biol. 109: 1–12. https://doi.org/10.1016/j.ibmb.2019.04.002

Melcarne, C., E. Ramond, J. Dudzic, A. J. Bretscher, E. Kurucz et al., 2019b ‘Two Nimrod receptors, NimC1 and Eater, synergistically contribute to bacterial phagocytosis in Drosophila melanogaster. FEB J. 286: 2670–2691. https://doi.org/10.1111/febs.14587
Meyer, S. N., M. Amoyel, C. Bergantinos, C. de la Cova, C. Schertel et al., 2014  An ancient defense system eliminates unfit cells from developing tissues during cell competition. Science 346: 1258236. https://doi.org/10.1126/science.1258236

Milan, M., S. Campuzano, and A. Garcia-Bellido, 1997  Developmental parameters of cell death in the wing disc of Drosophila. Proc. Natl. Acad. Sci. USA 94: 5691–5696. https://doi.org/10.1073/pnas.94.11.5691

Moreno, E., M. Yan, and K. Basler, 2002  Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the Drosophila homolog of the TNF superfamily. Curr. Biol. 12: 1263–1268. https://doi.org/10.1016/S0960-9822(02)00954-5

Narasimamurthy, R., P. Geuking, K. Ingold, L. Willen, P. Schneider et al., 2009  Structure-function analysis of Eiger, the Drosophila TNF homolog. Cell Res. 19: 392–394. https://doi.org/10.1038/cr.2009.16

Schneider, D. S., J. S. Ayres, S. M. Brandt, A. Costa, M. S. Dionne et al., 2007  Drosophila eiger mutants are sensitive to extracellular pathogens. PLoS Pathog. 3: e41. https://doi.org/10.1371/journal.ppat.0030041

Shklyar, B., F. Levy-Adam, K. Mishnaevski, and E. Kurant, 2013  Caspase activity is required for engulfment of apoptotic cells. Mol. Cell. Biol. 33: 3191–3201. https://doi.org/10.1128/MCB.00233-13

Somogyi, K., B. Sipos, Z. Penzes, E. Kurucz, J. Zsamboki et al., 2008  Evolution of genes and repeats in the Nimrod superfamily. Mol. Biol. Evol. 25: 2337–2347. https://doi.org/10.1093/molbev/msn180

Sonnenfeld, M. J., and J. R. Jacobs, 1995  Macrophages and glia participate in the removal of apoptotic neurons from the Drosophila embryonic nervous system. J. Comp. Neurol. 359: 644–652. https://doi.org/10.1002/cne.903590410

Communicating editor: C. Gonzalez