Drosophila TNF/TNFRs
At the crossroad between metabolism, immunity, and tissue homeostasis
Colombani, Julien; Andersen, Ditte S.

Published in:
FEBS Letters

DOI:
10.1002/1873-3468.14716

Publication date:
2023

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC-ND

Citation for published version (APA):
Drosophila TNF/TNFRs: At the crossroad between metabolism, immunity, and tissue homeostasis

Julien Colombani and Ditte S. Andersen

Department of Biology, University of Copenhagen, Copenhagen, Denmark

Correspondence
D. S. Andersen and J. Colombani, Department of Biology, University of Copenhagen, Universitetsparken 15, Building 3, 2100 Copenhagen, Denmark. Tel: +45 2785302. E-mail: ditte.andersen@bio.ku.dk and julien.colombani@bio.ku.dk

Ditte S. Andersen and Julien Colombani contributed equally to this article.

(Received 13 June 2023, revised 24 July 2023, accepted 25 July 2023, available online 26 August 2023)

do:10.1002/1873-3468.14716

Edited by Esther M. Verheyen

Tumor necrosis factor (TNF)-α is a highly conserved proinflammatory cytokine with important functions in immunity, tissue repair, and cellular homeostasis. Due to the simplicity of the Drosophila TNF-TNF receptor (TNFR) system and a broad genetic toolbox, the fly has played a pivotal role in deciphering the mechanisms underlying TNF-mediated physiological and pathological functions. In this review, we summarize the recent advances in our understanding of how local and systemic sources of Egr/TNF contribute to its antitumor and tumor-promoting properties, and its emerging functions in adaptive growth responses, sleep regulation, and adult tissue homeostasis. The recent annotation of TNF as an adipokine and its indisputable contribution to obesity- and cancer-associated metabolic diseases have provoked a new area of research focusing on its dual function in regulating immunity and energy homeostasis. Here, we discuss the role of TNFR signaling in coupling immune and metabolic processes and how this might be relevant in the adaptation of host to environmental stresses, or, in the case of obesity, promote metabolic derangements and disease.

Keywords: Drosophila; immunity; metabolism; TNF; tumor

Abbreviations
AMP antimicrobial peptides; AP1 activator protein 1; Atg autophagy-related protein; Avl Avalanche; BM basal membrane; BMP bone morphogenetic protein; Bnl branchless; Bsk basket; Btl Breathless; Cad99C cadherin 99C; Cas8 caspase 8; Cas9 CRISPR-associated endonuclease 9; CC cell competition; CIAP cellular inhibitor of apoptosis protein; CRISPR clustered regularly interspaced short palindromic repeats; CySCs somatic cyst stem cells; DD death domain; Dltp Drosophila insulin-like peptides; Dredd death-related ced-3/Nedd2-like caspase; DUOX dual oxidase; EBs enteroblasts; ECs enterocytes; EDA ectodysplasin; Egr Eiger; ERM ezrin/radixin/moesin; Fadd Fas-associating protein with death domain; FGFR fibroblast growth factor receptor; Gal4 galactose-responsive transcription factor 4; Grmd Grindelwald; GSC germ stem cell; GTase guanosine triphosphatase; IKK IkB kinase; IMD immune deficiency; InR insulin receptor; IPCs insulin-producing cells; IRS insulin receptor; SCs intestinal stem cells; lxB inhibitor of nuclear factor-κB; JNK c-Jun N-terminal kinase; lgl lethal-giant-larvae; MAPK mitogen-activated protein kinase; MAPKK MAPK kinase; MMP1 matrix metalloproteinase-1; myc myelocytomatosis oncogene; Mst mammalian sterile 20-like kinase; mTNF membrane-bound tumor necrosis factor; NADPH nicotinamide adenine dinucleotide phosphate; NF-κB nuclear factor-κB; NOPO no poles; PDGF platelet-derived growth factor; PGNs peptidoglycans; PGRPs peptidoglycan recognition proteins; Pvf1 PDGF- and VEGF-related factor 1; Rab Ras-associated binding protein; Rac1 Ras-related C3 botulinum substrate 1; Ras rat sarcoma viral oncogene homolog; RIPK1 receptor-interacting serine/threonine kinase 1; ROS reactive oxygen species; scribble; sTNF soluble tumor necrosis factor; TAB2 TGF-beta-activated kinase 1 and MAP3K7-binding protein 2; TACE tumor necrosis factor-α-converting enzyme; TAH tumor-adherent hemocytes; TAK1 transforming growth factor-β-activated kinase 1; TGF-β transforming growth factor-β; THD TNF homology domain; TNF tumor necrosis factor; TRAF tumor necrosis factor receptor; TOR target of rapamycin; TRADD TNFR1-associated death domain; TRAF2 TNFR-associated factor 2; TRIP TNFR-interacting protein; UAS upstream activating sequence; UBC13 ubiquitin-conjugating enzyme E2 13; UEV1a ubiquitin-conjugating enzyme E2 variant 1A; Upd unpaired; VEGF vascular endothelial growth factor; Wgn Wengen; Wnt wingless-related integration site; Zfh-1 zinc finger homeodomain protein 1.

2416 FEBS Letters 597 (2023) 2416-2432 © 2023 The Authors. FEBS Letters published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
The founding member of the tumor necrosis factor (TNF) superfamily, TNF-α, was first characterized as a macrophage-derived factor with the capacity to induce tumor necrosis, and hence was proposed to be part of an antitumor response [1]. Soon after, a serum-derived factor capable of triggering many of the metabolic derangements associated with cachexia, such as tissue wasting and weight loss, was discovered and named cachectin (reviewed in Ref. [2]). Only a decade later, in the wake of advances in molecular biology, TNF-α and cachectin were found to encode the same protein that is now commonly referred to as TNF-α or simply TNF [3,4]. Since then, several TNF-related ligands have been identified, and the mammalian TNF superfamily currently encompasses 19 TNF-related ligands each binding to one or more of the 29 related TNF receptors (TNFRs) to mediate diverse processes including cell proliferation, differentiation, apoptosis, and innate and adaptive immunity (reviewed in Ref. [5], Box 1). In contrast to the complex TNF-TNFR signaling network in mammals, the Drosophila TNF-TNFR system consists of one TNF ligand, Eiger (Egr), and two TNFRs, Wengen (Wgn) and Grindelwald (Grnd), and hence, flies provide a convenient genetic model for studying TNF-mediated processes in vivo. In the past two decades, work in Drosophila has shed light on the mechanism underlying the antitumor suppressor function of TNF/Egr and has identified previously unknown functions of TNF/Egr in coupling growth to environmental cues, coordinating tissue damage responses, and, more recently, revealed its implication in coupling immunity and metabolism in both physiological and pathological conditions. This review summarizes the progress that has been made in the field over the past two decades with a focus on the antitumor and tumor-promoting functions of TNF/Egr and its capacity to reprogram metabolism to cope with environmental stresses or, in certain contexts, promote metabolic disease.

The Drosophila TNF-TNFR system

The unique fly TNF, Egr, was identified in functional gain-of-function screens for molecules that induce apoptosis upon ectopic expression in the developing compound eye [6] and by two other studies employing molecular approaches [7,8]. Egr stands for ‘ectodysplasin (eda)-like cell death trigger’ as the TNF homology domain (THD) of Egr shares the highest homology with the human TNF-related ligand EDA-A2 [6–8]. Egr encodes a single transmembrane protein with an extracellular C-terminal THD present in other members of the TNF superfamily, and early studies showed

Box 1. TNF-TNFR signaling

The mammalian TNF-TNFR system encompasses 19 TNF-related ligands and 29 related TNF receptors. Depending on whether they possess a death domain (DD) or not, mammalian TNFRs are classified as death receptors or nondeath receptors, respectively. Nondeath receptors bind directly to TRAFs and activate signaling pathways implicated in cell survival, proliferation, and cytokine production, while death receptors bind adaptor proteins through their DD, which subsequently recruit TRAFs to activate proapoptotic and proinflammatory pathways. Hence, TNF-α employs TNFR2, a nondeath receptor, to promote cell survival and tissue regeneration, while it triggers apoptosis and inflammation through the death receptor, TNFR1.

In contrast to the complex mammalian TNF-TNFR signaling network, the fly TNF-TNFR system consists of one ligand, Egr, and two receptors, Grnd and Wgn. Intriguingly, while Grnd is localized at the apical membrane, the majority of Wgn resides in intracellular vesicles. However, recent studies suggest that Wgn cycles between the plasma membrane, where it might engage with Egr, and intracellular compartments. Binding of Egr to Grnd results in their internalization in vesicles, which precedes active signaling. Although Grnd does not possess a DD, it employs dTRAF2 to promote JNK-dependent apoptosis. Through its association with the subapical Crumbs complex, Grnd also couples loss of polarity with JNK-dependent neoplastic growth independently of its ligand, Egr. The function of Wgn has primarily been studied in the nervous system, where it was reported to have both Egr-dependent and Egr-independent functions. In tissues with high immune activity, such as the gut, Wgn employs dTRAF2 to suppress NF-κB-dependent immune processes, while it restricts dTRAF3-mediated lipolysis. The role of Wgn in coordinating immune activity and lipid catabolism may be relevant during an infection, where lipid catabolism could serve to fuel the energy-consuming task of fighting off pathogens. Furthermore, Egr and Wgn act in intestinal progenitor cells to promote infection-induced regenerative growth. It is tempting to speculate that Grnd, despite the absence of a DD, represents an ancestral death receptor, while Wgn, by virtue of its capacity to promote cell survival, proliferation, and regeneration, classifies as an ancestral nondeath receptor.
ligand trimers reminiscent of human TNF-TNFRs, the crystallographic structure of the extracellular positive vesicles[13].

That are labeled by Grnd and distinct from Wgn-vesicles, a prerequisite for Egr-induced apoptosis[11,12], was recently reported that the ectopic expression of developing eye compound[10]. Consistent with this, it imaginal disks (precursors of the adult wing) and the Egr to induce JNK-mediated apoptosis in both wing that Grnd, and not Wgn, is required downstream of foot of Mt. Eiger ([10], Box 2). The same study found that Grnd, and not Wgn, is required downstream of Egr to induce JNK-mediated apoptosis in both wing imaginal disks (precursors of the adult wing) and the developing eye compound [10]. Consistent with this, it was recently reported that the ectopic expression of Egr in wing disks results in its internalization in vesicles, a prerequisite for Egr-induced apoptosis [11,12], that are labeled by Grnd and distinct from Wgn-positive vesicles [13].

Despite poor homology of Grnd with other TNFRs, the crystallographic structure of the extracellular domain of Grnd in complex with Egr reveals a heterohexameric Egr:Grnd assembly around core ligand trimers reminiscent of human TNF-TNFR complexes [13]. While Wgn also engages with Egr in heterohexameric Egr:Wgn complexes, the affinity of Egr for Wgn is strongly reduced [13]. As a soluble form of Egr was used in this study, it is not clear whether the poor affinity of Egr for Wgn is specific for the soluble form of Egr [13]. Hence, while mammalian TNFR1 binds to both soluble TNF (sTNF) and membrane-bound TNF (mTNF) with high affinity, TNFR2 preferentially binds mTNF and displays the poor affinity for sTNF [14]. Interestingly, many of the Egr-dependent functions attributed to Wgn are in neurons and require a proximal source of Egr in neighboring cells, suggesting that Wgn might indeed preferentially bind membrane-bound Egr. In line with this, local, but not systemic, sources of Egr can activate Wgn in nociceptive sensory neurons to increase the nociceptive (pain) sensitivity response following UV-induced epidermal damage [15,16]. While Grnd is strongly expressed in developing epithelia, it displays a highly restricted expression pattern in neurons [17]. Therefore, there might be a tissue-specific requirement for Wgn to mediate Egr-dependent processes in the brain, which is also where Egr is the most highly expressed [18]. In line with this, Wgn is required in neurons to promote glia-derived Egr-mediated healing processes associated with brain injury as discussed below [15,19].
Physiological and pathophysiological functions of Egr

Role of Egr in host defense

Shortly after the identification of Egr, it was reported that egr mutant animals are differentially sensitive to infections [20,21]. While egr mutant animals are immunocompromised with respect to facultative extracellular pathogens, they display prolonged survival to facultative intracellular pathogens such as Salmonella typhimurium [20,21]. As the pathogenic load following an infection with S. typhimurium does not increase in egr mutants compared with wild-type flies, it was proposed that Egr might drive infection-associated pathogenesis [20,21]. This is reminiscent of mammalian TNF, which plays a pivotal role in innate and adaptive immunity but triggers septic shock that is detrimental to the host at high titers. Interestingly, knockdown of Egr specifically in the fat body recapitulates the prolonged survival to S. typhimurium infection observed for egr mutant animals, suggesting that fat body–derived Egr contributes to a systemic response that is fatal to the host [22].

Tissue healing and cytoprotective functions of Egr

Egr/TNF is triggered by tissue injury in both flies and mammals, and several studies in flies point to a key function of Egr in orchestrating the multiple processes associated with the healing response. Hence, in response to UV-induced epidermal damage, Egr is secreted by damaged epidermal cells and signals through Wgn in neighboring nociceptive sensory neurons to increase nociceptive (pain) sensitivity to increase withdrawal behaviors during tissue healing [15,16]. Another study showed that Egr is required to stimulate glial cell divisions in response to brain injury and programmed cell death, a phenomenon that triggers the elimination of excess neurons generated during development [19]. The physiological relevance of these newly generated glial cells is not known, but it was proposed that they might protect neural tissue by removing dead cells and/or secreting cytoprotective factors or simply by providing structural support to fill the void after neural loss [19]. A cytoprotective function of Egr was also observed in pigmented glial cells, where it suppresses reactive oxygen species (ROS)–dependent accumulation of peroxidated lipid droplets, which upon dissipation in aged animals cause glial and neuronal degeneration [23]. Contrary to this, degeneration of neuromuscular junctions caused by severe cytological stress of motoneurons is suppressed in egr mutant animals or upon depletion of Egr in the glia, suggesting that in the context of nonresolved axonal insult, Egr contributes to neuromuscular degeneration [18]. This is reminiscent of mammalian TNF that not only has a neuroprotective function in the glia but also causes neuroinflammation and degeneration in diseases such as Alzheimer’s and Parkinson’s disease [24]. In addition to its cytoprotective and cytotoxic properties, studies from the 80s noted a correlation between TNF titers and sleep patterns and established the role of TNF in promoting sleep [25,26]. Consistent with the evolutionary conserved role of TNF/Egr in regulating sleep, a recent study found that glia-derived Egr promotes sleep by acting nonautonomously on Wgn in neurons [27]. As cytokines are often upregulated following infections or fever, it was proposed that they might serve a beneficiary role by promoting sleepiness when sick [27].

Tumor suppressor functions of Egr

As mentioned above, TNF was originally proposed to be part of an antitumor response based on its capacity to induce tumor necrosis. In the past two decades, a flurry of studies has confirmed that Egr possesses antitumor suppressor functions, and the mechanism underlying Egr-mediated elimination of premalignant polarity-deficient cells has been particularly well characterized. While cells mutant of the scribble-group genes, scribble (scrib), lethal-giant-larvae (lgl), or disk large (dlg), are readily eliminated by apoptosis when surrounded by wild-type cells, they persist in whole mutant animals, where they form large neoplastic tumors (reviewed in Ref. [28]). This behavior fits the definition of cell competition (CC), which is thought to be triggered by mutations in genes that confer an advantage (supercompetition) or disadvantage (classical competition) to a subset of cells (Box 3). While loser cells are maintained in the tissue when surrounded by loser cells, for example, in whole mutant animals, they are readily eliminated when juxtaposed with fitter winner cells. Therefore, it was long considered that the elimination of polarity-deficient cells occurs through classical CC, although, as discussed below, this might not be the case [29].

The first hint that Egr/TNF signaling could be implicated in the elimination of polarity-deficient cells came from the observation that JNK is required to trigger apoptosis and elimination of clonal patches of scrib+/− mutant cells [30,31]. Shortly after, several independent studies showed that Egr is required for JNK activation and elimination of both dlg−/− and
Box 3. Cell competition

Cell competition can be thought of as a short-range fitness-sensing mechanism that ensures that suboptimal cells are eliminated from developing or adult tissues to ensure the overall fitness of the animal. Cell competition is underpinned by a fitness-sensing mechanism that detects differences in competitive parameters, such as growth rate, metabolic status, and mechanical properties, and triggers the elimination of the less fit cells (the loser cells) by apoptosis. Importantly, fitness is measured in relative terms, as less fit cells are eliminated when juxtaposed with fitter and faster growing cells (the winner cells), but survive in tissues that are entirely made up of loser cells. Therefore, wild-type cells can be turned into loser cells when surrounded by cells that have been given a growth advantage (referred to as supercompetitors). In this context, tumorigenic cells can be thought of as supercompetitors that grow at the expense of the surrounding wild-type tissue. Indeed, although cell competition is thought to be an ancient tumor suppressor mechanism that ensures the elimination of preoncogenic cells, tumors often hijack the very same mechanisms to promote tumor progression. While several signals and pathways that are involved in the recognition and elimination of loser cells have been identified, it is still not well understood how differences in relative fitness are sensed and coupled with these pathways to elicit differential outcomes in loser cells.

When these are assessable for ligand binding, hinting that the elimination of polarity-deficient cells might not occur by classical CC [29]. How could the discrepancy regarding the source of Egr be explained? [29,32] Interestingly, tumor-derived Egr activates platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) factor 1 (PvF1) downstream of JNK in wing disks, which acts nonautonomously to induce the proliferation of Spätzle-producing hemocytes. Hemocyte-derived Spätzle signals through Toll in adipocytes eliciting a nonautonomous program to induce cell death in polarity-deficient cells (Fig. 1A) [37]. Hence, Egr produced by polarity-deficient cells is essential for the amplification of hemocytes, which in turn triggers the elimination of polarity-deficient cells by providing a local source of Egr from adhering hemocytes and by activating a fat body–mediated response [32,37,38]. One intriguing possibility is that activation of Toll in the fat body triggers the production and/or secretion of Egr from adipocytes, which then facilitates the elimination of polarity-deficient or damaged cells when rupture of epithelial organization permits binding of Grnd from the basal surface facing the hemolymph (Fig. 1A). Consistent with multiple sources of Egr contributing to homeostatic processes such as cell death and wound healing, Egr is required in both wing disks, hemocytes, and the fat body for healing of mechanically disrupted wing disks [29].

While the elimination of premalignant cells by Egr is executed by JNK-mediated apoptosis, a recent study showed that if apoptosis is blocked by inhibition of effector caspases, Egr instead induces necrosis [39]. Similarly, inadequate initiator caspase 8 activity was reported to switch the outcome of TNF-α-TNFR1-mediated JNK-dependent signaling from apoptosis to necrosis, showing that the role of caspases in regulating apoptotic versus necrotic outcome is conserved from flies to mammals (reviewed in Ref. [40]). Li et al. [39] speculated that the switch between apoptotic and necrotic fates represents an ancient double-layered defense mechanism where, if apoptosis fails, necrosis ensures that premalignant or damaged cells are eliminated from the tissue.

Tumor-promoting functions of Egr

As discussed above, Egr constitutes a key component of an antitumor surveillance system that promotes elimination of premalignant cells from peripheral tissues; however, it can also be diverted into a protumor signal with deleterious consequences for the host. Hence, when an oncogenic form of Ras (RasV12) is expressed in polarity-deficient scrib−/− mutant cells,
Lessons from the *Drosophila* TNF/TNFR

(A) JNK-dependent elimination of polarity deficient *scrib*−/− cells

(B) JNK-dependent proliferation of *Ras*12, *scrib*−/− tumor cells
Fig. 1. (A) Loss of polarity triggers the autonomous production of Egr, which is required to induce Grnd/JNK-mediated production of Pvf1. Pvf1 signals to adjacent hemocytes to stimulate their proliferation and production of Egr and Spz. While hemocyte-derived Egr induces apoptosis in polarity-deficient cells, Spz binds to its receptor Toll in the fat body to increase levels of circulating Egr. Loss of polarity or mechanical wounding delocalizes Grnd to BM, making it accessible to hemocyte- and fat body-derived Egr. Binding of Egr to Grnd triggers the JNK-dependent expression of the proapoptotic genes, head involution defective (hid) and reaper (rpr), and elimination of polarity-deficient cells. Consistent with multiple sources of Egr contributing to homeostatic responses, such as wound healing or elimination of pre-malignant polarity-deficient cells, knockdown of Egr in wing disks, hemocytes, or the fat body compromises wound healing responses. (B) Expression of an oncogenic form of Ras (RasV12) in polarity-deficient scrib-/- cells suppresses the expression and activity of Hid and Rpr rendering RasV12/scrib-/- cells refractory to cell death. Instead, Egr derived from tumor-adherent hemocytes (TAHs) signals through Grnd to induce the JNK-dependent expression of matrix metalloproteinase 1 (MMP1), which promotes the invasive behavior of RasV12/scrib-/- tumors. Activation of JNK signaling also stimulates caspase-mediated ROS production, which acts as an attractant for hemocytes. In parallel, RasV12 drives growth and proliferation through the activation of myc and cyclin E (CycE), respectively.

these no longer undergo apoptosis, but instead produce massive tumors with an invasive and metastatic behavior [30,31]. The invasive behavior of RasV12/scrib-/- tumors is strictly dependent on Egr that signals through Grnd to active JNK signaling and the expression of the matrix metalloproteinase-1 (MMP1) (Fig. 1B). The JNK target MMP1 is required for the invasive behavior of RasV12/scrib-/- tumors, and therefore, in an egr mutant background, these tumors no longer invade neighboring tissues, although they still overgrow [32]. In this context, the source of Egr is provided by tumor-adherent hemocytes (TAHs), as knockdown of Egr specifically in the hemocytes prevents JNK activation and MMP1 expression [32]. Perez et al. later showed that the recruitment of hemocytes to RasV12/scrib-/- tumors involves an amplification feedback loop where the production of ROS downstream of JNK-mediated caspase activation attracts Egr-producing hemocytes, which further promotes tumor progression [38,41] (Fig. 1B).

Tumor cells are considered supercompetitors that grow at the expense of their wild-type neighbors triggering their elimination by apoptosis. Cells carrying an oncogenic form of Ras (RasV12) behave like supercompetitors massively overgrowing and expanding by triggering apoptosis in neighboring cells. Using RasV12 tumors to model the interaction between tumor cells and their microenvironment, Chabu et al. [42] found that oncogenic Ras elevates exocytosis of Egr, which not only permits oncogenic Ras cells to escape cell death but also triggers JNK-mediated cell death and cytokine production in surrounding cells, which further fuels tumor growth. Hence, a growth advantage of Ras tumors is achieved by exporting a proapoptotic signal to the surrounding tissues, which, in the process of dying, secretes cytokines that non-autonomously stimulate tumor expansion. Interestingly, the implication of Egr in supercompetition might be a more widespread phenomenon. It was recently demonstrated that JNK activation plays a role in metabolic reprogramming of loser (wild-type) cells, when these are juxtaposed with winner cells expressing higher levels of myelocytomatosis oncogene (myc) [43]. In short, Grnd/TNFR-mediated activation of JNK results in the metabolic rewiring of loser cells, which causes them to produce and transfer lactate to winner cells, a prerequisite for high-myc cells to acquire their winner status [43]. Although a requirement for Egr was not investigated in this study, these observations suggest that TNFR signaling might contribute to a mechanism whereby tissue fitness is optimized by eliminating cells with a metabolic disadvantage.

Role of TNF in modulating growth and adult tissue homeostasis

Recent research has revealed the role of Egr in regulating growth through local and systemic relays. One study showed that growth impairment in one compartment of the wing disk triggers Egr-Grnd-JNK-dependent production of ROS, which acts in a nonautonomous manner to reduce proliferation rates in the adjacent compartment allowing growth coordination across the tissue to ensure the development of adult structures with correct proportions [44]. In addition to its role in buffering local growth perturbations, Egr regulates growth systemically in response to nutrient stress. Agrawal et al. [17] found that in response to nutritional stress, Egr is secreted by the fat body and acts remotely on the insulin-producing cells (IPC) in the brain to suppress systemic insulin signaling and reduce larval growth. In this condition, cleavage of Egr into its soluble and secreted form is regulated by a metalloprotease of the tumor necrosis factor-α-converting enzyme (TACE) family, which is under the control of the nutrient-sensing target of rapamycin (TOR) pathway in the fat body [17].
In addition to its role in adapting developmental growth to internal perturbations or nutrient sparsity, Egr also influences adult tissue homeostasis. In response to prolonged protein deprivation, germ stem cell (GSC) numbers of the adult testis are maintained by nutrients released from dying spermatogonia, a process that is triggered by apoptosis of the surrounding somatic cyst cells and causes a strong reduction in the number of somatic cyst stem cells (CySCs) and early progenitors. Starvation-induced Egr in testicular smooth muscles plays an important role in mediating the recovery of CySCs and early cyst cells upon protein refeeding, a process that involves Grnd-mediated JNK signaling in somatic cyst cells [45]. Although muscle-derived Egr promotes fast recovery of CySCs upon refeeding, it also mediates the ectopic expression of Zfh-1 in differentiating cyst cells [45]. Zfh-1 expression is normally restricted to CySCs and early somatic cyst cells, and its expression in differentiating cyst cells nonautonomously impairs germ cell differentiation causing an accumulation of early germ cells [46]. Hence, Egr mediates both beneficial and detrimental effects during stem cell recovery from prolonged/chronic protein deprivation. Induction of Egr and ectopic Zfh-1 expression is only observed after prolonged starvation, and hence, it is possible that the Egr-mediated effects on Zfh-1 expression represent a pathophysiological response triggered by unresolved nutritional stress. Likewise, it was reported that Egr contributes to the reproduction-induced disruption of GSC homeostasis in aged, but not young, males [46]. The authors show that Egr is upregulated in the muscle sheath of the testes in mated aged males where it nonautonomously triggers the ectopic expression of Zfh-1 in differentiating cyst cells and accumulation of early immature progenitor cells [46]. Previous studies showed that reproduction is associated with a reduction in future fertility [47], and this led the authors to propose that Egr mediates both the reproduction-dependent disruption of GSC homeostasis and reduced fertility in aged males [46]. It is noteworthy that induction of Egr in response to both starvation and mating was only observed in aged males, suggesting that these processes must be coupled with age-associated events to trigger Egr-dependent disruption of testis homeostasis [45,46].

The adult gut is another adult tissue where Egr was reported to influence SC proliferation and tissue homeostasis. Egr is expressed in intestinal SCs (ISCs) and their immediate progenitors, enteroblasts (EBs), where it is required to mediate the proliferative regenerative response triggered by oral infection [48,49]. Although the expression of Egr is not triggered by infections, the ectopic expression of Egr in ISCs is sufficient to promote proliferation and increase tissue turnover in the absence of an infection and promote tumor formation upon aging [48,49]. Like Egr, Wgn/TNFR is required in progenitor cells to promote infection-induced regenerative growth, suggesting Egr acts through Wgn to promote ISC proliferation [50]. Furthermore, the misexpression of Egr in ISCs/EBs triggers the activation of JNK signaling in differentiating between EBs and early ECs, although the TNFR responsible for mediating this effect was not identified [48,49]. Hence, much like mammalian TNFs, Egr promotes regenerative growth and repair in adult tissues but is also a potent driver of dysplasia in aged individuals.

**TNF-TNFR signaling**

**Initiation of TNF-TNFR signaling**

Although the fly TNF-TNFR system is highly simplified compared with that in mammals, the molecular events underpinning Grnd and Wgn activation are only starting to emerge (Table 1). One of the best understood processes is the induction of apoptosis by the ectopic expression of Egr in imaginal disks. Here, Egr/TNF signaling is initiated by the binding of TNF/Egr to Grnd positioned at the apical membrane, which triggers their internalization into early endosomes, a step that precedes and is strictly required for JNK activation and apoptosis [11–13]. Hence, reducing levels of Ras-related C3 botulinum substrate 1 (Rac1), a small guanosine triphosphatase (GTPase) required for entry of Egr into early endosomes, suppresses Egr-induced JNK activation and apoptosis in the eye compound [11]. In contrast, trapping Egr in early endosomes by knocking down Rab5 or Rab7, both required for maturation of early endosomes into late endosomes, enhances Egr-induced apoptosis, suggesting that active Egr signaling requires the localization of ligand/receptor complexes in early endosomes [11,54]. In cultured mammalian cells, activation of the TNFR, p75NTR, triggers Rac1 activation, which is required for p75NTR-dependent JNK signaling and apoptosis [55], suggesting that the mechanism underlying TNF/TNF-mediated JNK activation is evolutionarily conserved. The position of Grnd at the apical membrane segregates it from circulating Egr/TNF derived from other tissues, and hence protects healthy cells from Egr-mediated apoptosis. As mentioned earlier, loss of polarity repositions Grnd at the basal membrane (BM) rendering it accessible to circulating Egr/TNF, thereby marking malignant cells for elimination [29]. Hence, Grnd signaling can be initiated both apically and...
basally depending on the source of Egr/TNF. Although Wgn is also expressed in imaginal disks, it is localized in intracellular vesicles and not required for Egr-mediated apoptosis. Consistent with this, the ectopic expression of Egr results in the internalization of Egr and Grnd in vesicles that are distinct from those labeled by Wgn [13]. While this shows that Grnd and Wgn have nonredundant cellular functions, the role of Wgn in mediating Egr-dependent processes in this tissue warrants further investigation. Importantly, Wgn was reported to mediate Egr-dependent processes in other tissues, where Grnd expression is highly restricted or absent, such as the brain and the gut. Common for all Wgn-mediated processes is that Egr is provided by a proximal source, which might reflect that Wgn displays a much-reduced affinity for soluble Egr compared with Grnd [13]. While Egr employs Grnd to mediate JNK-dependent proapoptotic responses, its activation of Wgn stimulates prosurvival behaviors and cytoprotective processes to combat infections and promote tissue healing ([15,16,50]; see below). Although the localization of Wgn in endosomes seems at odds with its function as a receptor for Egr, a fraction of Wgn is detected at the membrane

### Table 1. Overview of Eiger/TNF-dependent and Eiger/TNF-independent processes mediated by the two Drosophila TNFRs, Grindelwald and Wengen.

<table>
<thead>
<tr>
<th>Egr/TNF-dependent processes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNFR</strong></td>
</tr>
<tr>
<td>Grnd</td>
</tr>
<tr>
<td>Grnd</td>
</tr>
<tr>
<td>Grnd</td>
</tr>
<tr>
<td>Grnd</td>
</tr>
<tr>
<td>Grnd</td>
</tr>
<tr>
<td>Grnd</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>N.D.</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Egr/TNF-independent processes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNFR</strong></td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Wgn</td>
</tr>
<tr>
<td>Grnd</td>
</tr>
</tbody>
</table>

Grnd, Grindelwald; N.D., not determined; Wgn, Wengen.
upon forced expression, suggesting that it might be transiently available for ligand interaction [53]. Interestingly, in certain conditions, TNFRs are activated in ligand-independent fashions. Hence, Wgn controls developmental photoreceptor axon targeting through its association with the ezrin/radixin/moesin (ERM) family member, moesin, a process that does not depend on Egr [52]. In addition, as discussed in more detail below, Egr-independent functions of Wgn in regulating protein stability to restrict lipid catabolism in the gut and tracheal terminal cell differentiation are starting to emerge [50,53]. Likewise, knockdown of the Avalanche (Avl), a syntaxin required for an early step of endocytosis, results in JNK-driven neoplastic overgrowth, a process that does not require Egr [10]. Instead, the neoplastic growth triggered by Avl depletion is mediated by Grnd through its association with the subapical Crumbs complex [10]. In short, expansion of the apical domain triggered by Avl depletion permits the coupling of Crumbs with Grnd to promote JNK-mediated neoplastic growth in a ligand-independent fashion [10]. While mechanisms underlying ligand-independent TNFR activation are starting to emerge in flies, they have not been much studied in mammals. It will be important to address whether ligand-independent activation of TNFR signaling operates in mammals and if so, how this might interfere with anti-TNF therapeutic strategies.

**TNFR signaling pathways**

In mammals, binding of TNF to TNFR1 triggers their assembly into heterohexameric TNF:TNFR1 complexes consisting of trimers of ligands and receptors. Trimerization of TNFR1 permits binding of the adaptor protein, TNFR1-associated death domain (TRADD), and the subsequent recruitment of receptor-interacting serine/threonine kinase 1 (RIPK1), TNFR-associated factor 2 (TRAF2), and cellular inhibitor of apoptosis protein (cIAP) 1 and cIAP2 to form complex I (Fig. 2; reviewed in Refs. [5,40,56]). Of note, TNFR1 can bind RIPK1 independently of TRADD [57,58], and TNFR2, which does not possess a death domain (DD), binds directly to TRAF2 to recruit RIPK1 and cIAP1/2 [5]. TRAF2-mediated K63-linked ubiquitination of RIPK1 recruits the scaffolding proteins, transforming growth factor-β-activated kinase 1 (TAK1) and MAP3K7-binding protein (TAB) 2 and TAB3, and their binding partner TAK1. Upon its activation by autophosphorylation, TAK1 phosphorylates MAPKks and IκKβ to activate JNK/p38 and NF-κB signaling, respectively. The mechanism underlying TNF-mediated JNK activation is highly conserved in flies, where direct binding of TRAFs to TNFRs leads to the recruitment of dTAB2 and dTAK1 and activation of JNK (Fig. 2; reviewed in Refs. [6–8,59]). While TNF signaling plays a key role in NF-κB-mediated immunity in mammals, the ectopic expression of Egr does not trigger NF-κB signaling in flies. Therefore, the immune deficiency (IMD) pathway, which is activated upon binding of bacterially derived peptidoglycans to peptidoglycan recognition proteins, was long considered to be the principal regulator of Relish/NF-κB-mediated immunity (reviewed in Ref. [60,61]). Nevertheless, IMD is homologous to RIPK1, and the IMD pathway bears a lot of resemblance with the mammalian TNF/NF-κB pathway, suggesting that this pathway might be regulated by TNF/Egr and TNFRs (Fig. 2). Indeed, we found that knockdown of Wgn/TNFFR and dTRAF2 in enterocytes (ECs) of the adult gut activates JNK and Relish/NF-κB signaling to promote antimicrobial peptide (AMP) expression in an dTAK1- and Relish/ NF-κB-dependent manner [50]. Similarly, knockdown of Egr/TNF in progenitor cells, but not ECs, triggers the expression of AMPs, suggesting that progenitor-derived Egr signals nonautonomously through Wgn in ECs to restrict immunity [50]. Hence, the role of TNFRs in regulating innate immunity is conserved between flies and mammals, although the mechanism underlying TNF-mediated activation of dTAK1/TAK1-Relish/NF-κB differs. While binding of TNFs to TNFRs triggers the activation of immunity in mammals, Egr/TNF-Wgn/TNFR-dTRAF2 signaling restricts dTAK1-NF-κB/JNK-mediated immune responses in homeostatic conditions (Fig. 2).

**Metabolic functions of Egr/TNF**

In addition to its well-described proinflammatory properties, TNF is now emerging as an important metabolic messenger with the capacity to rewire systemic metabolism (reviewed in Ref. [62]). As mentioned earlier, TNF was originally identified as a cachectic factor based on its capacity to induce tissue catabolism and fatal weight loss (reviewed in Ref. [2]). Since then, several studies have established that TNF produced by the tumor and/or its microenvironment promotes many of the metabolic derangements associated with cachexia in later stages of cancer, including altered glucose metabolism, lipid atrophy, and muscle wasting [63]. A recent study showed that Wgn/TNF signaling mediates cachectic muscle wasting in RasV12/scribble−/− tumor-bearing animals, suggesting that the cachexia-promoting functions of Egr/TNF are conserved between flies and mammals [51]. In addition to its
cachectic properties, TNF also plays a role in the metabolic reprogramming underlying obesity-associated inflammatory diseases such as type 2 diabetes. In obese individuals, elevated TNF production in adipose tissues suppresses insulin sensitivity directly by inhibiting insulin-mediated phosphorylation and activation of its receptor (InR) and its binding partner, insulin receptor substrate (IRS), and indirectly by preventing the uptake of free fatty acids (FFAs), which further promotes insulin resistance in other tissues [64–67]. The capacity of TNF to trigger insulin resistance in peripheral tissues depends on JNK, as mutations in jnk1 alleviate the insulin resistance and adiposity in obese mice [68]. The adverse effects of TNF-α on metabolism raise the question of how metabolic properties of a proinflammatory cytokine might be physiologically relevant outside a pathological context. Several observations in flies suggest that Egr/TNF signaling might be part of a multifaceted stress response that adapts host metabolism to environmental stressors. As mentioned earlier, Egr is produced by the fat body in response to dietary restrictions and acts remotely on the IPCs in the brain to suppress Drosophila insulin-like peptide (Dilp) release and slow down larval growth [17]. The suppression of Dilp release is mediated by Grnd-dependent JNK activation in the IPCs [17]. Interestingly, JNK-mediated suppression of endocrine insulin signaling is also triggered in response to paraquat-induced oxidative stress in both larvae and adult flies, which opens the possibility that Egr might regulate

Fig. 2. Schematic showing the similarities between the Drosophila and mammalian TNF/TNFR pathways. Functional homologs are indicated by the same colors. Binding of TNF-α to TNFR1 triggers JNK/p38- and NF-κB-dependent survival and immune processes, respectively. In flies, Egr/TNF signaling and Wgn/TNFR signaling restrict immunity and cell proliferation in homeostatic conditions in highly active immune tissues, such as the adult gut. Egr is required nonautonomously in progenitor cells and possibly other tissues to suppress immunity in the gut [50]. While the IMD pathway might not have a regulatory function in homeostatic conditions, binding of bacteria-derived peptidoglycans to their receptors, peptidoglycan recognition proteins, activates the IMD-dTAK1-NF-κB pathway to promote immune processes in response to infections (red arrows). Most likely, this is accompanied by a derepression of Wgn-dTARF2-mediated dTAK1 inhibition, as the overexpression of dTARF2 suppresses infection-induced antimicrobial peptide expression [50].
growth and metabolism in response to a wide range of environmental stressors [69].

**Role of TNFRs and TRAFs in immunometabolism**

Another context where coupling proinflammatory responses with metabolic adaptations might be relevant is during an infection, where the transient mobilization of stores can be employed to fuel the energy-consuming task of mounting an immune response. Interestingly, the *Drosophila* TNFR-associated factor, dTraf3, was recently reported to couple energy expenditure with immunity in the adult gut [70]. Hence, the ectopic expression of dTraf3 in ECs triggers autophagy-mediated lipolysis to generate nicotinamide adenine dinucleotide phosphate (NADPH), which is required to produce ROS by the NADPH oxidase, dual oxidase (DUOX), and for host resistance to enteric infection [70]. The accumulation of dTraf3 in intracellular Cad99C-positive vesicles, a step that is required for dTraf3-mediated ROS production, is triggered by enteric infections allowing reallocation of energy to fuel immune processes in this condition [70]. Interestingly, dTraf3 also accumulates in intracellular vesicles upon knockdown of Wgn or the ubiquitin E3 ligase, NOPO [TNFR-interacting protein (TRIP) in mammals], in the absence of infection, and this triggers dTraf3-dependent lipid catabolism [50,70]. The role of Wgn in restricting dTraf3-mediated lipolysis does not depend on Egr, as egr null mutant animals do not display lipid depletion of the gut [50] (Fig. 3). Although it is not clear how Wgn controls dTraf3 levels, TNFRs were reported to interact with both TRAFs and TRIP, the mammalian homolog of NOPO, suggesting that Wgn might promote NOPO-mediated degradation of dTraf3 by bringing dTraf3 into proximity of NOPO. Interestingly, a recent study reported that Wgn is required in the embryonic tracheal system to promote degradation of the fibroblast growth factor receptor (FGFR), Breathless (Btl) [53]. Hence, knockdown of Wgn results in the accumulation of Btl bound to its ligand, branchless (Bnl), in intracellular vesicles triggering Bnl/Btl-dependent terminal cell differentiation [53]. This suggests that Wgn, and possibly other TNFRs, could have a more general function in regulating protein localization and stability and thereby controlling a broad range of processes independently of their canonical ligands. As mentioned above, EC-specific depletion of Wgn also activates Relish/NF-κB-dependent antimicrobial production [50], and hence, it is tempting to speculate that Wgn/TNFR behaves as a metabolic switch that triggers metabolic reprogramming toward energy expenditure in conditions associated with elevated immune activities (Fig. 3). The observation that EC-specific knockdown of Wgn reduces lifespan [50] shows that Wgn/TNFR signaling is required to maintain immunometabolism homeostasis in homeostatic conditions. Given that many of the functions attributed to TNFRs are
evolutionarily conserved, it will be interesting to test whether mammalian members of the TNFR superfamily might carry out similar protective functions in the gastrointestinal tract of healthy individuals. dTRAF2 (TRAF6 in mammals), another TNFR-associated factor, was recently reported to regulate cellular metabolism through its interaction with the autophagy protein Atg9 in response to oxidative stress [71]. Hence, infection- or oxidative stress–induced association of dTRAF2 with Atg9 triggers JNK-mediated autophagy and tissue repair in the adult gut [71]. In mammalian cells, TRAF6 and mAtg9a also interact and couple oxidative stress with JNK activation, suggesting a wider and evolutionarily conserved implication of TRAFs in coupling energy expenditure with immune and tissue repair processes [71]. As TRAFs have been identified as downstream adaptors of several receptor families with immunoregulatory functions, including TNFRs, Toll-like receptors, the interleukin-17 receptor, they likely coordinate immune and metabolic responses in a variety of conditions, and how TNF- and/or TNFR-dependent inputs contribute to immunometabolism homeostasis will be an exciting future direction of research.

Conclusions
The past two decades of fly research have proved successful in characterizing Egr/TNF-dependent processes adding new properties to a long list of functions attributed to this pleiotropic cytokine. Using genetic approaches, flies have significantly improved our understanding of how TNF/Egr detects and eliminates premalignant cells and how different sources contribute to this process. The capacity of TNF/Egr to eliminate cancerous cells by apoptosis or necrosis in both flies and mammals suggests that TNF represents a highly conserved antitumor surveillance system and confirms the antitumor properties that led to its initial discovery. TNF-mediated surveillance extends beyond its capacity to detect premalignant cells, as TNFs are triggered by tissue injury and external threats, such as malnutrition and pathogenic infections, to promote host recovery. Interestingly, recent research suggests that they do so not only through their direct action on immune processes and tissue repair but also indirectly by promoting sleep or heightening nociceptive sensitivity and avoidance behavior to facilitate wound healing. While Egr/TNF-mediated stress responses have evolved to cope with local growth perturbations or pathogenic intruders, it can, in the absence of temporal resolution, provoke systemic responses with fatal outcomes for the host. One illustration of this is when Egr/TNF-producing immune cells are hijacked by tumors that have become refractory to apoptotic effector to promote their growth and metastatic behavior.

Landmark studies from the 90s showing that adipocyte-derived TNF-α promotes obesity-associated insulin resistance gave birth to a new area of research referred to as immunometabolism. The emergence of immunometabolism, defined as the capacity of immune-derived signals to rewire the metabolic program of nonimmune cells, provided a framework to study the metabolic properties of TNF-α in physiological and pathological conditions. While studies in mammals have focused on the role of adipocyte-derived TNF-α as a driver of obesity-associated metabolic diseases, genetic approaches in flies have provided further insight into the metabolic properties of Egr/TNF, and how these contribute to physiological processes in non-pathological conditions. Studies in flies have uncovered the physiological role of Egr in adapting growth to internal perturbations and external stresses through an evolutionarily conserved coupling between Egr/TNF and insulin signaling. Intriguingly, recent findings point to a key function of Wgn/TNFR and TRAFs in coupling energy homeostasis with immune processes in the adult gut. This is likely to be relevant during an infection where energy stores are mobilized to fuel energetically costly affair of mounting an immune defense. These findings provide a framework for exploring whether other members of the TNFR superfamily regulate immunometabolism in the gastrointestinal tract, and whether this occurs in a ligand-dependent or ligand-independent fashion. There is no doubt that the emerging metabolic properties of Egr/TNF have paved the road for exploring its role in immunometabolism, which will be an exciting future area of research.

Future perspectives
While significant progress has been made in our understanding of how Egr/TNF couples local and systemic cues with spatially controlled activation of TNFRs, it is becoming increasingly clear that Drosophila TNFRs regulate a diverse range of processes independently of their canonical ligand. The observation that Wgn/TNFR controls Btl and dTRAF3 localization/stability independently of Egr/TNF in two unrelated tissues raises the exciting possibility that this might be a more general function of Wgn/TNFR [50, 53]. However, the mechanism underpinning Wgn/TNFR-mediated effects on protein localization/stability warrants further investigation. First, it is not clear how Wgn/TNFR-dependent effects on dTRAF3/Btl are regulated. The
overexpression of Wgn/TNFR does not rescue the infection-induced accumulation of dTRAF3 [50], suggesting that additional upstream signals are required to fully activate Wgn/TNFR-dependent effects on dTRAF3 localization/stability. Identifying such signals will be essential for deciphering the physiological and pathophysiologival conditions, where Egr/TNF-independent Wgn/TNFR-mediated effects might be relevant. Second, what are the molecular events involved in Wgn/TNFR-mediated dTRAF3 degradation, and in which compartment does this take place? As previously mentioned, the E3 ubiquitin-ligating enzyme, NOPO/TRIP, was recently reported to promote lysosome-dependent degradation of dTRAF3, and hence represents a promising candidate for mediating Wgn/TNFR-dependent dTRAF3 degradation [70]. Future research should aim at resolving in which compartment Wgn/TNFR resides and whether it employs NOPO/TRIP or another enzymatic activity to promote protein degradation. Third, how does Wgn/TNFR exert its opposing effects on different TRAFs within the same tissue? The observation that Wgn/TNFR activates dTRAF2 to restrict immune processes, while it suppresses dTRAF3 activity to limit lipid catabolism, suggests that Wgn/TNFR might recruit different ubiquitinating/deubiquitinating enzymes to promote or suppress protein stability. In line with this, the deubiquitinating enzyme, cylindromatosis (CYLD), was previously found to promote dTRAF2 stability by reducing ubiquitination-mediated degradation [72]. One possibility is that Egr/TNF triggers a conformational change in Wgn/TNFR favoring the binding of dTRAF2, and possibly the exchange of a ubiquitinating enzyme for a deubiquitinating enzyme, for example, CYLD, to promote dTRAF2 signaling. To fully understand how Wgn/TNFR carries out its Egr/TNF-dependent and Egr/TNF-independent functions, it will be essential to characterize the composition and enzymatic activity of the complexes assembled by Wgn/TNFR in the presence or absence of Egr/TNF. The mechanistic insight gained from resolving how Wgn/TNFR integrates Egr/TNF-dependent and Egr/TNF-independent cues to promote separate downstream responses will set the scene for exploring whether these findings can be extrapolated to other members of the TNFR superfamily. Given the broad use of anti-TNF drugs to treat various inflammatory conditions, it will be highly relevant to know whether TNF antagonists target only a subset of TNFR-mediated processes, and if so, whether decoupling of TNF-dependent and TNF-independent processes might provoke adverse effects.

Acknowledgements

DSA is funded by H2020 European Research Council (grant number 803630) and Novo Nordisk Foundation (grant number NNF18OC0033920). JC is funded by Novo Nordisk Foundation (grant number NNF200C0065395).

Author contributions

All the authors reviewed the available literature and wrote the manuscript.

Data accessibility

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

Lessons from the Drosophila TNF/TNFFR

J. Colombani and D. S. Andersen

30 Brumby AM and Richardson HE (2003) Scribble mutants cooperate with oncogenic Ras or notch to cause neoplastic overgrowth in drosophila. EMBO J 22, 5769–5779.


