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Leptin- and cytokine-like unpaired signaling in *Drosophila*

Meet Zandawala [a,b,*], Jayati Gera [a]

[a] Neurobiology and Genetics, Theodor-Boveri Institute, Biocenter, University of Würzburg, 97074, Würzburg, Germany
[b] Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, 89557, USA

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ABSTRACT

Animals have evolved a multitude of signaling pathways that enable them to orchestrate diverse physiological processes to tightly regulate systemic homeostasis. This signaling is mediated by various families of peptide hormones and cytokines that are conserved across the animal kingdom. In this review, we primarily focus on the unpaired (Upd) family of proteins in *Drosophila* which are evolutionarily related to mammalian leptin and the cytokine interleukin 6. We summarize expression patterns of Upd in *Drosophila* and discuss the parallels in structure, signaling pathway, and functions between Upd and their mammalian counterparts. In particular, we focus on the roles of Upd in governing metabolic homeostasis, growth and development, and immune responses. We aim to stimulate future studies on leptin-like signaling in other phyla which can help bridge the evolutionary gap between insect Upd and vertebrate leptin and cytokines like interleukin 6.

1. Introduction

Animals have evolved a multitude of signaling pathways that enable them to coordinate diverse physiological processes. This signaling is paramount to tightly regulate systemic homeostasis and is mediated by various families of peptide hormones and cytokines that are conserved across the animal kingdom. *Drosophila* has served as an excellent genetic model for deciphering conserved hormonal signaling pathways including insulin/insulin-like growth factor (IIS), Neuropeptide Y/F (NPY/F), and glucagon-like pathways amongst others. These pathways have been extensively reviewed previously so the readers are referred to the following reviews for additional details (see Ikeya et al., 2002, Rulifson et al., 2002, Colombani et al., 2003, Kim and Rulifson 2004, Lee and Park 2004, Broughton et al., 2005, Baker and Thummel 2007, Bharucha et al., 2008, Gronke et al., 2010, Haselton and Fridell 2010, Droujinine and Perrimon 2016, Nässel and Vanden Broeck 2016, Schoofs et al., 2017, Nässel and Zandawala 2019, Ahmad et al., 2020). We instead focus on leptin- and cytokine-like signaling in insects with a particular emphasis on recent advances in *Drosophila*.

Leptin, a product of the *obese* gene, was discovered in mice and humans in 1994 (Zhang et al., 1994). This landmark discovery of a hormone predominantly known for its role in regulating energy balance and body weight provided important insights into the genetic basis of obesity and metabolic diseases. Subsequent discoveries of leptin and its functions in other vertebrates have now established it as an anorexigenic hormone (see Ahima et al., 1996; Villanueva and Myers 2008, Denver et al., 2011; Zhou and Rui 2013, Cui et al., 2014; Michel et al., 2016). However, the presence of a direct leptin homolog in invertebrates, especially insects, is somewhat debatable. While the *Drosophila* genome does not encode a leptin ortholog, it contains three related unpaired (Upd) ligands (Upd1, Upd2 and Upd3) which are structurally similar to type I cytokines. Roles of Upd2 in regulating growth and altering energy metabolism have inevitably led to comparisons with leptin (Rajan and Perrimon 2012). Thus, we refer to Upd signaling in *Drosophila* as leptin-like to distinguish between orthology and functional analogy.

In this review, we provide an overview of the current knowledge on leptin-like signaling in *Drosophila*, emphasizing the conservation of key molecular components and their interplay with other metabolic pathways such as insulin signaling. We focus on the roles of Upd in governing metabolic homeostasis, growth and development, and immune responses. In addition, we summarize the tissue expression of Upd in *Drosophila* based on publicly available datasets. We aim for this review to motivate additional functional studies on leptin-like signaling in insects and other phyla which can help bridge the evolutionary gap

* Corresponding author. Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, 89557, USA.
E-mail address: mzandawala@unr.edu (M. Zandawala).

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between insect Upd and vertebrate leptin and interleukin 6 (IL-6).

2. Components of the Upd signaling pathway and their expression

2.1. Upd signaling pathway

Drosophila possesses all components of the Upd-mediated JAK/STAT (Janus kinases and signal transducer and activator of transcription proteins) signaling pathway that are found in mammals, but with reduced redundancy (Fig. 1) (Levy 1999). This lack of genetic redundancy makes *Drosophila* an excellent model to decipher the roles of this pathway during normal physiological and pathological conditions (Arbouzova and Zeidler 2006).

Drosophila has three JAK/STAT ligands called Upd1 (Harrison et al., 1998), Upd2 (Gilbert et al., 2005; Hombria et al., 2005) and Upd3 (Agaisse et al., 2003; Wright et al., 2011). Upd proteins are structurally and functionally similar to vertebrate leptins (Hombria et al., 2005; Rajan and Perrimon 2012, Londraville et al., 2017). All three Upd ligands bind to a single receptor called Domeless (Dome), which has sequence and functional similarities with mammalian cytokine class I receptors like the IL-6 receptor (Brown et al., 2001; Chen et al., 2002). For instance, while structural alignment of human leptin and Upd2 only has 8% homology, 9 out of 20 amino acids in leptin which contributed to receptor binding are also conserved in Upd2 (Londraville et al., 2017). Genes encoding Upds are clustered on the X chromosome and have no obvious homologs outside of *Drosophila* species (Londraville et al., 2017). However, their receptor Dome does have invertebrate homologs (Londraville et al., 2017). The endogenous ligands of Dome in non-*Drosophila* species are yet unknown.

Binding of any Upd ligand to Dome causes receptor dimerization as well as activation of hopscotch, a JAK tyrosine kinase (Binari and Perrimon 1994). Receptor dimerization brings JAK in close proximity to each other, allowing for transphosphorylation of tyrosine residues in JAK, thus activating them. Activated JAKs then subsequently phosphorylate tyrosine residues on the cytoplasmic portion of the receptor, which serves as a docking site for Src homology 2 domains in STAT (Hou et al., 1996; Yan et al., 1996). Next, JAKs phosphorylate STATs allowing them to form dimers and translocate into the nucleus. Lastly, STAT dimers bind to a palindromic response element and activate target gene expression (Hou et al., 1996; Yan et al., 1996) (Fig. 1). This signaling pathway is conserved in vertebrates; however, vertebrates possess four copies of JAK, seven STATs, and multiple ligands (Hu et al., 2021).

2.2. Upd signaling – cytokines vs hormones

Given the structural similarity of Upd with cytokines and functional similarity with a hormone, it is worthwhile to first compare these two modes of intercellular signaling (**Table 1**). In the strictest sense, hormones are signaling molecules secreted into the circulation by endocrine glands. Hormones target the brain and peripheral tissues to modulate

Table 1
Comparison between cytokines and hormones.

	Hormone	Cytokine
Definition	Intercellular signaling molecules modulating diverse physiology and behavior	Intercellular signaling proteins mediating immune responses
Type	Peptides, proteins, steroids, biogenic amines, etc.	Proteins
Typical source	Endocrine glands, neuroendocrine cells and peripheral tissues	Immune and non-immune cells
Drosophila homolog	Unpaired 1 (Upd1) and 2 (Upd2)	Unpaired 3 (Upd3)
Vertebrate homolog	Leptin	Interleukin 6 (IL-6)

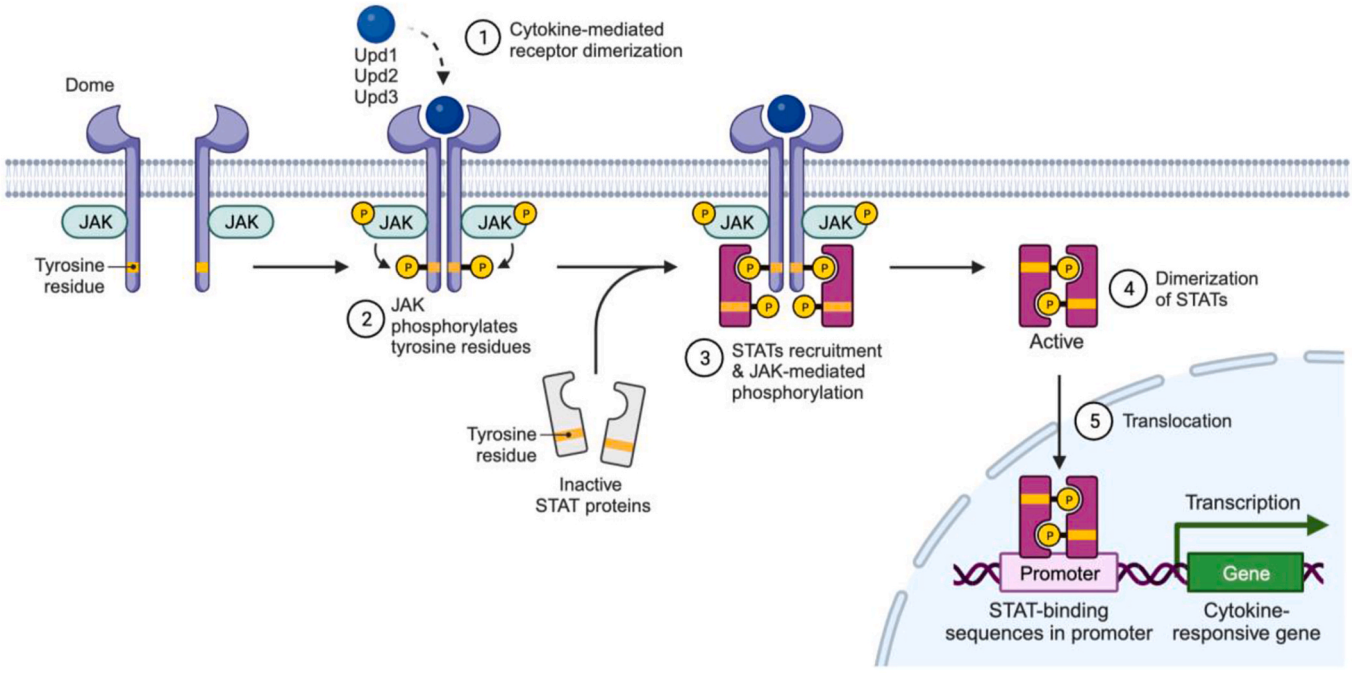

Fig. 1. Upd ligands activate the JAK-STAT signaling pathway. *Drosophila* unpaired 1, 2 and 3 (Upd1, Upd2 and Upd3) act as ligands for the cytokine receptor Domeless (Dome). Binding of Upd ligands to Dome (1), results in receptor dimerization and activation of intracellular Janus kinases and signal transducer and activator of transcription proteins, commonly referred to as the JAK-STAT pathway. Specifically, receptor dimerization causes transphosphorylation of tyrosine residues in JAK which increases their kinase activity. JAK then phosphorylates tyrosine residues on the receptor (2). This creates binding sites for STAT proteins which are recruited and phosphorylated by JAK (3). Following their dissociation, STATs undergo homo- or heterodimerization (4). Finally, STAT dimers translocate to the nucleus and induce gene transcription after binding to regulatory sequences (5). Adapted from "Cytokine Signaling through the JAK-STAT Pathway", by Iwasaki, A. (2020). Retrieved from https://app.biorender.com/biorender-templates/t-5fac3e99614e0c00aac4a356-cytokine-signaling-through-the-jak-stat-pathway.

diverse aspects of physiology and behavior. The molecular identity of hormones ranges from peptides and proteins to steroids and eicosanoids to biogenic amines. In contrast, cytokines are proteins which mediate immune responses following their release from immune and non-immune cells. However, this characteristic is not exclusive to cytokines, as hormones such as NPY can also be produced by immune cells (Schwarz et al., 1994) and mediate immune responses (Yu et al., 2022a). In addition, hormones can also be produced outside of endocrine glands by neuroendocrine cells and peripheral tissues (e.g. by the gut and adipocytes). Given the lack of a clear distinguishing feature between these modes of intercellular signaling, it is sometimes difficult to unambiguously categorize signaling molecules such as Upd. Nonetheless, Upd1 and 2 can be generally regarded as hormones and Upd3 a cytokine **(Table 1)** based on their functions discovered so far (see section 3).

2.3. *Upd expression in the brain and other tissues*

The source of Upd proteins has been investigated previously based on functional studies, genetic reagents, *in situ* hybridization, and antibodies (Wang and Huang 2009; Johnstone et al., 2013; Beshel et al., 2017; Recasens-Alvarez et al., 2017; Powers and Srivastava 2019). Antibody against Upd1 revealed sparse expression in the lateral brain neurons (Beshel et al., 2017). While an *upd2* promoter-based Gal4 was recently generated, its expression was only examined in the gut (Zhai et al., 2018). LacZ-based reporters have been commonly used to reveal *upd3* expression in diverse cell types (Zhou et al., 2013; Bunker et al., 2015; Zhai et al., 2018; Romao et al., 2021), including in pericardial nephrocytes (Gera et al., 2022) (Fig. 2A). *In situ* hybridization revealed co-expression of *upd3* with Dome in lymph glands of third instar larvae (Makki et al., 2010) (Fig. 2B). Using a similar approach, both *upd1* and *upd3* were shown to be expressed in eye imaginal discs and adult ovaries (Wang et al., 2014). While these studies using different approaches showcase broad expression, comprehensive mapping of Upd ligands (transcripts or proteins) have not yet been performed. Hence, we utilize the Fly Cell Atlas (Li et al., 2022) database to provide an overview of *upd and dome* expression across fly tissues (Fig. 2C). Based on our analysis, *upd1* is expressed in the testis and Malpighian tubules, *upd2* in the fat body, and *upd3* is expressed in the heart, trachea, gut and testis (Fig. 2C). In contrast, *dome* is highly expressed throughout the body, suggesting wide-spread effects of unpaired signaling. Examining further at cellular level, *upd1* expression is highest in the epidermal cells (Fig. 2D). *Upd2* appears to be broadly expressed, with highest expression in epidermal cells. It is also expressed in motor neurons, glial cells, hemocytes and oenocytes amongst other cell types. Lastly, *upd3* is also expressed in several cell types including ovarioles and hemocytes. *Upd1, upd2* and *upd3* are highly or moderately expressed in muscle, epithelial and epidermal cells, and to a lower extent in neurons, Malpighian tubules and germline cells. The three ligands could therefore be co-expressed in some cells and thus share overlapping functions.

In addition, we also examined *dome* expression using publicly available gene expression atlases for the red flour beetle *Tribolium castaneum* and the yellow fever mosquito *Aedes aegypti* (Hixson et al., 2022; Naseem et al., 2023). Similar to *Drosophila*, *T. castaneum* and *A. aegypti dome* is broadly expressed throughout the body, with the highest expression detected in Malpighian tubules and female gonads (not shown). It remains to be seen if *dome* expression is also conserved at the cellular level across these diverse insects.

3. Functions of Upd signaling in *Drosophila*

Upd signaling regulates diverse functions in *Drosophila.* Upd proteins can act as hormones, cytokines and myokines. Thus, they regulate diverse aspects of physiology ranging from growth and development to metabolism to immune response. Here, we summarize the functional roles of *Drosophila* Upd proteins and their interactions with other signaling pathways (Fig. 3).

3.1. *Growth and development*

Multiple protein hormones and neuropeptides have been implicated in regulating growth and development in *Drosophila*, with insulin-like growth factors being heavily investigated (Arquier et al., 2008, Ruaud

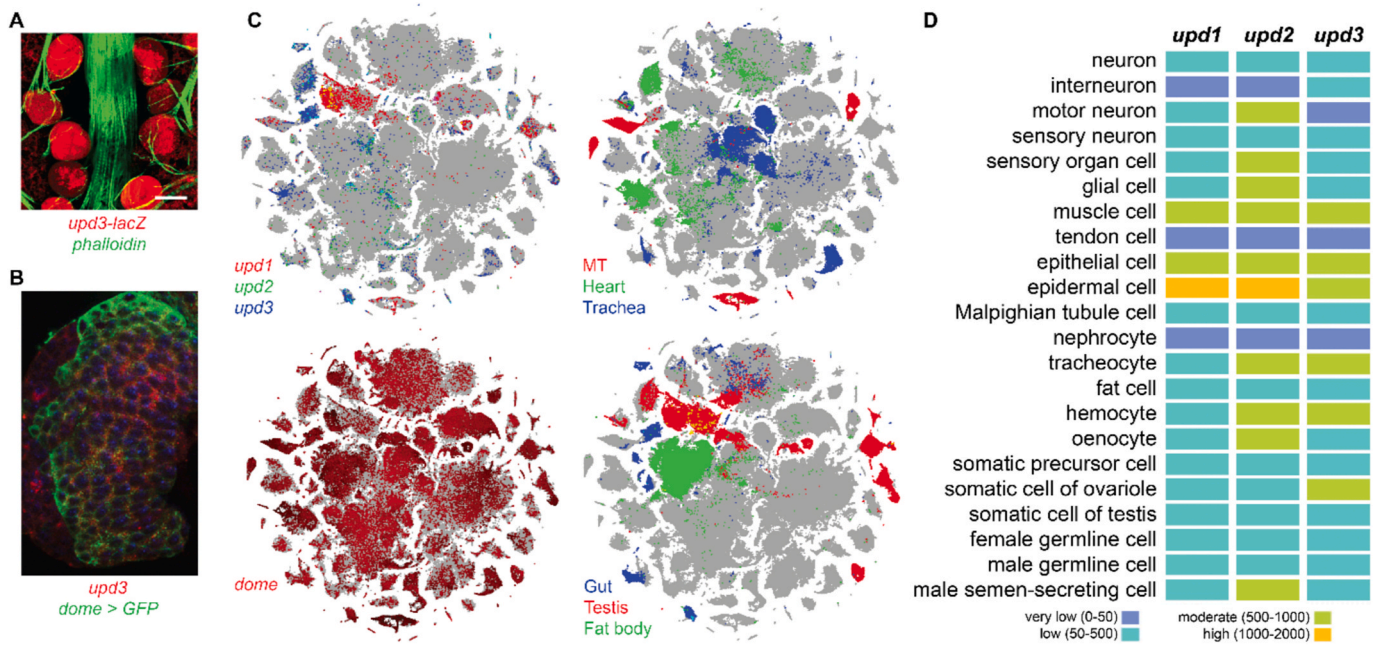

Fig. 2. Expression of *Drosophila unpaired* genes. *Unpaired 3* (*upd3*) is expressed in **(A)** the pericardial nephrocytes and **(B)** lymph glands (figure modified from (Makki et al., 2010)). Scale bar in **A** is 40 μm. **(C)** Mining the single nucleus transcriptomes of the adult fly reveals differential expression of *upd1*, *upd2* and *upd3,* and widespread expression of their receptor, *domeless* (*dome*). *Upd1* is expressed in the testis and Malpighian tubules (MT), *upd2* in the fat body, whereas *upd3* is expressed in the heart, trachea, gut and testis cell clusters. Data mined using https://scope.aertslab.org/#/FlyCellAtlas (Li et al., 2022). **(D)** Expression of *upd1*, *upd2* and *upd3* in different cell types. Data based on Fly Cell Atlas (Li et al., 2022) and retrieved from Flybase.com (Gramates et al., 2022).

Fig. 3. ***Drosophila*** **unpaired signaling pathways**. Summary of major pathways via which the three unpaired ligands (Upd1, Upd2 and Upd3) regulate diverse physiological processes and behaviors. For instance, neuronal Upd1 modulates post-feeding physiology and behaviors via inhibition of the neuropeptide F (NPF) pathway. Upd2 released from adipocytes regulates feeding, sleep, growth, metabolic physiology, and synaptic plasticity via direct and indirect modulation of insulin-producing cells (IPCs). Upd3 is produced and released by diverse immune cell-types and oenocytes to regulate immune response, growth and development, and metabolic physiology. Dashed arrows represent indirect pathway.

and Thummel 2008; Nässel and Winther 2010, Nässel et al., 2013, Sano et al., 2015, Buhler et al., 2018, Kannangara et al., 2020, Koyama et al., 2020, Oliveira et al., 2021). Here, we focus on the role of Upd-signaling in multiple aspects of development starting with axis formation, germ cell development, metamorphosis to reproductive tissue development. In terms of growth, we address the functions of Upd in cell differentiation and organ size determination under normal conditions as well as during injury and cancer.

3.1.1. Somatic tissue development

Drosophila has long served as an excellent model for developmental studies, where the conserved homeobox genes involved in axis formation were first discovered (Nusslein-Volhard and Wieschaus 1980, Garber et al., 1983). The process of axis specification in the developing embryo involves the interplay of numerous homeotic genes and signaling pathways including Wingless, JAK/STAT, transforming growth factor beta, and fibroblast growth factor families (Hajirnis and Mishra 2021). These axes encompass the lateral-medial (left-right), dorsoventral (back-belly), and anteroposterior (head-feet) orientations, which governs organismal symmetry and organization. The precise formation of a proximal-distal axis is fundamental for the development of the vertebrate limbs and invertebrate appendages. Hence, defects in formation of these axes are visible as deformities in adult structures. In insects, these adult structures originate from multiple imaginal discs (e.g. eye-antennal, wing and leg imaginal discs), which are sacs of epithelial cells. As larval holometabolous insects metamorphose into adults, extensive remodeling transforms these imaginal discs into adult appendages. Thus, the eye-antennal imaginal disc gives rise to the eyes and antenna. In *Drosophila*, Upd proteins play a vital role in axis formation during development. Wild type individuals display a characteristic wing posture, with their wings positioned parallel to the primary body axis. Originally named as “outstretched” based on the wing phenotype of its mutants, Upd1 expression is necessary in the proximal wing fold for normal wing development. Specifically, the wings of *upd1* mutants unfurl away from the main body axis, manifesting in dorsal hinge defects (Johnstone et al., 2013). Moreover, ectopic expression of *upd1* in the putative wing blade, results in underdeveloped and stunted wings (Ayala-Camargo et al., 2013). In addition to its role in wing development, Upd1 is involved in the formation of the compound eye from the larval eye imaginal disc. During the late larval stage, the onset of photoreceptor differentiation commences at the central point of the posterior margin (also known as the posterior center) and then progresses towards the anterior direction (Greenwood and Struhl 1999). This transformation is characterized by the presence of a row of cells known as the “morphogenetic furrow” (MF). MF undergoes apical constriction and sweeps through the eye disc from posterior to anterior end. Multiple factors promote whereas only the Wingless pathway suppresses MF initiation. Interestingly, *upd1* is transiently expressed at the posterior center and represses Wingless which is necessary for MF initiation as it sweeps towards the anterior eye disc (Tsai et al., 2007). Thus, Upd1 act as the growth modulator in the developing eye. Consequently, loss of Upd1 function leads to a reduction in eye size, while Upd1 overexpression causes eye enlargement (Vollmer et al., 2017). Further, Upd1 signaling is also crucial during cardiogenesis as *upd1* mutants have cardiac lumen defects, which result in dysfunctional hearts (Johnson et al., 2011). This is caused by reduced expression of a homeobox-containing transcription factor *tinman* which regulates proliferation, differentiation, and specification of cardiac precursor cells (Zaffran et al., 2002). Interestingly, mutations in the Nkx2.5 gene, the vertebrate homolog of *tinman*, is also associated with congenital heart defects (Zaffran et al., 2002). Lastly, Upd signaling is also implicated in cell-fate re-specification during development (Worley et al., 2018).

3.1.2. Germline and reproductive tissue development

All sexually reproducing animals are made up of germ cells and somatic cells. Germ cells are progenitors which give rise to eggs and sperms and their development is important as they are a vital component of transgenerational inheritance (Williamson and Lehmann 1996). Upd proteins play versatile roles during different aspects of germ cell development. In *Drosophila* gonads, the sex of the neighboring somatic cells determines the sexual identity of the germ cells. Thus, Upd1 from the male somatic cells determines male germline identity during gonad development (Wawersik et al., 2005; Casper and Van Doren 2009). This somatic to germline signaling is even more dominant than germline autonomous signaling during their sex determination. The identity and the nature of the germline sex-determination signal from the female somatic cells remains elusive. It is, however, possible that the female germline is determined by an absence of Upd1 signaling. In addition to determining male germline identity, Upd1 maintains high expression of *Sex-lethal,* a sex-determination gene, ensuring its autoregulation in the female embryos (Avila and Erickson 2007). Hence, Upd1 signaling is important for both germline and organismal sexual identity specification.

Following germline sex determination, eggs and sperms are produced via oogenesis and spermatogenesis, respectively. With respect to oogenesis, each of the two *Drosophila* ovaries can accommodate up to 18 ovarioles. The germarium, which houses both somatic and germline stem cells, is situated at the anterior tip of the ovariole. As oocytes develop and progress along the ovariole, they mature and eventually reach the posterior portion as fully developed eggs, ready for fertilization (Bastock and St Johnston 2008). Within the germarium, the germline stem cells engage in asymmetric cell division giving rise to one stem cell and a differentiating daughter cell. The differentiating daughter cell subsequently undergoes four rounds of mitotic cell division with incomplete cytokinesis, resulting in the formation of a cyst comprising 16 cells (Wu et al., 2008). Among these 16 cells, one assumes the role of the oocyte, while the remaining 15 cells become nurse cells, primarily dedicated to nourishing the oocyte. Moreover, in close proximity within the germarium are the follicle stem cells which give rise to follicle precursor cells (Margolis and Spradling 1995). Approximately 16 of these follicle precursor cells surround the cysts, where they halt their division and transform into pre-polar cells (Besse and Pret 2003). These pre-polar cells subsequently undergo further differentiation, ultimately assuming their roles as polar cells and stalk cells. The anterior polar cells attract and assemble a group of four to eight follicle cells around them, coalescing into a cluster known as the border cells (Ruohola et al., 1991). The formation as well as the migration of these borders is dependent on Upd1 signaling gradient. Upd1 is released from the polar cells and activates the neighboring follicle cells. The follicle cells in close proximity, but not the ones further away, receive high levels of Upd1 and resultant JAK/STAT activation. This activation results in the formation of border cells which migrate out of the cell layer. Conversely, cells with lower STAT activity remain within the follicular epithelium (Starz-Gaiano et al., 2009). This graded Upd1 signaling from polar cells is crucial for the precision of cell fate determination and is regulated by heparan sulfate proteoglycans (Hayashi et al., 2012). While the strength of Upd1 signaling is important during ovary development, polar cells releasing Upd1 are produced in excess. These cells undergo Upd1-dependent apoptosis during the later stages, eventually resulting in two polar cells per follicle (Borensztejn et al., 2013). Hence, polar cells regulate their own cell survival via Upd1 signaling. Lastly, Upd1-dependent JAK/STAT activity promotes the generation of stalk cells, which play a crucial role in the separation of developing egg chambers (McGregor et al., 2002).

Upd1 also plays an important role during spermatogenesis, specifically in the maintenance and differentiation of germ line stem cells. The maintenance and function of germ line stem cells is dependent on microenvironments known as niches or hubs. The hub is the source of Upd1 in *Drosophila* testes and is necessary for their self-renewal capacity (Tulina and Matunis 2001). Age-dependent decline in Upd1 levels is therefore associated with a decline in the number of stem cells in testes (Boyle et al., 2007).

While Upd1 signaling is important for tissue formation, and germline and reproductive tissue development, Upd3 also shares some of these functions, largely owing to overlapping expression patterns. Thus, *upd3* mutants have defects in eye and haltere formation. Moreover, Upd3 signaling is required for oogenesis and spermatogenesis. Particularly, Upd3 signaling affects border cell numbers, posterior cell distribution and follicle cell specification in ovaries and male fertility (Wang et al., 2014).

3.1.3. Gut maintenance

The gastrointestinal tract or the gut is the primary organ responsible for nutrient absorption. It is also as a major source of peptidergic hormones which regulate diverse behaviors and energy homeostasis (Song et al., 2014; Scopelliti et al., 2019; Zhou et al., 2020; Kim et al., 2021; Kubrak et al., 2022; Malita et al., 2022). The gut requires constant cellular turnover to replace the cells damaged by ingested pathogens and mechanical shear caused during food transit through the gut. The midgut forms the largest part of the gut and is comprised of intestinal stem cells (ISCs), enteroblasts, enterocytes, enteroendocrine cells and visceral muscles (Apidianakis and Rahme 2011, Buchon et al., 2013). Upd signaling is crucial in maintaining both the ISC population as well as differentiation of enteroblasts into other cell types. Specifically, Upd1 and Wingless from the muscular niche serve as paracrine signals and target the ISCs to maintain their self-renewal (Lin et al., 2010; Xu et al., 2011). In addition, Upd1 works in conjunction with Notch signaling to determine the fate of differentiating enteroblasts (Jiang et al., 2009). Hence, high Notch and low JAK/STAT signaling gives rise to enterocytes while low Notch and high JAK/STAT produces enteroendocrine cells (Ohlstein and Spradling 2007; Jiang and Edgar 2011). Therefore, JAK/STAT, Notch and Wingless signaling pathways govern ISC self-renewal and influence the binary fate selection of intestinal progenitor cells. JAK/STAT signaling also has similar effects on other stem cells, including the gastric stem cells (Singh et al., 2007) and those found within the Malpighian tubules, a core component of the *Drosophila* excretory system (Singh et al., 2011). Interestingly, enterocytes themselves can also release Upd1 during damage to promote ISC activation and gut repair (Staley and Irvine 2010). Similarly, Upd2 and Upd3 from the ISCs promote their proliferation in only aged flies (Osman et al., 2012). Interestingly, this pathway is hijacked following infection (see section 3.2) as well as during ISC tumor formation and basement membrane damage, resulting in Upd2 and Upd3 release from enterocytes which then promote tumor growth (Patel et al., 2015). Thus, Upd-mediated JAK/STAT signaling is crucial in overall gut homeostasis. This function of JAK/STAT signaling is also conserved across mammals, making *Drosophila* a valuable model for studying gut homeostasis (Apidianakis and Rahme 2011).

3.1.4. Metamorphosis

Holometabolous insects like *Drosophila* undergo metamorphosis as they transition from juvenile to adults. This process is controlled by the steroid hormone ecdysone which is produced by the prothoracic gland (Yamanaka et al., 2013). Moreover, the commitment to metamorphosis is an irreversible process and involves multiple checkpoints to ensure that this process is successful. One such checkpoint is the attainment of the critical weight needed before metamorphosis can commence (see Yamanaka et al., 2013). This critical weight is dependent on nutrient availability. Hence, developmental timing is increased in animals raised on poor nutrient conditions (Brown et al., 2019). Conversely, attainment of sufficient fat reserves and thus the critical weight, initiates the maturation process which is dependent on Upd2 release by the prothoracic glands (Juarez-Carreno et al., 2021). In addition, intrinsic JAK/STAT signaling within the prothoracic glands is crucial for their growth and consequently developmental timing (Cao et al., 2022). Other checkpoints besides critical weight include tissue damage and tumors which secrete Upd1 and Upd3 to delay metamorphosis. This delay is necessary to ensure that the organisms have sufficient time to recover (Katsuyama et al., 2015; Romao et al., 2021).

3.1.5. Aging

Lastly, Upd proteins play an important role in determining healthy lifespan. Conditional overexpression of *upd1* has tissue- and sex-specific effects on longevity (Moskalev et al., 2019). For example, elevated *upd1* levels in the intestine reduce lifespan in both males and females. However, activation of *upd1* in the nervous system extends only male lifespan. The cause of these sex-specific differences is still unclear.

3.2. Infection, wound healing and regeneration

In *Drosophila*, infection, wound healing and regeneration trigger humoral and cellular immune responses. The humoral immune response is triggered by bacterial or fungal infections, and result in the production of antimicrobial peptides by the fat body primarily via Nuclear factor-kB (NF-kB)-like signaling pathways. Conversely, the cellular immune response is mediated by macrophages and culminates with phagocytosis of invading microbes. Both these mechanisms, are considered to be part of the innate immune response, have been extensively investigated and reviewed previously (Hoffmann and Reichhart 2002; Govind 2008; Gold and Bruckner 2015; Vlisidou and Wood 2015, Yu et al., 2022b), Therefore, we focus specifically on the involvement of Upd signaling in these responses.

3.2.1. Infection

Upd signaling is involved in humoral immune responses via actions on multiple tissues. Both *upd2* and *upd3* are upregulated in hemocytes upon bacterial infection and activate the JAK/STAT pathway in the fat body and gut. Within the fat body, activation of JAK/STAT in conjunction with the NF-kB pathway leads to an increase in *Turandot A*, a stress-induced humoral factor, as well as *diptericin,* an antimicrobial peptide (Agaisse et al., 2003; Chakrabarti et al., 2016). In contrast, bacterial-infection dependent JAK/STAT activation in the gut stimulates ISC proliferation and an antimicrobial response via the expression of *Drosomycin*-like genes, which is important in reducing susceptibility to septic injuries. Hence, inter-organ signaling via Upd2 and Upd3 is crucial in mounting an effective humoral immune response (Chakrabarti et al., 2016). Interestingly, parasitoid wasp infection triggers Upd2 and Upd3 secretion from hemocytes to mount a cellular immune response via muscle cells (Yang et al., 2015). Additionally, Upd3-signaling is activated in the hemocytes following oxidative stress. The resultant JAK/STAT activation is crucial for fly survival under oxidative stress. While the pathways via which Upd3 mediates these effects have not been deciphered, the impact on survival could be due to altered energy mobilization and storage (Hersperger et al., 2023).

As mentioned earlier, Upd2 and Upd3 from the enterocytes are involved in ISC proliferation and differentiation. During septic injury, Upd proteins facilitate gut epithelial regeneration by promoting ISC division and differentiation (Jiang et al., 2009; Evans et al., 2022). Further, pathogenic infection results in gut remodeling because of enterocyte delamination, consequently compromising gut integrity (Buchon et al., 2010). Hence, in response, Upd3 is released from the enterocytes to control the expression of *vein*, a ligand for the epidermal growth factor receptor (EGFR) in visceral muscles. Subsequent activation of EGFR in ISCs stimulates their proliferation and generates new enterocytes to maintain gut homeostasis and integrity. Together, Upd proteins form part of a signaling network to control tissue repair following infection.

3.2.2. Wound healing and regeneration

Wounding is characterized by tissue damage which can be especially problematic in cases where epidermis, the primary physical barrier against the external environment, is compromised. Hence, effective wound healing is necessary to reduce risk of infections. This process is characterized by removal of debris from damaged cells, cell migration

and tissue growth. Moreover, the formation of syncytium by cell-cell fusion promotes wound closure. This process of cell fusion is under the influence of JNK and JAK/STAT signaling, where JNK promotes and Upd2 and Upd3 suppress cell fusion (Lee et al., 2017). The balance in these opposing pathways is essential for effective wound closure while preventing excessive cell fusion.

Additionally, Upd-signaling is important during tissue regeneration. For example, cell death triggers the release of reactive oxygen species (ROS) which propagates to nearby surviving cells, activating the JAK/STAT pathway. This activation induces Upd1 which results in cell proliferation, thus maintaining tissue homeostasis (Santabarbara-Ruiz et al., 2015).

Interestingly, aseptic or axenic injury triggers an inflammatory response to prepare the animal in anticipation of an immune challenge. Specifically, epidermal damage activates Toll and JNK signaling in the macrophages, causing them to differentiate into lamellocytes (Evans et al., 2022). In addition, Toll causes an induction of antimicrobial peptides and Upd3 in hemocytes. Hence, JAK/STAT, together with Toll and JNK pathways mount a proinflammatory response to injury. This response is evolutionary conserved as injury in mammals also promotes cytokine secretion and generation of specialized macrophages (Cronan et al., 2016).

3.2.3. Behavioral modulation during infection

Lastly, Upd signaling can also modulate behaviors that in turn lower the risk of infection. For instance, increased Upd2 and Upd3 release from the intestine in response to bacterial infection results in metabolic reprogramming of the ensheathing glia within the antennal lobe. Consequently, this reprogramming modulates olfactory sensitivity to reduce intake of bacteria-infected food. Ultimately, this adaptive behavior enhances fly survival (Cai et al., 2021). However, persistent intestinal inflammation in aged flies leads to constitutive activation of the Upd pathway which is associated with age-related decline in olfactory sensitivity (Cai et al., 2021). Additional behaviors modulated by Upd signaling will require comprehensive mapping of Dome in the nervous system which appears to be broadly expressed.

3.3. Inter-organ signaling regulating physiology and behavior

3.3.1. Metabolic physiology and feeding-related behaviors

Multicellular organisms possess different tissues or organs to perform highly specialized functions including feeding, nutrient uptake, storage and release, waste removal, and reproduction amongst others. These tissues are inter-dependent and therefore must communicate with each other. This signaling is crucial for maintaining overall homeostasis. Consequently, disruptions in inter-organ signaling, especially between tissues governing feeding and nutrient storage, can result in a range of eating and metabolic disorders including obesity, anorexia, and diabetes. In *Drosophila*, various neurohormone signaling pathways have recently been characterized that form the basis of inter-organ signaling to modulate metabolic physiology and associated behaviors (Nässel and Winther 2010; Hentze et al., 2015; Droujinine and Perrimon 2016; Zandawala et al., 2018; Fadda et al., 2019; Nässel and Zandawala 2019). Upd-mediated hormonal signaling through the JAK/STAT pathway one such pathway that plays a vital role in maintaining nutrient homeostasis, under both normal physiological conditions and during disease (Fig. 4).

Drosophila fat body is functionally analogous to the vertebrate liver and adipose tissue, and is the major site of lipid and carbohydrate storage. Fat body is under the hormonal control of *Drosophila* insulin-like peptides (DILPs) and glucagon-like adipokinetic hormone (AKH) which regulate lipid and carbohydrate homeostasis (Droujinine and Perrimon 2016; Nässel and Zandawala 2019). DILP2, 3 and 5 are produced and released from a group of approximately 14 insulin-producing cells (IPCs) in the pars intercerebralis of the brain. AKH on the other hand is

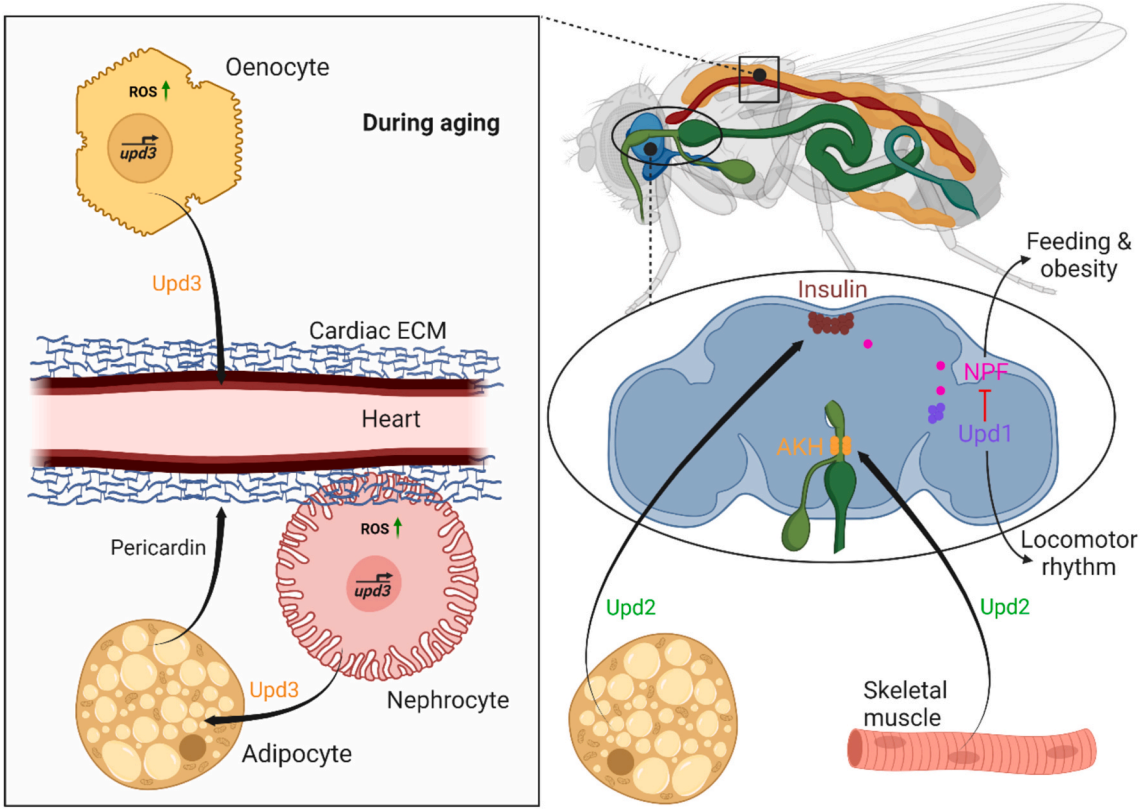

Fig. 4. Unpaired signaling regulates physiology and behavior. Select inter-organ and neuronal signaling pathways mediated by unpaired 1, 2 and 3 (Upd1, 2 and 3) which influence physiology and behavior. For clarity, only the relevant tissues are shown within the fly. Abbreviations: Reactive oxygen species, ROS; extracellular matrix, ECM; adipokinetic hormone, AKH; neuropeptide F, NPF.

produced by the corpora cardiaca gland which is closely associated to the aorta. Upd-signaling interacts with both insulin and AKH pathways to regulate nutrient homeostasis and related physiology. In healthy flies, insulin-signaling is normally low when flies are starved. Increased glucose levels post-feeding promote DILP release via at least three separate mechanisms. A subpopulation of IPCs can sense increased circulating glucose levels cell-autonomously and release DILPs (Oh et al., 2019). Additional populations of glucose-sensing neurons in the brain also signal to the IPCs to promote DILP release (Dus et al., 2015; Oh et al., 2019). Lastly, the fat body functions as a nutritional status sensor and indirectly stimulates DILP release via Upd2 signaling. Specifically, fat bodies of fed flies secrete Upd2 which signal to a population of brain GABAergic neurons that normally inhibit IPCs (Rajan and Perrimon 2012). Activation of JAK/STAT pathway in these GABA neurons relieves their inhibition of the IPCs, consequently stimulating DILP secretion. This inhibitory circuit motif is also conserved in vertebrates whereby leptin modulates hypothalamic circuits regulating feeding and obesity via GABAergic interneurons (Vong et al., 2011). Disrupting Upd2 signaling from the fat body is perceived as reduced energy, which consequently results in reduced growth. In this regard, Upd2 is functionally similar to human leptin which regulates metabolic homeostasis, feeding and related behaviors. Remarkably, ectopic expression of human leptin in fat body of *upd2* mutant flies can rescue growth and DILP-release defects (Rajan and Perrimon 2012). Hence, *Drosophila* Upd2 and human leptin also bear structural similarities in addition to partly conserved functions.

In addition, Upd2/leptin signaling is important for metabolic state dependent neuronal plasticity. Upd2 signaling sets the baseline activity of IPCs. It does so by altering the number of boutons and consequently the strength of inhibition from aforementioned GABAergic interneurons to IPCs (Brent and Rajan 2020). Reduction in bouton numbers during nutrient surplus lowers the baseline inhibition of IPCs, thus setting up a neural environment to promote DILP release. Intriguingly, insulin provides negative feedback on this same circuit. Insulin signaling resets the inhibitory tone by increasing bouton number and ultimately maintaining a state of negative tone in the circuit. Thus, two energy-regulating hormones, Upd2/leptin and insulin, act antagonistically to influence energy store-dependent neural circuit plasticity.

Since the level of Upd2/leptin signaling is proportional to fat stores, genetic downregulation of *Drosophila upd2* mimics a starved-like state. Under this state, flies modify their behavior which promotes nutrient homeostasis and survival. For instance, flies with *upd2* knockdown suppress sleep and increase feeding (Ertekin et al., 2020). Sleep reduction in starved animals aligns with increased hyperactivity/foraging during hunger to locate a food source. Moreover, *upd2* knockdown in the fat body also improves selective visual attention (Ertekin et al., 2020). This might be an ethologically relevant adaptation allowing flies to locate appropriate food resources during nutrient deprivation. Interestingly, these behaviors are modulated via Dome expressed in the IPCs. Hence, Upd2 regulates insulin signaling both directly and indirectly via GABAergic neurons. Further, high-fat diet stimulates Upd2 release from the fat body which subsequently suppresses fatty acid taste and increases sugar taste perception via sweet taste neurons (Zhao et al., 2023). Thus, Upd2 signaling promotes lipid homeostasis by modulating taste perception. The release of Upd2 from the fat body is controlled by AMP-activated protein kinase (AMPK) nutrient-sensing pathway via the tumor suppressor p53 (Ingaramo et al., 2020). There is also a possibility that Upd2 could influence its own release in an autocrine manner as knockdown of *Domeless* receptor in fat body cells inhibits *upd2* expression (Lourido et al., 2021). Taken together, fat body serves as a nutritional sensor and utilizes leptin-like Upd2 to influence energy homeostasis via orchestrating effects on insulin signaling, neural circuit plasticity and behavioral modulation.

Although Upd2 from the fat body acts as a hormone to stimulate insulin signaling and promote lipid storage, it can also function as a myokine. Interestingly, Upd2 released from the skeletal muscles increases AKH signaling which in turn promotes lipid mobilization from the fat body (Zhao and Karpac 2017). Upd2 has tissue-dependent and opposing effects on lipid levels via regulation of insulin and AKH signaling. Moreover, the expression of Upd2 in skeletal muscles is controlled by the metabolic transcription factor Forkhead box O (FOXO). Hence, Upd2 signaling forms the basis of an inter-organ network which influences lipid homeostasis in response to nutrient availability.

While Upd2 has been extensively investigated in the context of metabolic physiology under normal conditions and disease states, Upd1 and Upd3 also regulate different aspects of metabolism. For instance, *upd3* is upregulated in macrophages of flies raised on a lipid-rich diet (Woodcock et al., 2015). These flies exhibit elevated glucose and trehalose levels, reduced insulin sensitivity and lifespan. Interestingly, knockdown of *upd3* in the macrophages rescues effects on sugar levels, insulin sensitivity and lifespan, suggesting that increased Upd3 signaling is detrimental under lipid-rich diet. It remains to be seen if Upd3 has similar roles under normal physiological conditions. Upd1, on the other hand, is expressed in the brain. Upd1 in certain clock neurons is downstream of miR-279 and regulates locomotor rhythms. Although the precise neuronal targets of Upd1 which influence locomotion are not yet known at the moment (Luo and Sehgal 2012), it is possible that this effect could be mediated via neuropeptide F (NPF)-expressing neurons (Beshel et al., 2017). In addition, Upd1 inhibits NPF neurons to increase food odor-attraction, feeding, lipid levels and weight. Surprisingly, reintroduction of Upd1, human leptin, and even Upd2 in neurons rescued these phenotypes, further highlighting structural conservation between these related ligands. Moreover, the Upd1-NPF pathway is also conserved in mammals where leptin inhibits NPY-positive hypothalamic cells responsible for signaling satiety (Stephens et al., 1995).

3.3.2. *Cardiac physiology*

Inter-organ signaling via Upd is also important in maintaining other aspects of physiology, including cardiac function. Normal cardiac function requires optimum levels of the extracellular matrix protein, Pericardin, a type-IV collagen-like protein (Chartier et al., 2002). Hence, both increased and decreased levels of Pericardin and collagen result in cardiac arrhythmicity in both flies and mammals, respectively (Vaughan et al., 2018; Cowling et al., 2019; Gera et al., 2022). ROS, long recognized for their potentially harmful impact owing to their highly reactive nature, have lately emerged as important signaling molecules, regulating numerous physiological processes (see Sies and Jones 2020). Cardiac physiology is one of these processes which they regulate via two parallel pathways. Firstly, ROS-mediated pericardial paracrine signaling regulates cardiac function (Lim et al., 2014). Secondly, ROS stimulates *upd3* expression in the pericardial nephrocytes, which are functionally related to the podocytes in the mammalian kidney (Gera et al., 2022). Upd3, in turn, controls fat body–specific expression of Pericardin to influence cardiac physiology (Fig. 4). Hence, nephrocyte-heart axis is analogous to the kidney-heart axis observed in higher vertebrates (Husain-Syed et al., 2015; Liu 2019; Boorsma et al., 2022). Whether cytokine signaling is involved in this axis in vertebrates remains to be explored. Lastly, Upd3 signaling also forms the basis of cardiac arrhythmia during aging. Increased risk for cardiovascular diseases during aging can be a result of chronic low-grade inflammation (Huang et al., 2020). Upd3 functions as a pro-inflammatory factor during aging to disrupt cardiac function. Interestingly, the source of this age-dependent Upd3 are oenocytes, which are part of the fat body in *Drosophila* and function as hepatocytes.

4. Functional similarities between *Drosophila* Upds and vertebrate leptin and IL-6

Having summarized the roles of *Drosophila* Upds in regulating metabolic physiology, growth and development, immune responses, and feeding-related behaviors in the previous sections, we now draw

Table 2
Functional similarities between *Drosophila* Upds and vertebrate leptin and IL-6.

Drosophila Upd	Vertebrate functional analog	Physiological process	Function	References
Upd1	Leptin	Ovary development and ovulation	Affects maturation of follicles and oocyte in humans; controls the growth of follicles in mice and ovarian development in chicks	(Swain et al., 2004; Perez-Perez et al., 2015; Shaikat et al., 2021)
Upd1	Leptin	Germ cell development	Regulates the proliferation, differentiation, and apoptosis of germ cells in mouse testis	(El-Hefnawy et al., 2000; Bhat et al., 2006)
Upd2	Leptin	Glucose homeostasis and feeding	Regulates insulin secretion, glucose metabolism and appetite in humans	(Mantzoros et al., 2011; Amitani et al., 2013)
Upd1 and Upd2	Leptin	Feeding and obesity	Modulates NPY-positive and GABAergic neurons in the mouse hypothalamus to regulate food intake and body weight	(Stephens et al., 1995; Vong et al., 2011)
Upd2	Leptin	Lipid metabolism and obesity	Regulates food intake, energy expenditure and body fat in rodents and humans	(Harris 2014, Pereira et al., 2021; Minokoshi et al., 2012)
Upd2 and Upd3	Leptin and IL-6	Gut homeostasis	Activates MAPK pathways promoting gut repair in response to tissue injury in rodents	Kim and Kim (2021)
Upd3	IL-6	Immune response and inflammation	Contributes to host defense against bacterial infections and tissue injury in mice and humans	(Tanaka et al., 2014)
Upd3	IL-6	Cancer	Regulates cell survival, proliferation, and migration, thus promoting metastasis in human cancer cell lines	Hirano (2021)
Upd3	IL-6	Autoimmunity	Produces other pro-inflammatory cytokines and growth factors leading to autoimmune diseases and cancer in mice and humans	Hirano (2021)
Upd3	IL-6	Hematopoiesis	Regulates hematopoiesis leading to blood pathologies in mice	(Jenkins et al., 2007)
Upd3	IL-6	Wound healing	Promotes inflammatory responses, macrophage activation and collagen production in mice and humans	(Johnson et al., 2020)
Upd3	IL-6	Cardiac function	Regulates fibroblast proliferation and ECM deposition which affects cardiac function in rodents and humans	(Fontes et al., 2015; Feng et al., 2022)

comparisons with their vertebrate functional analogs, leptin and IL-6 (**Table 2**). In general, Upd1 and Upd2 participate in similar physiological processes and are functionally comparable to vertebrate leptin. Similar to the role of Upd1 in *Drosophila*, leptin also influences ovulation and ovary development in vertebrates (Swain et al., 2004; Perez-Perez et al., 2015; Shaikat et al., 2021). In addition, both Upd1 and mouse leptin regulate germ cell development (El-Hefnawy et al., 2000; Tulina and Matunis 2001, Bhat et al., 2006). Interestingly, the functions as well as some neural pathways via which Upd2 and human leptin modulate insulin secretion, metabolic physiology and appetite are conserved. However, the function of leptin as an anorexigenic hormone is not conserved across all vertebrates such as birds. Particularly, leptin is not expressed in the adipose tissue of birds and does not function as an adipostat (Friedman-Einat and Seroussi 2019). Hence, avian leptin may have evolved other functions in birds. Additionally, in fish, leptin is primarily expressed in the liver. Despite differences in their expression pattern, most studies indicate that fish leptin is also anorexigenic (Blanco and Soengas 2021). These examples highlight differences in leptin signaling and functions across diverse vertebrates. For additional species-specific differences in functions of vertebrate leptins, the readers are referred to the following reviews (Copeland et al., 2011, Friedman-Einat and Seroussi 2019; Blanco and Soengas 2021).

Although Upd1/Upd2 and leptin share several roles during reproduction, development, and metabolic physiology, it remains to be seen if Upds have also acquired other functions of leptin. For instance, leptin in mammals and fish promotes reproduction by stimulating the release of reproductive hormones such as gonadotropin releasing hormone (GnRH) from the hypothalamus, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary (Copeland et al., 2011). *Drosophila* possess a GnRH ortholog (AKH) but lack orthologs of LH and FSH (Nässel and Zandawala 2020). In addition, AKH does not directly regulate reproduction in *Drosophila* (Nässel and Zandawala 2019). Hence, it remains to be seen if *Drosophila* Upds regulate reproduction and via which hormones. In addition, Upds could play a similar role as leptin during aging. For example, leptin resistance is strongly associated with aging in vertebrates. Leptin resistance is caused by prolonged increase in leptin levels such as those observed during obesity (Izquierdo et al., 2019; Obradovic et al., 2021). Decreased leptin sensing can thus lead to increased adiposity as the animal is unable to sense the peripheral energy status. Further, leptin resistance and increased adiposity are both linked to aging in humans and other vertebrates (Scarpace et al., 2000; Wang et al., 2001; Ma et al., 2002; Carter et al., 2013). However, it is still unclear whether leptin resistance is a result of aging or whether it plays a causal role in accelerating age-associated weight gain. Future studies using the *Drosophila* model can help resolve this issue if Upd signaling also changes with age.

In contrast to Upd1 and Upd2, Upd3 appears to be the functional analog of IL-6 as both proteins are implicated in immune response, inflammation, cancer, wound healing, and cardiac physiology (**Table 2**). For example, IL-6 is a well-known regulator of inflammation (Tanaka et al., 2014; Hirano 2021). In response to an infection or tissue damage, mammalian IL-6 is upregulated to activate the host defense mechanisms. It does so by promoting antibody production by B cells as well as activation and differentiation of the naive T cells, thus linking innate and acquired immune responses (Tanaka et al., 2014; Hirano 2021). Several studies in mice have also shown the involvement of IL-6 in triggering autoimmune diseases such as arthritis and autoantibody production, resulting in B cell malignancies (Alonzi et al., 1998; Ohshima et al., 1998; Richards et al., 1999). In addition, like *Drosophila* Upd3, IL-6 mediated STAT signaling causes several hematopoietic and lymphoid pathologies in mice (Jenkins et al., 2007). Further, impaired IL-6 signaling can affect the production of multiple growth factors thereby leading to the development and progression of human cancer cell lines (Hirano 2021). IL-6 also plays a major role during wound healing in both mice and humans. Besides contributing to the inflammatory responses, IL-6 regulates macrophage activation and collagen production, thereby promoting wound healing (Johnson et al., 2020). Finally, Upd3 and mammalian IL-6 both regulate cardiac physiology by modulating ECM deposition around the cardiac tissue (Fontes et al., 2015; Feng et al., 2022; Gera et al., 2022). Taken together, Upds serve as a good model to unravel leptin- and cytokine-like signaling pathways in *Drosophila*.

5. Concluding remarks and future perspectives

The discovery of Upd proteins in *Drosophila*, as well as their structural and functional conservation in part with mammalian leptin and cytokines, suggest that this signaling pathway may be broadly conserved

across animals. The absence of redundancy in this pathway in *Drosophila* has enabled functional studies examining its role in diverse aspects of physiology. While much progress has been made on this front, several open questions remain regarding their evolution, functions and mechanisms of action. Due to the lack of comprehensive phylogenetic analysis of Upd signaling components, the precise evolutionary relationships between *Drosophila* and mammalian counterparts are still unclear. Moreover, Upd1 and Upd2 have been shown to be functionally similar to mammalian leptin. Hence, leptin can rescue the phenotypes of *upd1* and *upd2* mutants. It would be intriguing to see if mammalian IL-6 (which is thought to be similar to Upd3) can rescue *upd3* mutant phenotypes. There are several parallels between the insulin and Upd signaling pathways in *Drosophila* in terms of ligand diversity and function. For instance, both these pathways are characterized by multiple ligands which all act on the same receptor. Further, some ligands are co-expressed while others have unique expression domains. However, the lack of receptor binding studies for both insulin and Upd proteins have eluded mechanisms by which ligand specificity is achieved at the receptor level. Future Cryo-electron microscopy studies examining the receptor structure with ligands can help determine if the ligands have unique binding sites on the receptor (Viola et al., 2023). Another aspect of Upd signaling that is not clear is how secreted Upd proteins can achieve tissue level specificity given that there is only one receptor which appears to be ubiquitously expressed (Fig. 2C). Since *dome* does not encode multiple isoforms, it remains to be seen which intracellular components in the JAK/STAT pathway are responsible for differential responses following Dome activation. These and other questions surrounding Upd signaling would highly benefit from more advanced genetic reagents to track the release, transport and effects of Upd at the receptor level. One such possibility would be to adapt the Tango assay for Dome to monitor its activation under different contexts *in vivo* (Barnea et al., 2008; Inagaki, Ben-Tabou de-Leon et al., 2012). In conclusion, *Drosophila* Upd pathway presents an attractive genetic model to decipher leptin- and cytokine-like signaling pathways and examine their roles in regulating physiology, behavior, as well as their importance in disease states.

CRediT authorship contribution statement

Meet Zandawala: Conceptualization, Data curation, Funding acquisition, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Jayati Gera:** Conceptualization, Visualization, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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