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Surviving nutritional deprivation during development: neuronal intracellular calcium signaling is critical

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ABSTRACT Developing cells and tissues in a growing animal need to sense food quality and integrate this information with on-going time-bound developmental programs. The integration of metabolism with development requires cellular and systemic coordination. Work in our laboratory has focused on Ca^{2+} signaling arising from the release of Ca^{2+} stored in the endoplasmic reticulum (ER), which triggers store-operated Ca^{2+} entry. We describe a role for ER-store Ca^{2+} that operates at the cellular level in various classes of neurons, and eventually drives the systemic coordination required to survive and complete development under conditions of nutritional deprivation. In the model system *Drosophila melanogaster,* we have developed a paradigm to induce nutritional stress during the larval stage and used pupariation as a read-out for development. Applying the vast genetic tool kit available in *Drosophila* to this paradigm, we have uncovered novel roles for intracellular Ca^{2+} signaling in regulating neuronal activity, at the level of transcription in glutamatergic neurons, and translation in neuropeptidergic neurons. We find that such regulation of cellular processes is critical for integrating information across a neural circuit at multiple levels, starting from the point of sensing systemic and environmental levels of amino acids to finally connecting with neuropeptide secreting neurons, that communicate with the prothoracic gland, an organ that makes the key developmental hormone, ecdysone. This work underscores the importance of ER-store Ca^{2+} for neuronal health, with consequences for animal development.

KEY WORDS: *ER-Ca^{2+}, IP_3R, neuropeptide, malnutrition, glutamatergic, Drosophila*

Introduction

Nutritious food is indispensable for proper animal development and growth. Two major processes, metabolism and development, need to converge at the systemic as well as cellular level, to complete the formation of a fully mature adult animal. Metabolic pathways are tuned by developmental cues to supply the necessary nutrients, while developmental pathways rely on metabolic pathways to fuel cellular differentiation and build organ systems. The two pathways are thus deeply entwined, and understanding how they are coordinated would help us understand how developing animals cope with sub-optimal nutrition. This is particularly relevant in a world where fetal and early childhood malnutrition is still rife. To investigate biological mechanisms that underpin nutrition and development, we need model systems. *Drosophila melanogaster* (abbreviated herein as *Drosophila*), the vinegar fly, has emerged as one such powerful model system for studying how dietary input is sensed and integrated with the developmental program (Boulan *et al.*, 2015; Edgar, 2006; Mirth and Shingleton, 2012). We discuss our work in this area in the context of intracellular Ca^{2+} signaling.

Drosophila as a model system to study how nutrition and development are linked

A holometabolous insect, *Drosophila* exhibits four distinct life stages beginning from the egg (~24h), followed by three larval stages, L1, L2 and L3 (~96 h), pupa (~120 h) and adult (Fig.1). The egg and pupa are non-feeding stages during which development is fueled by stored nutrients. In contrast, the larva and adult are feeding stages, acutely sensitive to the availability of nutritious food. However, feeding patterns of the larva and adult differ significantly: larvae appear to feed continuously, except for brief periods of

Abbreviations used in this paper: Drosophila, Drosophila melanogaster; ER, endoplasmic reticulum; InR, insulin receptor; IPC, insulin-producing cell; IP_3R, inositol 1,4,5-trisphosphate receptor, STIM, stromal interacting molecule; NR, nutrient restriction; NP, neuropeptide; NE, neuroendocrine; PG prothoracic gland.

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moulting, whereas adults feed intermittently. Importantly, all growth occurs during the larval stages. Consequently, adult organismal size is determined by the final larval size. Because the systemic output in larvae is growth and maturation, whereas in adults it is survival and reproduction, it is likely that nutrient sensing pathways are tuned differently in the two stages. Thus, mechanisms derived from studying the effect of nutrition on adult flies may not necessarily be relevant or similarly important in larvae.

Laboratory fly food typically consists of the three major macronutrients: proteins, carbohydrates and lipids. In our laboratory, yeast serves as the major source of proteins, in addition to supplying precursors for sterol and lipid biosynthesis, whereas sugars and corn flour provide the bulk of the carbohydrates. While there are descriptions of completely synthetic fly media composed of amino acids, sugars and select micronutrients (Piper *et al.*, 2013), in general, nutrient content can be altered by changing the proportion of yeast and sugar in the fly media. Thus protein deficiency is easily accomplished by reducing the amount of yeast.

Holometabolous insect larvae feed and reach an established critical weight after which starvation does not affect their ability to complete development (Robertson, 1963). For the laboratory grown *w1118* strain of *Drosophila* this happens ~8 h after transition to L3, with a weight checkpoint of ~0.8mg (Koyama *et al.*, 2014; Stieper *et al.*, 2008). Starvation after reaching critical weight does not prevent development to pupae and further, to adults, except that a major cost is imposed on the size of the resulting adult. Changing the nutrient value of fly food after critical weight and before wandering stage, during which larvae crawl up the vial and stop feeding, yields a time window where integration of nutritional deficiency with development can be studied, in the absence of survival being a major issue (Fig. 1).

Signaling mechanisms that integrate growth and development in *Drosophila*

The key hormonal event in *Drosophila* that triggers development to the next stage and ultimately metamorphosis, is release of the steroid hormone ecdysone, from the prothoracic gland (PG) (Caldwell *et al.*, 2005; Colombani *et al.*, 2005; Mirth *et al.*, 2005). The PG senses growth and coordinated development of various larval parts through multiple ways. Importantly, these pathways ultimately regulate pupariation timing by regulating ecdysone biosynthesis. Signaling pathways known to regulate PG function include insulin receptor (InR) signaling (Colombani *et al.*, 2005; Mirth *et al.*, 2005), TOR signaling (Layalle *et al.*, 2008; Ohhara *et al.*, 2017), TGFβ/activin pathway (McBrayer *et al.*, 2007), PTTH peptide secreted by a pair of neurons in the brain (Rewitz *et al.*, 2009), circulating hedgehog released by enterocytes (Rodenfels *et al.*, 2014), dILP8 secreted by damaged imaginal discs (Colombani *et al.*, 2012; Garelli *et al.*, 2015) and more recently, circulating Dpp from peripheral tissues (Setiawan *et al.*, 2018). Of these, the InR and TOR signaling pathways are perhaps the major ones that link nutrient availability to cellular growth (Britton *et al.*, 2002; Layalle *et al.*, 2008). Both pathways respond to local and systemic nutrient levels to promote protein synthesis and therefore, increase in cellular size and proliferation. Importantly, the molecular components of these signaling systems are conserved genetically and functionally between mammals and *Drosophila* (Das and Dobens, 2015).

InR activation is reliant on the availability of its ligand, insulin. *Drosophila* possess several insulin-like peptides (dILPs 1-8) of which the transcription and release of dILPs 2, 3 and 5 are known to be regulated by nutrient availability (Broughton *et al.*, 2005; Kim and Neufeld, 2015; Okamoto and Nishimura, 2015; Slaidina *et al.*, 2009). Unlike mammals though, insulin-like peptides in *Drosophila* are made in the central nervous system, in neurons called insulin-producing cells (IPCs) (Rulifson *et al.*, 2002). IPCs release insulin peptides into circulation and also directly innervate the PG, thereby regulating ecdysone synthesis through non–cell autonomous as well as cell-autonomous mechanisms (Mirth *et al.*, 2005). IPCs themselves appear to sense nutrient availability through a number of secreted factors that include, Neural Lazarillo (Pasco and Léopold, 2012), Upd2 (Rajan and Perrimon, 2012), CCHamide 2 (Sano *et al.*, 2015), Eiger (Agrawal *et al.*, 2016), Stunted (Delanoue *et al.*, 2016) and Growth-Blocking Peptides (Koyama and Mirth, 2016), with the number of factors regulating IPCs set to grow. Together, the InR and TOR pathways are essential for integrating nutritional inputs such as sub-optimal protein conditions with growth and development. They operate at the level of peripheral tissues as well as on the PG. Such growth signaling pathways explain how nutritional input is converted to optimal cellular proliferation and growth. However they fail to explain how the animal is able to complete development in the absence of nutritional inputs, as in the case of *Drosophila*, where after reaching critical weight, larval to pupal development continues even in the absence of food. It is in this niche that our studies on ER-Store Ca^{2+} signaling have unexpectedly made a contribution.

IP_3R regulated Ca^{2+} signaling in development and metabolism

In the early 1990s, it became apparent that Ca^{2+} released from the ER is utilized by cells in order to respond to external stimuli

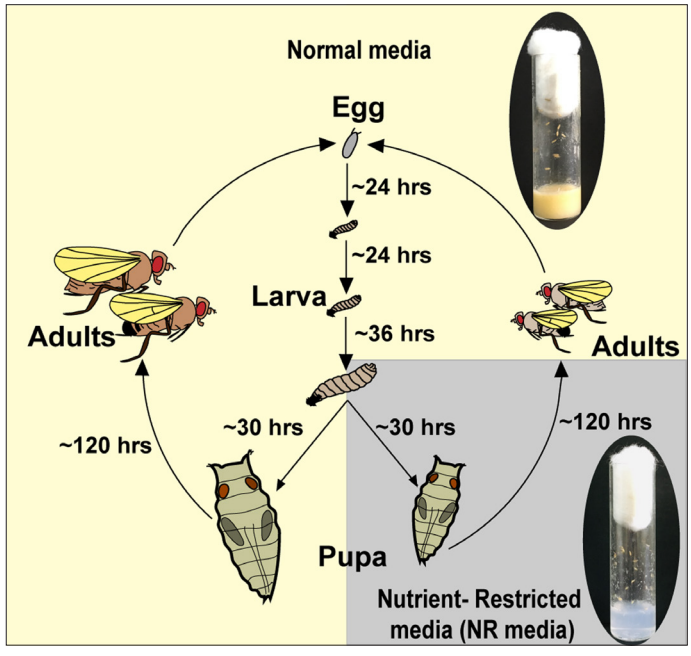

Fig. 1. Nutrient restriction (NR) assay during development. Drosophila *larvae in mid-third instar (~84 hours after egg laying) are taken from normal food conditions and moved to either 100mM Sucrose (NR media; inset) or back into normal media (inset). The number of pupae that develop are measured as a readout for development.*

(Clapham, 1995). Basal cytosolic Ca^{2+} levels are tightly maintained, at concentrations that are 100-500 fold lower than in the ER, as well as the extracellular milieu. This maintenance is enabled in part by an ATP-dependent channel on the endoplasmic reticulum (ER) called SERCA, which continuously pumps Ca^{2+} from the cytosol in to the ER (Clapham *et al.*, 2007). Dynamic changes in the levels of cytosolic Ca^{2+} ions, either via release of Ca^{2+} from the ER or entry from the extracellular milieu, thus provides metazoan cells a mechanism to utilize Ca^{2+} as a second messenger to connect external stimuli to cellular function (Berridge *et al.*, 2003; Clapham *et al.*, 2007). The two known ligand-gated receptors on the ER that release ER-Store Ca^{2+} are the ryanodine receptor and the inositol 1,4,5-trisphosphate receptor (IP_3R). Homologs of both intracellular Ca^{2+} release channels were identified in *Drosophila* by reverse genetic methods (Hasan and Rosbash, 1992). Subsequently, studies from our lab have focused exclusively on the IP_3R, whose activation lies downstream of G-protein coupled receptors (GPCRs) of the Gαq subtype. The IP_3R is present in all metazoan cell types and given its pivotal role in gating ER-Store Ca^{2+} it is not surprising that it plays an important role in many different cellular functions. The temporality and amplitude of the stimuli, as well as the presence of cell-type specific proteins deliver outputs of intracellular Ca^{2+} signaling that vary according to cell-type. Thus there exists a breadth of cell signaling events where the IP_3R is important: fertilization, muscle contraction, neurotransmitter release, vesicle secretion, transcription and mitochondrial dynamics, amongst others (Mikoshiba, 2015). Here we focus on the participation of IP_3R in cellular mechanisms that culminate in development and metabolic homeostasis.

Complete loss of the single IP_3R gene (*itpr*) in *Drosophila* resulted in early larval lethality. Hence, to investigate IP_3R function at later stages of development and in adults, an EMS-based mutagenesis screen was performed. This screen yielded several single *itpr* point mutants which in homoallelic combinations resulted in larval lethality, while in some heteroallelic combinations yielded animals with varying levels of survival to adulthood (Joshi *et al.*, 2004). The latter provided a genetic tool kit to study IP_3R function at various stages of development. Genetic analysis showed that IP_3-mediated Ca^{2+} release in the PG lies upstream of either ecdysone synthesis and/or release, as feeding ecdysone could rescue development of IP_3R mutants (Venkatesh and Hasan, 1997). This finding was validated independently in 2015, when a role for IP_3R in releasing ecdysone from the PG was shown (Yamanaka *et al.*, 2015).

Mammals have three isoforms of the IP_3R whose expression varies across tissue types. Mice lacking IP_3R1 are mostly embryonic lethal (Matsumoto *et al.*, 1996) with survivors ultimately succumbing to ataxia and seizures, while mice lacking $IP_3R2/3$ die early due to poor secretion of digestive enzymes (Futatsugi *et al.*, 2005). In humans, a number of mutations in the IP_3R genes have been associated with disease (Terry *et al.*, 2018) of which two that have parallels with mice studies are spinocerebellar ataxia (SCA), that affects motor coordination (van de Leemput *et al.*, 2007), and Sjögren's Syndrome where salivary secretion is compromised (Teos *et al.*, 2015). In regards to development, in mammals the earliest role for IP_3R is in fertilization (Miyazaki *et al.*, 1992; Miyazaki *et al.*, 1993) followed by a role in specifying the dorso-ventral axis (Kume *et al.*, 1997). While there is no single hormone responsible for coordinating overall development in vertebrates akin to ecdysone, the oscillatory pattern of Ca^{2+} release induced by IP_3R activation is necessary for the secretion of hormones such as the gonadotropes follicle-stimulating hormone and luteinising hormone (Durán-Pastén and Fiordelisio, 2013), as well as oxytocin (Ludwig *et al.*, 2002), whose downstream targets ultimately control sexual maturation, reproduction and social behavior. Thus, while the details vary from species to species, IP_3R mediated Ca^{2+} release is important for the release of certain hormones and overall animal development.

In so far as nervous system development is concerned, an early study showed that IP_3R is enriched in growth cones of neurons in the chick dorsal root ganglion and required there for neurite extension (Takei *et al.*, 1998). In *Drosophila*, our heteroallelic IP_3R mutants were useful in demonstrating a neuronal requirement for IP_3R in viability (Joshi *et al.*, 2004) and flight circuit maturation (Banerjee *et al.*, 2006). These studies established physiological consequences of a loss in IP_3R function in neurons during development. At that time, this line of investigation was particularly novel. A major focus of Ca^{2+} signaling in neurons was the characterization of activity-dependent Ca^{2+} channels on the cell surface. That ER-Store Ca^{2+} too can contribute to, and modulate neuronal activity, was less well studied. Our group has pioneered this aspect of intracellular Ca^{2+} signaling and neuronal function to a significant extent.

At the cellular level, there is evidence that IP_3R regulates metabolism most notably by regulating the transfer of Ca^{2+} from the ER to the mitochondria (Cárdenas *et al.*, 2010). The IP_3R is enriched at ER-Mitochondria contact sites and this coupling is important to allow for efficient transfer of Ca^{2+} from the ER to the mitochondria via the mitochondrial Ca^{2+} uniporter (Rizzuto *et al.*, 2012). Ca^{2+} thus imported under basal conditions is required for the proper functioning of Ca^{2+}-dependent enzymes of the TCA cycle and in cancerous cells, is crucial for cell survival (Cardenas *et al.*, 2016). At the systemic level, in mice hepatocytes, there is evidence that IP_3R, phosphorylated upon glucagon induction, leads to gluconeogenic gene expression, which ultimately contributes to systemic glucose homeostasis during fasting (Wang *et al.*, 2012). This process is dysregulated in diabetes. Additionally a role for IP_3R in regulating lipid droplet formation in mice hepatocytes in the context of fatty liver disease has been described (Feriod *et al.*, 2017). The contribution of IP_3R to metabolism in neuronal cells, in tissue or primary culture, has been poorly explored. Our explorations in this area are detailed in the next section.

Store-operated Ca^{2+} entry and neuronal function

In 2005, the discovery of Stromal Interacting Molecule (STIM) an ER-Ca^{2+} sensor and in 2006, of Orai, a calcium selective ion channel on the plasma membrane, ushered in the molecular study of intracellular Ca^{2+} signaling characterized as Store-operated Ca^{2+} entry (SOCE) (Hogan and Rao, 2015). When GPCRs activate the IP_3R, it leads to a release of ER-Ca^{2+} and concomitant decrease in ER-Ca^{2+} levels that is sensed by STIM (Fig. 2). Loss of ER-Ca^{2+} triggers a conformational change in STIM followed by STIM dimerization and the exposure of Orai-binding sites. Bound by STIM, the channel pore of Orai opens, leading to an influx of Ca^{2+} ions from the extracellular milieu into the cytosol. Increased cytosolic Ca^{2+} then leads to the activation of varied cell specific processes. Thus, SOCE offers cells yet another means to modulate the frequency and amplitude of Ca^{2+} signaling in the cytosol. Functional studies have shown a role for SOCE in cell types as different as immune cells (Shaw and Feske, 2012), various cancer cells (Vashisht *et*

al., 2015) and neural progenitor cells (Gopurappilly *et al.*, 2018; Somasundaram *et al.*, 2014; Toth *et al.*, 2016), with the list growing as new functions for STIM and Orai are discovered. Of note, both STIM and Orai have been implicated in systemic metabolism in the maintenance of lipid homeostasis; STIM in fat body of flies (Baumbach *et al.*, 2014; Xu *et al.*, 2019), and both STIM and Orai in mammals (Maus *et al.*, 2017).

Capitalizing on single gene homologs of *STIM* and *Orai* in the *Drosophila* genome, our lab demonstrated that IP_3-mediated Ca^{2+} release is followed by SOCE in *Drosophila* neurons (Venkiteswaran and Hasan, 2009). Importantly, by using flight as a behavioral readout, we showed that SOCE in neurons is required for maturation of the flight neuronal circuit during pupal development (Agrawal *et al.*, 2010; Venkiteswaran and Hasan, 2009). Thus, not only do *Drosophila* neurons display SOCE signaling, but also, this signaling has functional consequences during neural development. Next we delved into the molecular basis of how neuronal function is regulated by SOCE. A transcriptomic screen in pupal brains identified many genes that are regulated by STIM and are required for flight circuit maturation (Richhariya *et al.*, 2017). We are now exploring how these molecular changes differ in various neuronal subtypes, and how they affect cellular output both at the level of the neuron, and in the neural circuit required for flight behavior.

Unlike the investigations on flight circuit maturation, our investigations on SOCE in the context of nutrition and development were somewhat serendipitous. Adult flies carrying hypomorphic mutations for the IP_3R exhibit hyperphagia and altered lipid metabolism, with systemic consequences such as increased weight and higher levels of Triacylglycerides (TAGs) (Subramanian *et al.*, 2013). To understand if adult metabolism could be reset by manipulating the nutritional input of larvae, IP_3R mutants were subjected to nutrient restriction (NR) in the late larval stage. We devised an NR paradigm that involved shifting mid to late stage 3rd instar larvae to a protein deficient diet consisting of 100mM Sucrose, up until pupariation (Fig. 1). While control larvae can successfully complete development in the NR paradigm, surprisingly, IP_3R mutant larvae cannot. This despite the fact that both control and mutant larvae were placed under NR after crossing critical weight, and much after critical time, beyond which the development of *Drosophila* typically becomes independent of food quality. Why do *itpr* mutants fail to pupariate under NR conditions? It is this question that led to insights on the neuronal requirement of intracellular Ca^{2+} signaling and SOCE for development, in the NR paradigm.

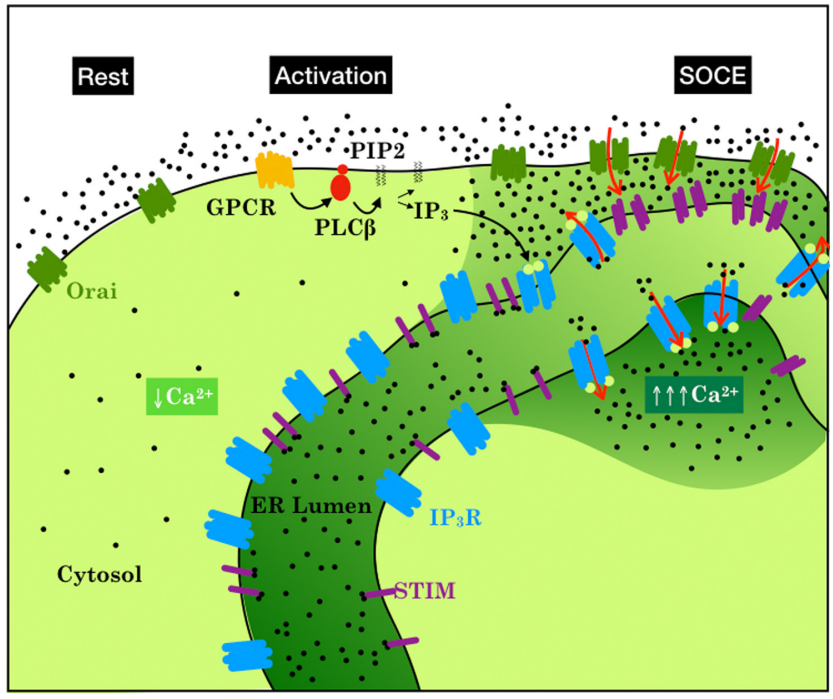

Fig. 2. SOCE (Store-operated Ca^{2+} entry) in neurons. *At rest, cytosolic Ca^{2+} levels are low and range between 100-400nM. Generation of IP_3, downstream of GPCR activation, leads to opening of the IP_3R channel and release of ER-store Ca^{2+}. A drop in ER Ca^{2+} concentration causes STIM to dimerize, cluster at ER-PM junctions and bind to the plasma-membrane localized SOCE channel Orai. Binding of STIM with Orai leads to an influx of Ca^{2+} from the extracellular milieu. Increased cytosolic Ca^{2+} regulates translation in peptidergic neurons. In glutamatergic neurons it directly modulates neuronal activity and in addition, regulates transcription leading to long-term changes in activity of neural circuits. Regulation of basic cellular processes and neuronal function by intracellular calcium signaling underpins the ability of larvae to pupariate under nutrient stress.*

Neuronal control of development under conditions of nutritional stress

Because key components of IP_3 signaling and SOCE, like the IP_3R, STIM and Orai, are expressed ubiquitously in multiple cell types, the cellular basis of pupariation needed further investigation. The *GAL4-UAS* system (Brand and Perrimon, 1993) was used to perform a genetic screen in which GAL4s specific to various organs and cell types were employed to reduce IP_3R expression using a transgenic RNAi construct referred to as *UAS-itprIR* (Agrawal *et al.*, 2010). The resulting larvae were tested for pupariation on NR. This screen identified a strong requirement for IP_3R in neuronal tissues and as expected in the PG, for larval to pupal development on an NR diet (Jayakumar *et al.*, 2016; Megha and Hasan, 2017). Based on the strength of the NR phenotype observed we focused on two neuronal subtypes, the neuroendocrine (NE) cells and a subset of glutamatergic neurons of the ventral nerve cord referred to as *VGN6341* neurons, for further study.

Neuroendocrine (NE) cells produce neuropeptides (NPs), a class of signaling agents, which can act either locally on neural circuits or systemically on other organs, to ultimately regulate a large number of behaviors and metabolism (Nassel and Winther, 2010). The broad role played by NPs and the poor characterization of SOCE molecules in modulating NE cell activity, fueled our interest in this neuronal subtype. We observed that reducing SOCE activity by manipulating IP_3R, STIM or Orai in NE cells reduced pupariation on NR (Megha and Hasan, 2017). A parallel observation at the systemic level was that IP_3R mutants were sensitive to levels of dietary protein and displayed lower protein/TAG ratio suggesting a protein metabolism defect. Because InR and TOR signaling pathways are known regulators of protein metabolism we combined the two observations to design a genetic experiment to test if molecules of the InR and/or TOR pathway compensated

for IP_3R function. Indeed, over-expression of either InR or TOR signaling in NE cells was sufficient to rescue pupariation of IP_3R mutants on NR, as well as systemic protein/TAG ratio. Conversely, over-expression of IP_3R could overcome loss of InR signaling in NE cells. These experiments led to the hypothesis that the InR and IP_3R pathways are functionally complementary in NE cells. To test this, we measured levels of protein translation in NE cells in various genetic conditions. IP_3R mutant NE cells displayed decreased protein translation and this could be restored to normal levels by the over-expression of wild type IP_3R. Together, these experiments describe a novel role for IP_3R in protein translation in NE cells and hence as an important determinant of development on NR (Megha and Hasan, 2017). Unlike InR or TOR pathways, the IP_3R signaling pathway is nutrient-independent. We speculate that this type of nutrient-independent control of protein translation is required in NE cells, perhaps because peptides made by these neurons are required in NR conditions to coordinate development. Reduced nutrient availability during NR likely reduces the activity of InR and TOR pathways in NE cells and a positive nutrient-independent regulator of protein translation, such as IP_3R, is required to maintain a certain level of protein translation and therefore, peptide levels. This speculation was based on a study of how neural stem cells (NSCs), which usually rely on InR and TOR signaling to exit quiescence and begin proliferation, continue to do so under NR conditions by switching to another ligand-receptor system that helps to maintain active signaling downstream of InR and TOR (Cheng *et al.*, 2011).

Each NE neuron secretes one or more distinct neuropeptide (NP) and together the NE subset tested in the previous project make at least 20 known NPs. To understand which neuropeptide(s) made in these cells is required in the context of SOCE to survive NR a curated genetic screen was performed. This screen yielded several candidate NPs, of which we decided to pursue Corazonin (Crz) and short Neuropeptide F (sNPF). Crz and sNPF are co-expressed in small set of neurons (~6; Dorsolateropeptidergic; DLPs) in the larval brain, providing a system where STIM's role in cellular neuropeptide biology could be easily studied. Immunohistochemistry and mass spectrometry revealed that loss of STIM perturbed peptide levels in DLP neurons: in the fed state, peptide levels were increased, while upon starvation, they remained unaltered while in the control they increased. Additional genetic experiments suggest two possible points at which STIM acts on neuropeptides – secretion and synthesis. Ultimately, loss of STIM in DLPs, resulted in reduced systemic Crz signaling which correlated with an inability of larvae to survive nutritional stress (Megha *et al.*, 2018).

Nutrient restriction stimulates a neuropeptide dependent glutamatergic neuron–insulin-producing cell circuit for stimulating ecdysone synthesis

In addition to regulating the synthesis and secretion of neuropeptides, experiments on a set of glutamatergic interneurons (the *VGN6341* neurons) in the context of development on NR have established a role for neuropeptide and acetylcholine stimulated intracellular Ca^{2+} signaling through the IP_3R in regulating neuronal gene transcription as well as neuronal activity (Jayakumar *et al.*, 2016). *VGN6341* neurons are located in the mid-ventral ganglion and IP_3R activity in this glutamatergic interneuron subset was found to be necessary for survival on NR. Because IP_3R operates downstream of GPCRs, a genetic screen was conducted to identify receptors on *VGN6341* neurons required in the NR paradigm. Interestingly, amongst the receptors identified, the largest class belonged to neuropeptide receptors, further underscoring the importance of neuropeptides to animal development under NR conditions. In *VGN6341* neurons neuropeptides stimulate specific aspects of intracellular Ca^{2+} signaling (see next paragraph). Additionally, the identification of muscarinic acetylcholine receptor (mAChR) suggested that cholinergic neurons were involved in transmitting NR information to *VGN6341* neurons. Here, the genetic toolkit available to *Drosophila* researchers came handy; by testing a series of GAL4 lines known to mark various subsets of cholinergic neurons, we identified multidendritic sensory cholinergic neurons (MSNs) located on the larval body wall as the source of NR sensing. Subsequently, we discovered that MSNs can sense nutrients directly through the amino acid transporter *slimfast* and also respond to the loss of essential amino acids, including arginine, in the food media (Jayakumar *et al.*, 2018). The MSNs convey this information to the glutamatergic neuronal subset covered by *VGN6341-GAL4*. A role for glutamatergic neurons in nutrient sensing is novel and required better understanding. Mapping of neuronal projections from the *VGN6341* neurons identified projections from the VNC that travel up to the larval brain lobes and reach the medial neurosecretory cells (mNSCs), a set of NE cells that make and secrete a number of NPs, and includes the IPCs. Others have demonstrated that ILPs secreted from the IPCs stimulates ecdysone synthesis from the PG (Colombani *et al.*, 2005; Mirth *et al.*, 2005). Thus, glutamatergic neurons in the ventral ganglion receive cholinergic inputs from the MSNs present on the larval body wall and transmit this information to the mNSCs in the brain. Signals received by the mNSCs stimulate ILP synthesis and/or release under protein-deprived conditions. Overall, this neural circuit up-regulates the transcription of ecdysone synthesizing genes in the PG (Jayakumar *et al.*, 2016).

In addition to the circuit, we examined how IP_3R regulates activity in the *VGN6341* neurons using *ex-vivo* brain preparations (Jayakumar *et al.*, 2018). Oscillating and long lasting Ca^{2+} signals were produced in the glutamatergic neurons upon loss of arginine that were dependent on intracellular Ca^{2+} signaling. Whereas initiation of Ca^{2+} signals required cholinergic stimulation of the mAchR, subsequent oscillations depended on NP receptor activation. To understand how neuronal properties were altered in IP_3R mutants, differentially regulated genes were identified by an RNAseq and validated in fluorescent activator-sorted *VGN6341* neurons. Many genes coding for voltage-gated ion channels including *NaCP60E* (Sodium channel), *Hk, eag* (Potassium channel), *cacophony, Caaplha1D* (Calcium channels) were down-regulated in the IP_3R mutants as well as upon *dSTIM* and *IP3R* knockdown. Changes in intracellular Ca^{2+} signaling thus modulate neuronal receptivity to acetylcholine and neuropeptides, and also leads to chronic changes in gene expression that can alter neuronal properties of excitability.

Together, these studies show that intracellular Ca^{2+} signaling plays an important and necessary role in regulating neuronal activity at the level of transcription as well as translation (Fig 2). Importantly, this regulation appears to be a fail-safe mechanism because it does not affect development when sufficient food resources are present. In times of nutritional stress, after the animal has sufficient internal resources to reach adult hood, this neuronal regulation takes on an important role. It ensures that nutrient-sensitive circuits and neuropeptidergic outputs continue to

adequately provide measures to over-ride loss of nutrition signals and allow development to proceed.

Future studies

There are two main directions we see emerging from these studies: further identification of molecular changes that are brought about by intracellular Ca^{2+} signaling in neurons during neural circuit maturation, and adapting the NR paradigm to ask questions that resonate with public health investigations on malnutrition.

In psychiatric disorders with developmental origins such as autism, no single gene correlates with major disease risk. Instead, the emerging hypothesis is that a change in balance of excitatory and inhibitory stimuli experienced by developing neural circuits might underlie disease manifestation (Nelson and Valakh, 2015). We propose that intracellular Ca^{2+} signaling can be contributory in maintaining a balance for such activation and silencing of neurons. Cumulatively, our work in *Drosophila* shows that intracellular Ca^{2+} signaling can perturb basic cellular processes in neurons, such as translation and transcription, providing broad mechanisms by which this balance may be controlled. Transcriptomic analysis of *Drosop*hila pupal neurons has already provided us a list of genes whose expression is sensitive to intracellular Ca^{2+} signaling (Richhariya *et al.*, 2017). Interestingly, mutations in *rala*, a modulator of synaptic vesicle release validated from this screen (Richhariya *et al.*, 2018), were recently identified as a genetic cause of intellectual disability (Hiatt *et al.*, 2018). Transcriptional regulation by intracellular calcium signaling also sets the stage to uncover how such gene expression changes are regulated: either directly perhaps by a common transcription factor whose activity is controlled by cytosolic Ca^{2+} levels, or other mechanisms of gene expressions, such as chromatin remodeling. The latter is suggested by another gene identified in the transcriptomic screen for SOCE-regulated genes, *dSet2,* a gene encoding a histone methyltransferase.

Scaling from these molecular studies to understanding how nutrition can re-program neurons and therefore behavior, requires a whole organism approach for which *Drosophila* is ideally suited. The molecular players involved in development as well as metabolism are strongly conserved between humans and animals, including *Drosophila*. There exist multiple classes of genetic tools that allow for scaling from molecules to organ systems to individuals, in *Drosophila*. Moreover, it is relatively cost-effective as compared to mammalian model organisms, easy to breed and its dietary input can be easily manipulated. We are at present characterizing the locomotory behavior (flight, walking, climbing, daily activity rhythms) in flies that have been subject to early life protein deprivation as well investigating their metabolism in response to a variety of diets in adulthood. These experiments will hopefully help identify adult phenotypes that correlate with early life nutritional stress, whose molecular basis can then be investigated by working on neural circuits described for these phenotypes. In humans, severe to moderate protein deficiency during childhood is associated with growth retardation, along with long-term consequences for mental health and metabolism. The molecular basis of how early life nutrition manifests into disease and health risks later in adult life is not well understood. Given the high prevalence of protein malnutrition in India, model systems to pursue this biology are therefore, the need of the hour, a niche that *Drosophila* may fulfil.

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References

AGRAWAL N, DELANOUE R, MAURI A, BASCO D, PASCO M, THORENS B, LEOPOLD P (2016). The *Drosophila* TNF Eiger Is an Adipokine that Acts on Insulin-Producing Cells to Mediate Nutrient Response. *Cell Metab* 23: 675–684.

AGRAWAL N, VENKITESWARAN G, SADAF S, PADMANABHAN N, BANERJEE S, HASAN G (2010). Inositol 1,4,5-trisphosphate receptor and dSTIM function in *Drosophila* insulin-producing neurons regulates systemic intracellular calcium homeostasis and flight. *J Neurosci* 30: 1301–1313.

BANERJEE S, JOSHI R, VENKITESWARAN G, AGRAWAL N, SRIKANTH S, ALAM F, HASAN G (2006). Compensation of inositol 1,4,5-trisphosphate receptor function by altering sarco-endoplasmic reticulum calcium ATPase activity in the *Drosophila* flight circuit. *J Neurosci* 26: 8278–8288.

BAUMBACH J, HUMMEL P, BICKMEYER I, KOWALCZYK KMM, FRANK M, KNORR K, HILDEBRANDT A, RIEDEL D, JÄCKLE H, KÜHNLEIN RP, JACKLE H, KUHNLEIN RP (2014). A *Drosophila in vivo* screen identifies store-operated calcium entry as a key regulator of adiposity. *Cell Metab* 19: 331–343.

BERRIDGE MJ, BOOTMAN MD, RODERICK HL (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 4: 517.

BOULAN L, MILAN M, LEOPOLD P (2015). The Systemic Control of Growth. *Cold Spring Harb Perspect Biol* 7. 7(12). pii: a019117

BRAND AH, PERRIMON N (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118: 401–415.

BRITTON JS, LOCKWOOD WK, LI L, COHEN SM, EDGAR BA (2002). *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev Cell* 2: 239–249.

BROUGHTON SJ, PIPER MD, IKEYA T, BASS TM, JACOBSON J, DRIEGE Y, MARTINEZ P, HAFEN E, WITHERS DJ, LEEVERS SJ, PARTRIDGE L (2005). Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc Natl Acad Sci USA* 102: 3105–3110.

CALDWELL PE, WALKIEWICZ M, STERN M (2005). Ras activity in the *Drosophila* prothoracic gland regulates body size and developmental rate via ecdysone release. *Curr Biol* 15: 1785–1795.

CÁRDENAS C, MILLER RA, SMITH I, BUI T, MOLGÓ J, MÜLLER M, VAIS H, CHEUNG K-H, YANG J, PARKER I, THOMPSON C, BIRNBAUM M, HALLOWS KR, FOSKETT JK (2010). Essential Regulation of Cell Bioenergetics By Constitutive InsP(3) Receptor Ca(2+) Transfer to Mitochondria. *Cell* 142: 270–283.

CARDENAS C, MULLER M, MCNEAL A, LOVY A, JANA F, BUSTOS G, URRA F, SMITH N, MOLGO J, DIEHL JA, RIDKY TW, FOSKETT JK (2016). Selective Vulnerability of Cancer Cells by Inhibition of Ca(2+) Transfer from Endoplasmic Reticulum to Mitochondria. *Cell Rep* 15: 219–220.

CHENG LY, BAILEY AP, LEEVERS SJ, RAGAN TJ, DRISCOLL PC, GOULD AP (2011). Anaplastic lymphoma kinase spares organ growth during nutrient restriction in *Drosophila*. *Cell* 146: 435–447.

CLAPHAM DE (1995). Calcium signaling. *Cell* 80: 259–268.

CLAPHAM DE, YEROMIN AV, ZHANG XH, YU Y, SAFRINA O, PENNA A, ROOS J, STAUDERMAN KA, CAHALAN MD, TAKEMORI H, AL. et (2007). Calcium signaling. *Cell* 131: 1047–1058.

COLOMBANI J, ANDERSEN DS, LEOPOLD P (2012). Secreted peptide Dilp8 coordinates *Drosophila* tissue growth with developmental timing. *Science* 336: 582–585.

COLOMBANI J, BIANCHINI L, LAYALLE S, PONDEVILLE E, DAUPHIN-VILLEMANT C, ANTONIEWSKI C, CARRE C, NOSELLI S, LEOPOLD P (2005). Antagonistic actions of ecdysone and insulins determine final size in *Drosophila*. *Science* 310: 667–670.

DAS R, DOBENS LL (2015). Conservation of gene and tissue networks regulating insulin signalling in flies and vertebrates. *Biochem Soc Trans* 43.

DELANOUE R, MESCHI E, AGRAWAL N, MAURI A, TSATSKIS Y, MCNEILL H, LÉOPOLD P (2016). *Drosophila* insulin release is triggered by adipose Stunted ligand to brain Methuselah receptor. *Science (80-)* 353: 1553 LP – 1556.

DURÁN-PASTÉN ML, FIORDELISIO T (2013). GnRH-Induced Ca2+ Signaling Patterns and Gonadotropin Secretion in Pituitary Gonadotrophs. Functional Adaptations to Both Ordinary and Extraordinary Physiological Demands. *Front Endocrinol* 4: 127.

EDGAR BA (2006). How flies get their size: genetics meets physiology. *Nat Rev Genet* 7: 907–916.

FERIOD CN, OLIVEIRA AG, GUERRA MT, NGUYEN L, RICHARDS KM, JURCZAK MJ, RUAN H-B, CAMPOREZ JP, YANG X, SHULMAN GI, BENNETT AM, NATHANSON MH, EHRLICH BE (2017). Hepatic Inositol 1,4,5 Trisphosphate Receptor Type 1 Mediates Fatty Liver. *Hepatol Commun* 1: 23–35.

FUTATSUGI A, NAKAMURA T, YAMADA MK, EBISUI E, NAKAMURA K, UCHIDA K, KITAGUCHI T, TAKAHASHI-IWANAGA H, NODA T, ARUGA J, MIKOSHIBA K (2005). IP3 receptor types 2 and 3 mediate exocrine secretion underlying energy metabolism. *Science (80-)* 309: 2232–2234.

GARELLI A, HEREDIA F, CASIMIRO AP, MACEDO A, NUNES C, GARCEZ M, DIAS ARM, VOLONTE YA, UHLMANN T, CAPARROS E, KOYAMA T, GONTIJO AM (2015). Dilp8 requires the neuronal relaxin receptor Lgr3 to couple growth to developmental timing. *Nat Commun* 6: 8732.

GOPURAPPILLY R, DEB BK, CHAKRABORTY P, HASAN G (2018). Stable STIM1 Knockdown in Self-Renewing Human Neural Precursors Promotes Premature Neural Differentiation. *Front Mol Neurosci* 11: 178.

HASAN G, ROSBASH M (1992). *Drosophila* homologs of two mammalian intracellular Ca(2+)-release channels: identification and expression patterns of the inositol 1,4,5-triphosphate and the ryanodine receptor genes. *Development* 116: 967–975.

HIATT SM, NEU MB, RAMAKER RC, HARDIGAN AA, PROKOP JW, HANCAROVA M, PRCHALOVA D, HAVLOVICOVA M, PRCHAL J, STRANECKY V, *et al.,* (2018). De novo mutations in the GTP/GDP-binding region of RALA, a RAS-like small GTPase, cause intellectual disability and developmental delay. *PLOS Genet* 14: e1007671.

HOGAN PG, RAO A (2015). Store-operated calcium entry: Mechanisms and modulation. *Biochem Biophys Res Commun* 460: 40–49.

JAYAKUMAR S, RICHHARIYA S, DEB BK, HASAN G (2018). A Multicomponent Neuronal Response Encodes the Larval Decision to Pupariate upon Amino Acid Starvation. *J Neurosci* 38: 10202–10219.

JAYAKUMAR S, RICHHARIYA S, REDDY OV, TEXADA MJM, HASAN G, RICHARIYA S, REDDY VO, TEXADA MJM, HASAN G (2016). *Drosophila* larval to pupal switch under nutrient stress requires IP3R/Ca2+ signalling in glutamatergic interneurons. *Elife* 5: e17.

JOSHI R, VENKATESH K, SRINIVAS R, NAIR S, HASAN G (2004). Genetic dissection of itpr gene function reveals a vital requirement in aminergic cells of *Drosophila* larvae. *Genetics* 166: 225–236.

KIM J, NEUFELD TP (2015). Dietary sugar promotes systemic TOR activation in *Drosophila* through AKH-dependent selective secretion of Dilp3. *Nat Commun* 6: 6846.

KOYAMA T, MIRTH CK (2016). Growth-Blocking Peptides As Nutrition-Sensitive Signals for Insulin Secretion and Body Size Regulation. *PLOS Biol* 14: e1002392.

KOYAMA T, RODRIGUES MA, ATHANASIADIS A, SHINGLETON AW, MIRTH CK (2014). Nutritional control of body size through FoxO-Ultraspiracle mediated ecdysone biosynthesis Ed. H McNeill. *Elife* 3: e03091.

KUME S, MUTO A, INOUE T, SUGA K, OKANO H, MIKOSHIBA K (1997). Role of inositol 1,4,5-trisphosphate receptor in ventral signaling in *Xenopus* embryos. *Science* 278: 1940–1943.

LAYALLE S, ARQUIER N, LEOPOLD P (2008). The TOR pathway couples nutrition and developmental timing in *Drosophila*. *Dev Cell* 15: 568–577.

VAN DE LEEMPUT J, CHANDRAN J, KNIGHT MA, HOLTZCLAW LA, SCHOLZ S, COOKSON MR, HOULDEN H, GWINN-HARDY K, FUNG H-C, LIN X, *et al.,* (2007). Deletion at ITPR1 underlies ataxia in mice and spinocerebellar ataxia 15 in humans. *PLoS Genet* 3: e108.

LUDWIG M, SABATIER N, BULL PM, LANDGRAF R, DAYANITHI G, LENG G (2002). Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 418: 85–89.

MEGHA, CHRISTIAN WEGENER and GAITI HASAN (2019) Neuropeptides required for Drosophila development under nutritional stress are regulated by the ER-Ca2+ sensor STIM. *PLoS One.* 14(7):e0219719.

MATSUMOTO M, NAKAGAWA T, INOUE T, NAGATA E, TANAKA K, TAKANO H, MINOWA O, KUNO J, SAKAKIBARA S, YAMADA M, YONESHIMA H, MIYAWAKI A, FUKUUCHI Y, FURUICHI T, OKANO H, MIKOSHIBA K, NODA T (1996). Ataxia and epileptic seizures in mice lacking type 1 inositol 1,4,5-trisphosphate receptor. *Nature* 379: 168–171.

MAUS M, CUK M, PATEL B, LIAN J, OUIMET M, KAUFMANN U, YANG J, HORVATH R, HORNIG-DO H-T, CHRZANOWSKA-LIGHTOWLERS ZM, MOORE KJ, CUERVO AM, FESKE S (2017). Store-Operated Ca(2+) Entry Controls Induction of Lipolysis and the Transcriptional Reprogramming to Lipid Metabolism. *Cell Metab* 25: 698–712.

MCBRAYER Z, ONO H, SHIMELL M, PARVY JP, BECKSTEAD RB, WARREN JT, THUMMEL CS, DAUPHIN-VILLEMANT C, GILBERT LI, O'CONNOR MB (2007). Prothoracicotropic Hormone Regulates Developmental Timing and Body Size in *Drosophila*. *Dev Cell* 13: 857–871.

MEGHA, HASAN G (2017). IP3R-mediated Ca2+ release regulates protein metabolism in *Drosophila* neuroendocrine cells: implications for development under nutrient stress. *Development* 144: 1484–1489.

MIKOSHIBA K (2015). Role of IP3 receptor signaling in cell functions and diseases. *Adv Biol Regul* 57: 217–227.

MIRTH C, TRUMAN JW, RIDDIFORD LM (2005). The role of the prothoracic gland in determining critical weight for metamorphosis in *Drosophila melanogaster*. *Curr Biol* 15: 1796–1807.

MIRTH CK, SHINGLETON AW (2012). Integrating body and organ size in Drosophila: Recent advances and outstanding problems. *Front Endocrinol* 3:49.

MIYAZAKI S, SHIRAKAWA H, NAKADA K, HONDA Y (1993). Essential role of the inositol 1,4,5-trisphosphate receptor/Ca2+ release channel in Ca2+ waves and Ca2+ oscillations at fertilization of mammalian eggs. *Dev Biol* 158: 62–78.

MIYAZAKI S, YUZAKI M, NAKADA K, SHIRAKAWA H, NAKANISHI S, NAKADE S, MIKOSHIBA K (1992). Block of Ca2+ wave and Ca2+ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science* 257: 251–255.

NASSEL DR, WINTHER AM (2010). *Drosophila* neuropeptides in regulation of physiology and behavior. *Prog Neurobiol* 92: 42–104.

NELSON SB, VALAKH V (2015). Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders. *Neuron* 87: 684–698.

OHHARA Y, KOBAYASHI S, YAMANAKA N (2017). Nutrient-Dependent Endocycling in Steroidogenic Tissue Dictates Timing of Metamorphosis in *Drosophila melanogaster*. *PLoS Genet* 13: e1006583.

OKAMOTO N, NISHIMURA T (2015). Signaling from Glia and Cholinergic Neurons Controls Nutrient-Dependent Production of an Insulin-like Peptide for *Drosophila* Body Growth. *Dev Cell* 35: 295–310.

PASCO MY, LÉOPOLD P (2012). High Sugar-Induced Insulin Resistance in *Drosophila* Relies on the Lipocalin Neural Lazarillo. *PLoS One* 7: e36583.

PIPER MDW, BLANC E, LEITÃO-GONÇALVES R, YANG M, HE X, LINFORD NJ, HODDINOTT MP, HOPFEN C, SOULTOUKIS GA, NIEMEYER C, KERR F, PLETCHER SD, RIBEIRO C, PARTRIDGE L (2013). A holidic medium for *Drosophila melanogaster*. *Nat Methods* 11: 100.

RAJAN A, PERRIMON N (2012). *Drosophila* cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* 151: 123–137.

REWITZ KF, YAMANAKA N, GILBERT LI, O'CONNOR MB (2009). The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis. *Science* 326: 1403–1405.

RICHHARIYA S, JAYAKUMAR S, ABRUZZI K, ROSBASH M, HASAN G (2017). A pupal transcriptomic screen identifies Ral as a target of store-operated calcium entry in *Drosophila* neurons. *Sci Rep* 7: 42586.

RIZZUTO R, DE STEFANI D, RAFFAELLO A, MAMMUCARI C (2012). Mitochondria as sensors and regulators of calcium signalling. *Nat Rev Mol Cell Biol* 13: 566–578.

ROBERTSON FW (1963). The ecological genetics of growth in *Drosophila* 6. The genetic correlation between the duration of the larval period and body size in relation to larval diet. *Genet Res* 4: 74–92.

RODENFELS J, LAVRYNENKO O, AYCIRIEX S, SAMPAIO JL, CARVALHO M, SHEVCHENKO A, EATON S (2014). Production of systemically circulating Hedgehog by the intestine couples nutrition to growth and development. *Genes Dev* 28: 2636–2651.

RULIFSON EJ, KIM SK, NUSSE R (2002). Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science (80-)* 296: 1118–1120.

SANO H, NAKAMURA A, TEXADA MJ, TRUMAN JW, ISHIMOTO H, KAMIKOUCHI A, NIBU Y, KUME K, IDA T, KOJIMA M (2015). The Nutrient-Responsive Hormone CCHamide-2 Controls Growth by Regulating Insulin-like Peptides in the Brain of *Drosophila melanogaster*. *PLOS Genet* 11: e1005209.

SETIAWAN L, PAN X, WOODS AL, O'CONNOR MB, HARIHARAN IK (2018). The BMP2/4 ortholog Dpp can function as an inter-organ signal that regulates developmental timing. *Life Sci Alliance* 1: e201800216.

SHAW PJ, FESKE S (2012). Physiological and pathophysiological functions of SOCE in the immune system. *Front Biosci (Elite Ed)* 4: 2253–2268.

SLAIDINA M, DELANOUE R, GRONKE S, PARTRIDGE L, LEOPOLD P (2009). A *Drosophila* insulin-like peptide promotes growth during nonfeeding states. *Dev Cell* 17: 874–884.

SOMASUNDARAM A, SHUM AK, MCBRIDE HJ, KESSLER JA, FESKE S, MILLER RJ, PRAKRIYA M (2014). Store-operated CRAC channels regulate gene expression and proliferation in neural progenitor cells. *J Neurosci* 34: 9107–9123.

STIEPER BC, KUPERSHTOK M, DRISCOLL M V., SHINGLETON AW (2008). Imaginal discs regulate developmental timing in *Drosophila melanogaster*. *Dev Biol* 321: 18–26.

SUBRAMANIAN M, METYA SK, SADAF S, KUMAR S, SCHWUDKE D, HASAN G (2013). Altered lipid homeostasis in *Drosophila* InsP3 receptor mutants leads to obesity and hyperphagia. *Dis Model Mech* 6: 734–44.

TAKEI K, SHIN RM, INOUE T, KATO K, MIKOSHIBA K (1998). Regulation of nerve growth mediated by inositol 1,4,5-trisphosphate receptors in growth cones. *Science* 282: 1705—1708.

TEOS LY, ZHANG Y, COTRIM AP, SWAIM W, WON JH, AMBRUS J, SHEN L, BEBRIS L, GRISIUS M, JANG S-I, YULE DI, AMBUDKAR IS, ALEVIZOS I (2015). IP3R deficit underlies loss of salivary fluid secretion in Sjogren's Syndrome. *Sci Rep* 5: 13953.

TERRY LE, ALZAYADY KJ, FURATI E, YULE DI (2018). Inositol 1,4,5-trisphosphate Receptor Mutations associated with Human Disease. *Messenger (Los Angeles, Calif Print)* 6: 29–44.

TOTH AB, SHUM AK, PRAKRIYA M (2016). Regulation of neurogenesis by calcium signaling. *Cell Calcium* 59: 124–134.

VASHISHT A, TREBAK M, MOTIANI RK (2015). STIM and Orai proteins as novel targets for cancer therapy. A Review in the Theme: Cell and Molecular Processes in Cancer Metastasis. *Am J Physiol Cell Physiol* 309: C457–C469.

VENKATESH K, HASAN G (1997). Disruption of the IP3 receptor gene of *Drosophila* affects larval metamorphosis and ecdysone release. *Curr Biol* 7: 500–509.

VENKITESWARAN G, HASAN G (2009). Intracellular Ca2+ signaling and store-operated Ca2+ entry are required in *Drosophila* neurons for flight. *Proc Natl Acad Sci USA* 106: 10326–10331.

WANG Y, LI G, GOODE J, PAZ JC, OUYANG K, SCREATON R, FISCHER WH, CHEN J, TABAS I, MONTMINY M (2012). Inositol-1,4,5-trisphosphate receptor regulates hepatic gluconeogenesis in fasting and diabetes. *Nature* 485: 128–132.

XU Y, BORCHERDING AF, HEIER C, TIAN G, ROEDER T, KÜHNLEIN RP (2019). Chronic dysfunction of Stromal interaction molecule by pulsed RNAi induction in fat tissue impairs organismal energy homeostasis in *Drosophila*. *Sci Rep* 9: 6989.

YAMANAKA N, MARQUES G, O'CONNOR MB (2015). Vesicle-Mediated Steroid Hormone Secretion in *Drosophila melanogaster*. *Cell* 163: 907–919.

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