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1 **Growth regulation by amino acid transporters in *Drosophila* larvae**

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26 **Summary:**

27 *Drosophila* larvae need to adapt their metabolism to reach a critical body size to pupate.
28 This process needs food resources and has to be tightly adjusted in order to control
29 metamorphosis timing and adult size. Nutrients such as amino acids either directly present in
30 the food or obtained via protein digestion play key regulatory roles in controlling metabolism
31 and growth. Amino acids act especially on two organs, the fat body and the brain, to control
32 larval growth, body size developmental timing and pupariation. The expression of specific
33 amino acid transporters in fat body cells, and in the brain through specific neurons and glial
34 cells are essential to activate downstream molecular signaling pathways in response to amino
35 acid levels. In this review, we highlight some of these specific networks dependent on amino
36 acid diet to control DILP levels, and by consequence larval metabolism and growth.

37

38

39 **Keywords:** neuron, glia, physiology, molecular signal, insulin producing cells, LAT1

40 **Manuscript:**

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42 In order to grow and to survive, animals must constantly adapt to their environment where
43 they have to find all the essential components they need such as water, oxygen, and food. In
44 particular, they must find in their diet all the elements necessary to cover their daily
45 nutritional needs and to develop. To ensure an optimal supply of nutrients, animals constantly
46 adjust their food intake to their nutritional status [1]. The ingested food is broken down during
47 digestion into various nutrients such as fatty acids, sugars and amino acids, which are
48 essential for energy production and cellular functioning, division, growth and renewal [2-4].
49 Among these, essential amino acids are key nutrients that animals cannot synthesize and need
50 to find from their food [5]. Most amino acids also represent signaling molecules that control
51 animal metabolism. They need to be finely regulated to assess vital requirements such as
52 energy balance, protein synthesis, and cell and tissue development [1]. In *Drosophila*, growth
53 occurs during four morphologically distinct developmental forms: embryo, larva (three instar
54 stages), pupa, and adult. The most important body size increase prevails during third larval
55 instar. This spectacular growth is concluded when a critical weight is reached, which
56 mobilizes hormonal signals such as PTTH, ecdysone, juvenile hormone, and insulin-like
57 peptides [6-9]. As a consequence, larvae must eat a lot during this third instar in order to gain
58 weight. This review will focus on the role of amino acids transporters affecting this larval
59 growth.

60 In *Drosophila* larvae, nutrients like amino acids go across the gut wall from the lumen of
61 the digestive tract to the hemolymph [4,10]. Thus, amino acids circulate in the whole body
62 before being detected and used by cells for protein synthesis or metabolic purposes. Organs
63 that are able to detect them include the fat body, and some neurons and glial cells located in
64 the brain. As in mammals, a permanent dialogue between these tissues exists to maintain an
65 amino acid homeostasis necessary for development and growth of *Drosophila* larvae [11].
66 Amino acids can thus modify the physiology of the organism by direct cell-autonomous, or
67 indirect non-cell-autonomous detection (Fig. 1).

68 In *Drosophila* larvae, the regulation of metabolism involves many hormones including
69 insulin-like peptides [12]. Eight insulin-like coding genes (*dilp1-8*) have been identified so
70 far. DILPs 1–7 are thought to act through a single *Drosophila* insulin/IGF receptor (InR),
71 whereas DILP8 acts through the neuronal relaxin receptor *Igr-3* [13-17]. The corresponding
72 peptides are expressed in many tissues such as the nervous system and the digestive system
73 [14-15]. During the larval stage, when maggots feed, DILPs produced and secreted by the

74 brain couple nutrient uptake with systemic growth. DILP2 and DILP5 are specifically
75 expressed in 7 cell-pairs (IPCs, Insulin-Producing Cells) of the brain *pars intercerebralis*
76 region [14]. These cells can be compared to the mammalian pancreatic β cells that produce
77 and secrete insulin [18, 19]. It has been shown that the genetic ablation of IPCs alone has a
78 major impact on metabolism and development [20, 21]. In particular, the level of circulating
79 sugars in the hemolymph is drastically increased when IPCs are ablated [21]. Another DILP,
80 DILP6 is expressed in the fat body and in glial cells from the blood-brain barrier [22-25].
81 During post-feeding development, DILP6 plays an important role in growth regulation [22,
82 23].

83

84 **Amino acid sensing and secretion of hormones by the fat body**

85 The *Drosophila* fat body is considered to be the equivalent of both vertebrate liver and
86 adipose tissue [26]. It plays a key role in energy storage by converting fatty acids, proteins
87 and sugars into triglycerides that are stored in lipid droplets [27]. The fat body can then
88 mobilize these droplets when the animal needs high energy and compounds for cellular
89 functioning or larval growth. For this purpose, the fat body is able to detect nutrient variations
90 in the hemolymph, including amino acids. Sensing of amino acid in the fat body depends on
91 the activity of TORC1 (target of rapamycin complex 1) signaling, which then indirectly
92 induces the activation of the insulin pathway (InR/Pi3k) in salivary glands and imaginal discs
93 [28]. The fat body then secretes hormonal factors, which act on different organs to control
94 development and to adjust the metabolic status [28].

95 The role of two amino-acid transporters, Minidisks (Mnd) and Slimfast (Slif), has been
96 characterized in the fat body (Fig. 2). Mnd is an amino acid transporter belonging to the L-
97 type Amino Acid Transporter (LAT1) family [29-32], which catalyzes the cross-membrane
98 flux of large neutral amino acids. Mnd is expressed in several tissues including the fat body,
99 and is involved in the larval development of imaginal discs [33]. *Mnd* mutant imaginal discs
100 transplanted into a wild type larva continue to develop. This result indicates that Mnd action
101 on imaginal discs development is non-cell autonomous, and it suggests the existence of
102 messengers from other organs expressing Mnd [33]. Slif belongs to the cationic amino acid
103 family (CAT) and plays a major role in amino-acid transport in the fat body, leading to
104 TORC1 activation [34]. The inhibition of *slif* expression in the fat body causes a larval
105 development defect that mimics an amino-acid deprivation and leads to smaller adults at
106 emergence (Fig. 2) [28].

107 In response to amino acids levels in the hemolymph, different factors are secreted from
108 the fat body and induce the release of insulin-like peptides by IPCs to regulate growth. These
109 factors have been revealed by brain and fat body co-cultures [35, 36]. Fat bodies from larvae
110 fed with a rich amino-acid medium (tryptone) are capable to stimulate the release of DILPs
111 that are normally stored in the IPCs during starvation conditions [35]. Depending on the
112 protein diet, two cytokines Eiger and Sun can be released by the fat body (Fig. 2A-B) [37,
113 38].

114 In low protein conditions, the fat body expresses and releases the transmembrane protein
115 Eiger, a cytokine homologous to TNF- α in mammals [37]. Eiger is cleaved by the TNF- α
116 converting enzyme (TACE). From this reaction a soluble form of Eiger can circulate in the
117 hemolymph (Fig. 2A). TACE is under the control of the TORC1 pathway that is sensitive to
118 the presence of amino acids in the hemolymph. Eiger then binds to its receptor Grindelwald
119 (Gnrd), a TNF- α receptor localized on the IPCs, and allows the activation of the Jun N-
120 terminal Kinase (JNK) pathway. Then activated JNK inhibits *dilp2* and *dilp5* gene expression
121 and therefore blocks larval growth (Fig. 2A) [37].

122 In a rich protein diet, TORC1 inhibits TACE, thus, Eiger is not cleaved and remains
123 attached to the adipocyte membrane leading to normal growth. Another cytokine, Stunted
124 (Sun), is secreted by the fat body into the hemolymph after high amino-acid diet (Fig. 2B)
125 [38]. This release is dependent on the TORC1 pathway. Sun then binds to its receptor
126 Methuselah, a secretin-incretin receptor expressed in IPCs. This binding permits the release of
127 DILP2 and DILP5, which in turn activates systemic organ growth [38].

128 In response to a rich amino-acid diet and to the activation of TORC1 pathway, the fat
129 body secretes two other factors called GBP1 and GBP2 (Growth Blocking Peptide) that
130 indirectly induce the release of DILP2 and DILP5 by IPCs (Fig. 2B) [36, 39]. Secreted GBP1
131 binds to its receptor, a transmembrane EGF receptor located on inhibitory neurons (IPC-
132 connecting neurons or ICNs) that synapse with IPCs [39]. The binding of GBP1 to its
133 receptor removes the inhibition of IPCs by ICNs and thus allows the release of DILPs (Fig.
134 2B) [39]. Thus, the intake of food or a rich amino-acid diet acts through the fat body and
135 inhibitory neurons to indirectly cause the release of DILPs by IPCs.

136

137

138 **Direct amino acid sensing by brain cells**

139 During the larval stages, food intake and the detection of amino acids are under the
140 control of the nervous system allowing the larvae to distinguish balanced and imbalanced

141 amino-acid diets (Fig. 3). This control depends on a cluster of dopaminergic neurons of the
142 dorsolateral cluster 1 (DA DL1 neurons) located in the larval brain [40]. Larvae avoid food
143 with low essential amino acids (EAA) content and prefer food with balanced amino acids.
144 The circulating amino acids in the hemolymph are directly detected by the DA DL1 neurons.
145 These dopaminergic neurons have an inhibitory role in food intake since the release of
146 dopamine reduces food consumption (Fig. 3).

147 In balanced amino-acid diet, GABA receptors located on DA DL1 neurons repress the
148 secretion of dopamine allowing food intake [40]. The inhibition of the expression of the
149 amino-acid transporter Slif in DA DL1 neurons reduces food intake in the presence of a rich
150 diet (Fig. 3A) [40].

151 Similarly, an imbalanced amino-acid diet detected by DA DL1 neurons decreases food
152 intake (Fig. 3B). In the DA DL1 neurons, an amino-acid sensor, conserved GC
153 nonderepressing 2 (GCN2) kinase, is activated in the absence of EAA and, in turn, leads to
154 the activation of the ATF4 transcription factor. Then ATF4 binds to and inactivates GABAR1
155 and GABAR2 receptors. The inactivation of GABA receptors allows the secretion of
156 dopamine and leads to a drop of food intake (Fig. 3B) [40].

157 Levels of essential amino acids can thus regulate food intake and therefore animal growth
158 via dopaminergic DL1 neurons.

159

160 In mammals, amino acids such as leucine and isoleucine, which are BCAA-type amino
161 acids (Branched Chain Amino Acids), are transported by LAT-1-type amino-acid transporters
162 and stimulate insulin release from pancreatic β -cells [41]. In *Drosophila* larvae, homologous
163 cells of pancreatic β -cells are the 7 pairs of IPCs located in the median region of the *pars*
164 *intercerebralis* [14, 18, 19]. In fasting *Drosophila* larvae, leucine and isoleucine are able to
165 directly regulate the neuronal activity of these IPCs [31, 42]. Effectively, in *ex vivo* cultured
166 brains from fasting larvae, leucine induces the release of DILP2 and DILP5 by IPCs (Fig. 4).
167 The function of two amino-acid transporters of the LAT-1 family, Mnd and Juvenile hormone
168 Inducible-21 (JhI-21), has been characterized in IPCs. Mnd is mostly present in the
169 endoplasmic reticulum of IPCs, while JhI-21 appears to be preferentially localized at the
170 plasma membrane of IPCs (Fig. 4) [31, 42]. Knockdown of Mnd or JhI-21 or both
171 transporters in IPCs impairs DILPs release in the presence of leucine and has an impact on
172 metabolism and on larval growth [31, 42]. Therefore, as in mammals, leucine stimulates a
173 leucine sensitive enzyme GDH located in the IPCs to promote insulin release in *Drosophila*
174 larvae (Fig. 4) [31].

175

176 In the brain, glial cells are also able to detect amino acids and regulate cell growth and
177 larval development (Fig. 5). They can directly react to amino acids transported by the amino-
178 acid transporter, Slif. In response to amino acids, glial cells release an insulin-like peptide,
179 DILP6. Then, DILP6 binds to InR located on some acetylcholinergic neurons in the brain.
180 This neuronal activation allows the release of the Jelly belly (Jeb) peptide (Fig. 5) [43]. The
181 mechanism of Jeb secretion is unknown but is different from Acetylcholine release. Jeb
182 peptide binds to Alk (Anaplastic lymphoma kinase receptor) expressed by IPCs. It allows the
183 activation of a PI3K pathway that leads to the phosphorylation of the transcription factor
184 FoxO. The phosphorylated inactive form of FoxO remains in the cytoplasm allowing the
185 expression of *dilp5*. Finally, the secretion of DILP5 into the hemolymph promotes larval
186 growth (Fig. 5). In low protein diet condition, the transcription factor FoxO represses the
187 expression of *dilp5* gene in IPCs. Some glial cells expressing the InR receptor are sensitive to
188 DILP5 and other DILPs and express *dilp6* generating a feedback loop (Fig. 5) [43].

189

190 Larval development coordination involves also many hormones including the
191 prothoracicotropic hormone (PTTH) and ecdysone, a steroidogenic hormone [44]. These
192 hormones must be finely regulated to control the duration of the different larval stages and the
193 transition to pupal stage. Before each larval or pupal molt, the increase of PTTH synthesis and
194 secretion by PTTH neurons located in the larval brain activates ecdysone secretion by cells
195 localized in the Ring Gland (Fig. 5). This ecdysone production may also depend on the
196 presence of various nutrients such as amino acids since it is under the control of DILPs [45].
197 Moreover, it has been shown that the amino acid transporter coding gene *Sobremesa* (Sbm)
198 expressed in glial cells is involved in the timing of larval and brain development [46]. Sbm
199 downregulation causes an extension of the duration of the last larval instar together with an
200 increased body size, but leads to smaller brain lobes. Downregulation of Sbm in glia cells
201 does not affect *dilp6* expression in glia but reduces PTTH levels (Fig. 5) [46]. This drop of
202 PTTH might lead to a decrease of ecdysone synthesis inducing a developmental delay and a
203 reduction of the brain size due to less neuroblast division [47]. The mechanism of how glial
204 cells govern PTTH level in PTTH neurons remains elusive. In glial cells, Sbm could
205 participate to the transport of circulating amino acids in hemolymph to promote PTTH release
206 necessary for ecdysone synthesis required to regulate developmental timing [46].

207 During the *Drosophila* larval development, some cells like neuroblasts remain quiescent
208 in the brain until they enter cell division to generate two cell types, neurons and glial cells

209 [48]. The activation of neuroblast division depends on amino acids present in the food
210 whereas differentiation of neuroblasts into neurons and/or glial cells is food independent [24,
211 25, 49]. The fat body senses the amino acids via the TORC1 pathway and produces an
212 unknown signal. This signal acts on brain glia cells which in turn release DILP6 [24, 25].
213 DILP6 binds to InR located on neuroblast membranes, inducing neuroblast reactivation and
214 division (Fig. 5) [24, 25].

215

216 **Conclusion and outlook**

217 Amino acids are essential as basic components for protein synthesis that are involved in
218 many cellular functions (membrane activities, enzymes, transports, signaling, and gene
219 expression). Amino acids can also act as neurotransmitters like glutamate and participate as
220 precursors in the synthesis of neurotransmitters (tryptophan for serotonin or phenylalanine for
221 dopamine). Some amino acids like glutamate can be synthesized by the cellular enzymatic
222 machinery. The so-called essential amino acids like tryptophan, phenylalanine or leucine are
223 not synthesized by the organism, and must absolutely be found in the food. Thus, among the
224 nutrients capable of regulating larval growth and development, amino acids play a major role.

225 Here, we highlight known internal detection mechanisms and pathways depending on
226 amino acids that regulate larval and tissue growth. Once in larval hemolymph, circulating
227 amino acids are mainly detected by the fat body, and by neurons and glial cells in the brain.
228 This detection leads to the release of various factors, which allow the coordination of larval
229 systemic growth. Usually to study the role of amino acids in *Drosophila*, a mix of amino
230 acids like yeast or peptone extracts are used as a source of amino acids [35, 50]. Few essential
231 amino acids have been shown to play a key role in development or in food intake [31, 40, 42].

232 Other cells than the specific neurons and glial cell we described upper are also sensitive to
233 amino acids in larvae. For example, the fat body cells are sensitive to these nutrients, which in
234 turn can influence larval growth. In this regard, during mid-third instar Class IV
235 multidendritic nociceptive ppk peripheral neurons react to the loss of environmental arginine
236 via Slif. Then, they activate glutamatergic neurons in the ventral chord, which in turn
237 stimulate Dilp2 release by IPCs [51, 52]. Imaginal discs seem also to be sensitive to the
238 presence of nutrients including amino acids via the activity of the tumor suppressor PTEN
239 (phosphatase and tensin homolog deleted on chromosome 10). This effect of nutrients on
240 imaginal discs can potentially influence their growth and proliferation in specific conditions
241 [34].

242 In *Drosophila* adults, nutrients and amino acids are also crucial since flies need food
243 resources to regulate their life cycle, to survive, to have social interactions and to reproduce.
244 In adults, the essential amino acid threonine promotes sleep via GABA neurons in the brain
245 and three other amino acids (L-Glutamate, L-Alanine and L-Aspartate) are able to directly
246 stimulate DH44+ brain neurons to increase in food consumption via a putative amino acid
247 transporter, CG13248 [53, 54]. In adult females, nutrients play also an important role in
248 germline stem cells development. This requires specific nutrient-responsive signaling
249 pathways which includes insulin/IGF signaling, TOR signaling and GCN2-dependent amino
250 sensing [55]. This indicates that similar molecular pathways can be mobilized all along
251 *Drosophila* life cycle to sense amino acids in different cell types.

252 In mammals, amino acids are detected by the digestive tract to release gut hormones like
253 cholecystinin hormone (CCK) to regulate food intake. Surprisingly, in *Drosophila*, such
254 mechanism of detection of amino acids by the gut to regulate DILPs or food intake has not
255 been discovered yet. However, a recent study highlights the presence of intestinal cells
256 capable to detect amino acids but the role of these cells remains unknown [56]. Further
257 characterizations and analyzes are necessary to understand the exact role of amino acids on
258 larval development and growth.

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514 **Figure Legends:**

515

516 **Fig. 1 Internal amino acid sensing, and regulation of larval metabolism and growth**

517 Amino acids and others nutrients go through the intestine wall into the hemolymph. Once in
518 the hemolymph, these molecules can circulate in the whole body and are detected by the fat
519 body or by the brain. Then, these two organs secrete and exchange many signaling molecules
520 required to regulate metabolism and growth.

521

522 **Fig. 2 Amino acid sensing by the larval fat body**

523 **A/** In low-protein diets, the fat body secretes Eiger cytokine, which is cleaved by the enzyme
524 TACE. Then, Eiger binds to its receptor Grindelwald (Gnrd) located on Insulin Producing
525 Cells (IPCs) to repress expression of *dilp2* and *dilp5* genes through the Jun N-terminal kinase
526 (JNK) activation. In addition, IPC-connecting neurons (ICNs) inhibit IPC neuronal activities
527 and thus inhibit the release of DILPs. In consequence, metabolism and larval growth are
528 reduced.

529 **B/** In high-amino acids diets, TORC1 (Target of Rapamycin) inhibits TACE and induces the
530 release of two growth blocking peptides (GBP1 and GBP2). These peptides repress the
531 inhibitory activity of ICNs allowing the release of DILPs and larval growth. TORC1 also
532 promotes the secretion of Stunted (Sun) cytokine. Sun binding to its receptor Methuselah
533 (Mth) promotes the release of DILPs, which influences metabolism and larval growth.

534 AA: amino acids, DILP: Drosophila Insulin-Like Peptide, EGFR: EGF Receptor, GBP:
535 Growth-Blocking Peptide, Gnrd: Grindelwald, ICNs: IPC-connecting neurons, IPCs: Insulin-
536 Producing Cells, JNK: Jun N-terminal Kinase, Mnd: Minidiscs, Mth: Methuselah, Slif:
537 Slimfast, Sun: Stunted, TACE: TNF α -Converting Enzyme, TORC1: Target Of Rapamycin.

538

539 **Fig. 3 Amino acid sensing by larval dopaminergic neurons**

540 In larva, food intake is under the control of dopaminergic neurons (DA DL1 neurons).

541 **A/** In balanced amino-acid diet, the inhibition of dopamine release by GABA receptors on DA
542 DL1 neurons allows food intake and growth.

543 **B/** In imbalanced amino-acid condition, dopaminergic neurons detect amino acids transported
544 by Slimfast (Slif) transporter. A sensor of amino acids GCN2 represses GABA receptors. The
545 inhibition of GABA receptors promotes the release of dopamine and thus blocks food intake.

546 AA: amino acids, GABAR: GABA Receptor, GCN2: GC nonderepressing 2 kinase, GDH:
547 Glutamate dehydrogenase, LAT-1: Large neutral Amino-acid Transporter, Mnd: Minidiscs,
548 Slif: Slimfast.

549

550 **Fig. 4 Amino acid sensing by larval Insulin Producing Cells**

551 Two LAT-1 transporters (Minidiscs (Mnd) and Juvenile hormone Inducible-21 (JhI-21) are
552 expressed in IPCs. Mnd is located on the endoplasmic reticulum whereas JhI-21 is on the
553 plasma membrane. They allow the transport of branched amino acids (Leucine and
554 Isoleucine) that leads to the release of DILPs. DILPs are involved in metabolism regulation
555 and in growth control.

556 AA: amino acids, ER: Endoplasmic Reticulum, GDH: Glutamate dehydrogenase, LAT-1:
557 Large neutral Amino Acid Transporter, Mnd: Minidiscs.

558

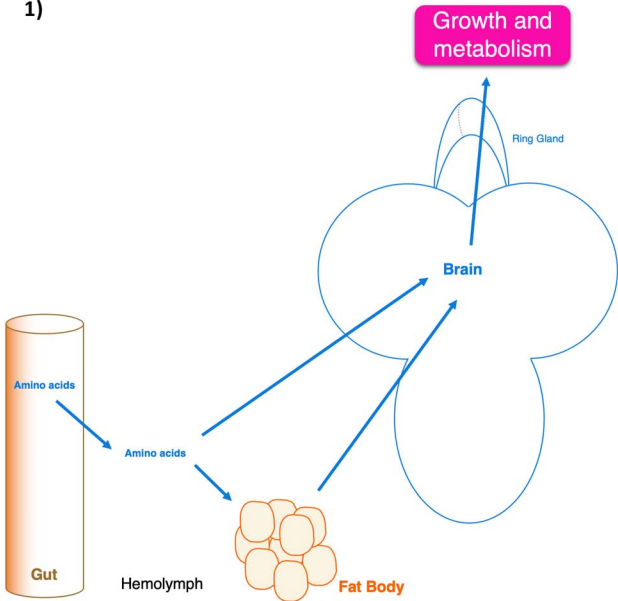
559 **Fig. 5 Amino acid sensing by glial cells**

560 Glial cells detect amino acids to control metabolism and growth.

561 Comparison of low- (5A) and high-protein diet (5B) situations shows that in a high-protein
562 diet, amino acids activate the secretion of an unknown factor by the fat body that induces the
563 release of DILP6 by glial cells. Furthermore, glial cells can directly detect amino acids by
564 TORC1 via the Slif amino-acid transporter and secrete DILP6. DILP6 promotes the
565 neuroblast reactivation and the release of Jelly belly (Jeb) by acetylcholinergic neurons. Jeb
566 promotes the FoxO phosphorylation in IPCs to allow the *dilp5* gene expression and DILPs
567 release. Glial cells express another amino-acid transporter Sobremesa (Sbm). Sobremesa is
568 involved in larval development by regulating PTTH secretion and therefore ecdysone.

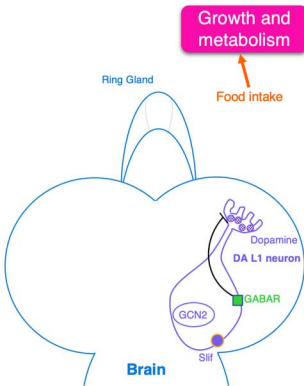
569 AA: amino acids, Ach neuron: Acetylcholinergic neurons, Alk: Anaplastic lymphoma kinase
570 receptor, IPCs: Insulin Producing Cells, InR: Insulin Receptor, Jeb: Jelly belly, PTTH:
571 Prothoracicotropic Hormone, Sbm: Sobremesa, Slif: Slimfast, TORC1: Target of Rapamycin.

1)



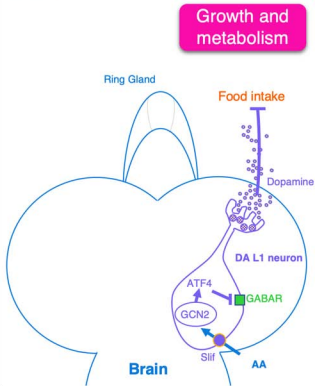
3A)

Balanced amino acid diet



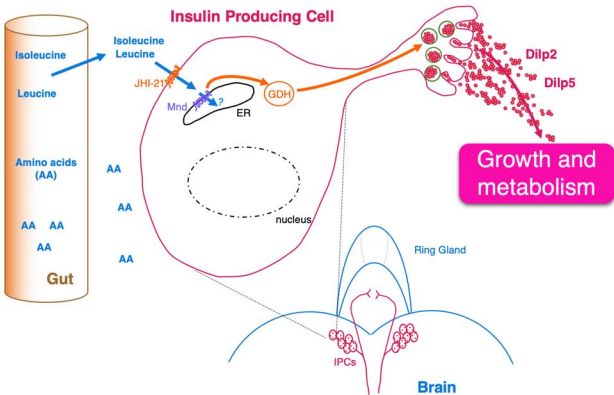
3B)

Imbalanced amino acid diet



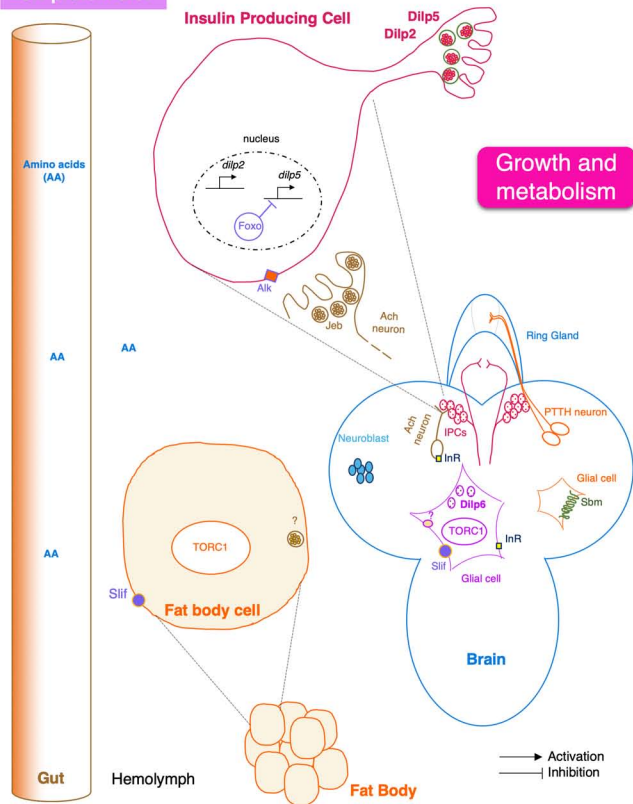
4)

High amino acid diet



5A)

Low protein diet



5B)

High protein diet

