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Nutritional control of insect reproduction

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The amino acid–Target of Rapamycin (AA/TOR) and insulin pathways play a pivotal role in reproduction of female insects, serving as regulatory checkpoints that guarantee the sufficiency of nutrients for developing eggs. Being evolutionary older, the AA/TOR pathway functions as an initial nutritional sensor that not only activates nutritional responses in a tissue-specific manner, but is also involved in the control of insect insulin-like peptides (ILPs) secretion. Insulin and AA/TOR pathways also assert their nutritionally linked influence on reproductive events by contributing to the control of biosynthesis and secretion of juvenile hormone and ecdysone. This review covers the present status of our understanding of the contributions of AA/TOR and insulin pathways in insect reproduction.

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Introduction

Development of chorionated eggs with a large quantity of nutrient reserves represents one of the evolutionary advances of insects that is responsible for their extraordinary success as terrestrial animals. Hence, reproductive events of female insects require a massive input of nutritionally-rich and energy-rich resources. The regulatory checkpoints exemplified by the amino acid–Target of Rapamycin (AA/TOR) and insulin pathways ensure the proper influx of nutrients for developing eggs. These pathways are obligatory for reproduction of all insects. Moreover, the AA/TOR as an evolutionary older pathway serves as a primary nutritional sensor activating secretion of insect insulin-like peptides (ILPs) and also triggering nutritional responses in the tissue-specific manner. The insulin/TOR pathway is involved in controlling biosynthesis of juvenile hormone (JH) and ecdysone (E), which in turn initiate yolk protein production (vitellogenesis) and egg maturation. The relative contribution of above mentioned signaling pathways differs depending on insects with various life strategies. The AA/TOR and insulin pathways play dual roles as mediators of nutritional status, one by directly affecting reproductive tissues, and another by controlling biosynthesis and secretion of juvenile hormone and ecdysteroids (Fig. 1).

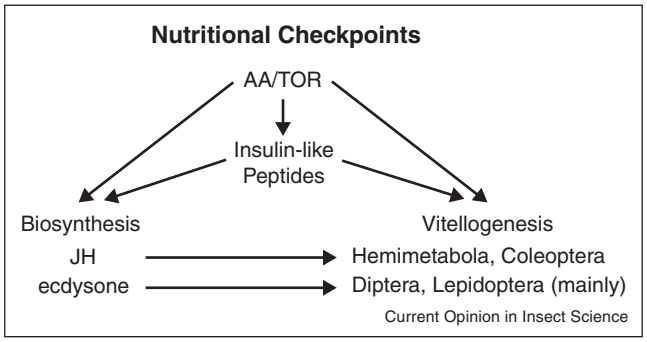
The role of insulin and TOR in nutritional regulation of insect growth and development, particularly that of *Drosophila melanogaster*, has been studied in great detail [1–3]. These studies have laid a foundation for our understanding of the mechanisms underlying the nutritional control. In the last several years, progress has also been made in elucidating the mechanisms of the nutritional control of insect reproduction. These advances have provided important insights into the role of insulin and AA/TOR as nutritional sensors in multiple reproductive events.

The amino acid/TOR pathway as a nutritional sensor

The serine/threonine kinase TOR being the center of nutritional signaling is linked to nutritional sensing through AAs [4–7]. The presence of the AA/TOR pathway in unicellular Eukaryotes, such as yeast, indicates its early evolutionary origin [5–9]. Research suggests that in addition to glucose, AA/TOR controls synthesis and secretion of ILPs [10••,11]. In adult *Drosophila*, ILP secretion is at least in part under the remote control of cytokine unpaired 2 produced by the fat body in response to nutritional signals [11,12•]. Thus, it appears that AAs signaling through TOR represents a first order of nutritional signaling, particularly for insects requiring a protein meal for the initiation of egg production (Fig. 2). TOR signaling is partitioned into two different pathways, TORC1 and TORC2, with only TORC1 being nutritionally sensitive [4–6]. AAs connect to TORC1 (hereafter TOR) through transmembrane AA transporters and the intracellular pathway that includes Ras-related small GTP-binding protein GTPases or Rags [7,8]. Two types of Rags, Rag A/B and Rag C/D are involved in mediating AA signaling [8]. Ras-homolog enriched in brain GTPase (Rheb) is also an integral part of the pathway that activates TOR in response to AAs [7,9]. In conjunction with phospholipase D1 and upon its loading with GTP, Rheb promotes TOR phosphorylation and stimulation [9].

Despite of its importance, the relative contribution of the AA/TOR pathway compared to that of the insulin one in nutritional sensing has not been investigated in detail in insect reproduction. In many insects, intake of proteins serves as a key trigger for the initiation of egg development. It is particularly pronounced in blood-feeding

Fig. 1



Insect nutritional checkpoints. The serine/threonine kinase TOR pathway represents primary nutritional checkpoint via sensing amino acids (AAs). In *Drosophila*, AA/TOR regulates synthesis and secretion of insulin-like peptides (ILPs) and together with ILPs participates on biosynthesis of lipophilic hormones Juvenile hormone (JH) and ecdysone. AA/TOR, ILPs, JH and ecdysone regulate production of yolk proteins and their uptake into the oocytes, marked here as vitellogenesis. Contributions of particular players differ substantially among insect orders. Vitellogenesis is controlled mainly by JH in Hemimetabola and Coleoptera and by ecdysone (20-hydroxyecdysone) in Diptera and Lepidoptera orders.

species. In mosquitoes, in which only females feed on blood, egg development is arrested until a female takes a blood meal. Understanding of this phenomenon came from a realization that the AA signaling via TOR is responsible for de-repression of the egg developmental arrest [13••,14]. AA transporters of the solute carrier 7 family are involved in the AA sensing mechanism, as shown by the resulting decrease in TOR signaling and fertility caused by RNA interference silencing of any family member in female mosquitoes [15•,16,17]. Upon blood intake by *Aedes aegypti* female mosquitoes, the influx of signaling AAs, such as leucine, leads to activation of TOR, phosphorylating the translational activator S6K and the translational repressor 4E-BP [18]. Rheb silencing in *A. aegypti* females downregulates S6K phosphorylation and subsequently *vitellogenin* (*Vg*) gene expression [19•], while 4E-BP phosphorylation inhibits its translational repression function and allows protein synthesis and progression of vitellogenesis [18,19•]. In the red flour beetle *Tribolium castaneum*, RNAi-mediated silencing of most members of the insulin and TOR signaling pathways either decreases expression of *Vg2* or severely affects egg production. However, knockdown of *Rheb* lowers *Vg2* mRNA levels by only 10–30%, suggesting that the insulin rather than the AA branch of the TOR pathway is essential for signaling in this insect [20].

Nutrient-sensitive TOR-mediated activation of S6K leads to translation, resulting in cell growth and differentiation and triggering various aspects of egg development in reproducing female insects. The TOR and S6K are involved in the regulation of the *Vg* gene expression by providing transcriptional and translational machineries required for this central reproductive event [14,15•]. Park *et al.* [21••] have revealed that the TOR signaling pathway regulates the translation of a GATA transcription factor, which is an activator of the *Vg* gene, in a Rapamycin-dependent and AA-dependent manner in *A. aegypti*. Upon blood ingestion by the female mosquito, massive translation of AaGATA occurs in the fat body and AaGATA binds to the *Vg* gene promoter, activating its transcription (Fig. 3).

TOR plays an important role in regulating developmental or starvation-induced autophagy that are important for either programmed cell remodeling or tissue catabolism, respectively [22–24]. TOR interacts with the initiator of autophagy, Autophagy related 1 (ATG1), inhibiting its action in the presence of sufficient nutrients. However, the situation is reversed in the case of starvation, during which ATG1 suppresses TOR and initiates autophagy. Interestingly, at the end of the female *A. aegypti* reproductive cycle fat body undergoes programmed autophagy. TOR represses autophagy during the vitellogenic phase, preventing its premature triggering. Activation of programmed autophagy leading to the remodeling of the fat body is required for a normal switch to the second reproductive cycle (Fig. 4) [25•]. Further studies should demonstrate whether a similar programmed autophagy occurs in other insects with cyclical reproduction.

A recent study has shown a possible crosstalk between wingless (Wnt) and TOR signaling pathways in *A. aegypti* vitellogenesis [26]. RNAi depletion of *Frizzled 2*, the transmembrane Wnt receptor that is predominantly expressed in the mosquito fat body after a blood meal, causes a significant reduction in S6K phosphorylation and subsequently in *Vg* expression [27].

Insulin-like peptides (ILPs) as nutritional sensors

TOR is linked to nutritional sensing through not only AAs but also the insulin pathway, which is conserved in Protostome and Deutorostome animals [27,28]. Insects possess multiple insulin-like peptides (ILPs), the number of which varies among different species [3,27–33]. Although, some ILPs are functionally analogous to vertebrate insulin, the complete repertoire of their actions remains to be elucidated, particularly during reproduction. *Drosophila* ILPs regulate germline stem cell division, and germline cyst development rate and progression through vitellogenesis [34••]. In the mosquito *A. aegypti*, ILP3 is involved in stimulation of egg production following the intake of vertebrate blood [35•,36]. Only one, in dipteran and lepidopteran, or two, in hymenopteran and hemipteran, tyrosine kinase transmembrane insulin receptors (InR) exist in insects [3,27,37]. The negative effect of *InR* RNAi-mediated silencing on reproductive events has been observed in several insects, suggesting