HHS Public Access

Author manuscript

Curr Opin Insect Sci. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Curr Opin Insect Sci. 2015 October 1; 11: 31–38. doi:10.1016/j.cois.2015.08.003.

Nutritional Control of Insect Reproduction

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Abstract

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.

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-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 (Figure 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, 2, 3]. These

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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 (Figure 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 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- and AA-dependent manner in *A. aegypti*. Upon blood ingestion by the female mosquito, massive translation of Aa-GATA occurs in the fat body and AaGATA binds to the *Vg* gene promoter, activating its transcription (Figure 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, 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 (Figure 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]. 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 involvement of the insulin pathway in the control of reproduction [20, 35*, 36, 38].

The insulin signaling that is conserved in the animal kingdom converges onto the main effector Akt/protein kinase B, which in turn suppresses the negative regulators of TOR, Tuberous Sclerosis Complex 1 and 2 (TSC1/2), thereby activating the TOR via its

phosphorylation (Figure 2) [4, 5, 39]. Insulin/TOR regulates cell growth, protein synthesis and metabolism. In ovaries of *D. melanogaster*, insulin and TOR control the development of niche-stem units in a tissue-specific manner [40*, 41**, 42–43]. This ovary-specific action occurs via coupling of the insulin signaling with TOR by phosphorylating the 40-kDa proline-rich Akt substrate TOR inhibitor (PRAS40) [40*]. Moreover, this action of insulin signaling in *Drosophila* is required for ovarian production of yolk proteins, and the ovarian vitellogenesis is autonomous for the ovary independently from juvenile hormone and ecdysteroids [43].

The role of insulin and TOR in control of JH and ecdysone biosynthesis

Insulin/TOR pathway also asserts its nutritionally linked influence on reproductive events by contributing to the control of biosynthesis and secretion of JH and ecdysone.

In the German cockroach *Blatella germanica*, in which JH plays a role of major gonadotrophic hormone, TOR connects the nutritional status with JH biosynthesis and as a consequence vitellogenesis. TOR RNAi knockdown resulted in a severe inhibition of JH synthesis in adult female corpora allata (CA) mimicking starvation conditions. Under both TOR silencing and starvation, there was a significant reduction in mRNA levels of JH biosynthetic enzymes, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase-1, HMG-CoA synthase-2, and HMG-CoA reductase [44*]. Moreover, a similar negative effect on JH biosynthesis and adult vitellogenesis has been obtained by means of InR RNAi in the penultimate and last instar nymphs of the same insect [38]. In female mosquitoes, JH controls posteclosion maturation and leads to reproductive competency and ability to feed on blood. The insulin/TOR pathway plays a role in the transduction of the nutritional information that controls JH synthesis in mosquitoes [45]. This pathway (partially) mediates transcription of the genes encoding JH biosynthetic enzymes regulating JH production [46*]. Incubation of Corpus Cardiacum-CA complexes from *A. aegypti* females in the presence of bovine insulin increases JH synthesis by 2- to 3-fold, but this is blocked by incubating with LY294002 or with rapamycin, insulin and TOR signaling inhibitors, respectively [46*]. In adult *Drosophila*, mutations in InR cause a decrease of JH titer [47].

The role of brain factors (neuropeptides) in activation of ecdysone production by ovaries in dipterans, in which ecdysteroids are the primary effectors of vitellogenesis and egg maturation, has been well established [48]. More recently, ILPs have been implicated in regulation of ovarian ecdysteroidogenesis [28, 35*, 49]. Female *D. melanogaster* mutants for InR exhibit reduced ecdysteroid synthesis [49]. Wen *et al.* [50**] have shown that two mosquito ILPs, ILP3 and ILP4, exhibit gonadotropic activity in blood-fed females, including the stimulation of ovaries to produce ecdysteroids and the uptake of yolk by primary oocytes. However, ILP3 and ILP4 do not cross compete in binding assays; ILP3 interacts with InR and ILP4 with an uncharacterized 54-kDa membrane protein. In addition, in female *A. aegypti* mosquitoes blood feeding triggers the release of ILPs and ovary ecdysteroidogenic hormone (OEH) from the brain. OEH binds to the recently identified putative receptor tyrosine kinase (RTK) orphan receptor AAEL001915 in *A. aegypti* ovarian follicle cells [51] and activates the insulin pathway protein kinase B/Akt in an InR-

independent manner [52]. Moreover, AAs with either OEH or ILP3 stimulate synthesis and release of ecdysteroids from *A. aegypti* ovaries [52].

Conclusions and future directions

Recent progress has revealed critical roles of AA/TOR and insulin in the control of female insect reproduction. These pathways serve as sensors of the nutritional status of a reproducing insect. Research has shown that these pathways act at multiple levels affecting reproductive organs directly and indirectly through the control of gonadotrophic hormones, JH and ecdysone. However, more studies are required in non-Drosophilid and non-dipteran insects to evaluate cell- and tissue-autonomous and systemic functions of these pathways.

AAs act through TOR independently from insulin, connecting AA signaling to reproduction as well as triggering release of ILPs. Relative contributions of insulin and AA/TOR as nutritional sensors in different insects are unclear but most likely are tightly bound to various insect life strategies. It is conceivable that AA/TOR plays a more significant role in insects, in which protein intake is required for the initiation of egg production. More studies are required to clarify this question.

Our understanding of the nutritional regulation of insect reproduction is still incomplete. Despite the exceptional value of *D. melanogaster* as a model insect, it cannot serve as a universal research system because reproductive events in insects vary dramatically. There is an urgent need for the development of genetic tools and their applications for investigating nutritional regulation of reproduction in insects other than *Drosophila*. Limited genetic tools have been established for *A aegypti*, *Tcastaneum*, *Bombyx mori* and *Apis mellifera* [53–56]. However, thus far genetic tools have found only narrow use in the reproductive biology of non-Drosophilid insects [57–60]. Most investigations of these insects discussed above have been performed using systemic RNA interference for gene silencing. Although such studies have been valuable for initial understanding of insect reproductive biology, deeper insights are required utilizing novel genetic tools. Recent advancements in genetic engineering such as CRISPR/Cas9 [61–63] provide a new prospective in research of insect reproduction.

Acknowledgments

This work was supported by NIH/NIAID Award 5R01 AI036959 to ASR. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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* of special interest

** of outstanding interest

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Highlights

- Amino acids/Target of Rapamycin pathway serves as a nutritional sensor that is essential for reproduction in female insects requiring protein-rich diet
- Amino acids/Target of Rapamycin pathway regulates synthesis and secretion of ILPs as shown for *Drosophila*
- A second nutritional checkpoint is represented by ILPs that are involved in regulation of vitellogenesis and metabolism of reproducing female insects
- ILPs control the development of ovarian niche stem units in an autonomous manner, independent of JH and ecdysone as demonstrated in *Drosophila*
- Insulin and TOR signaling pathways regulate JH and ecdysone biosynthesis providing a link to the nutritional status of a reproducing insect

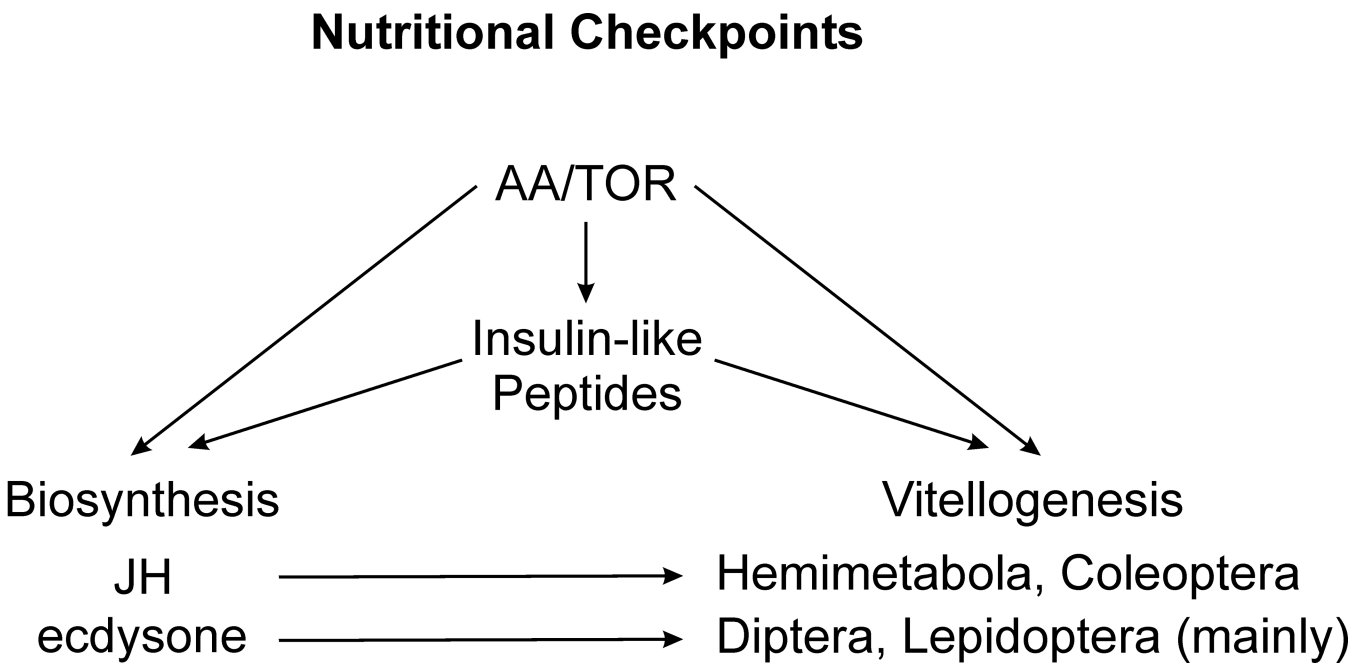

Figure 1.

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.

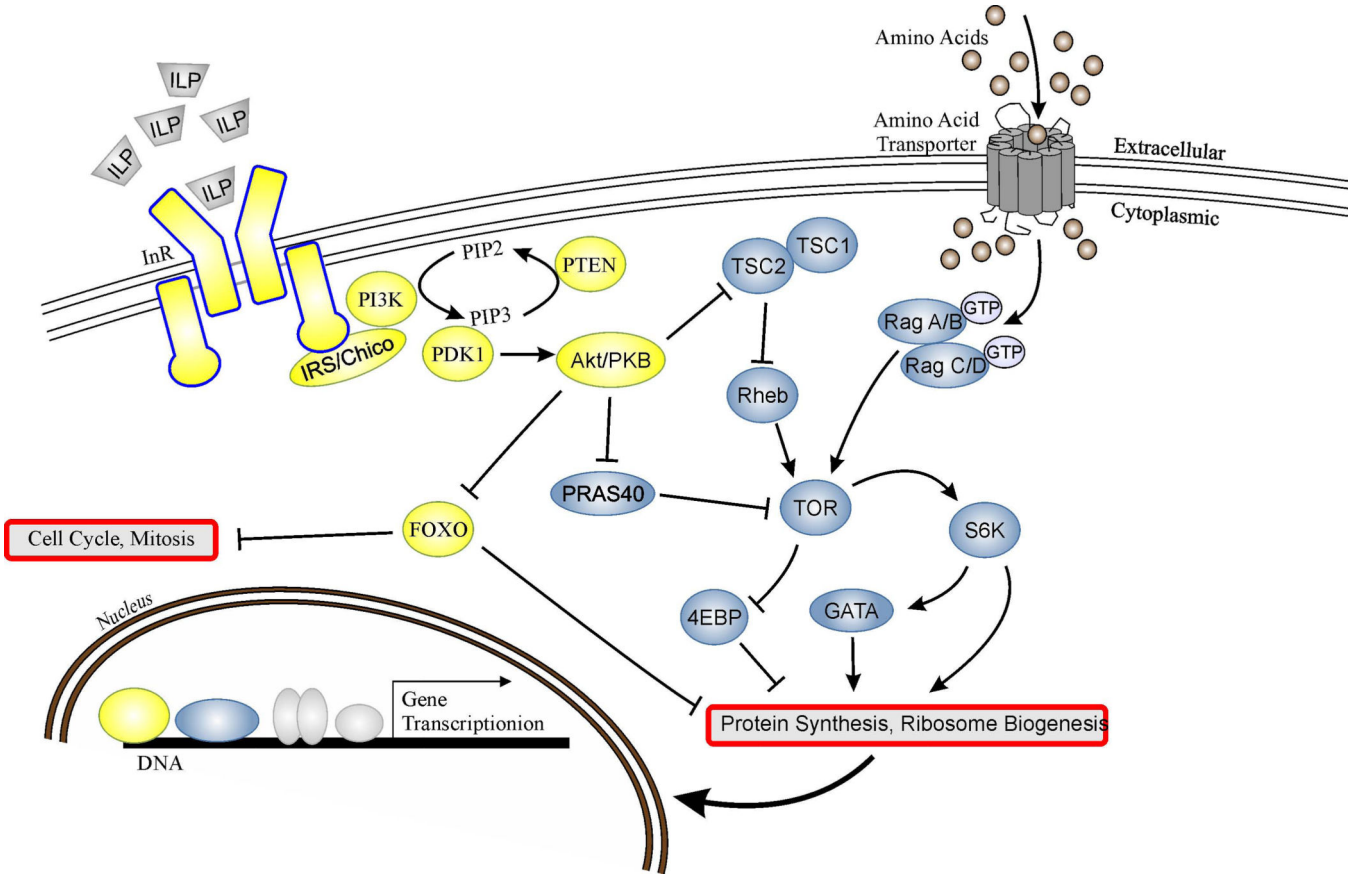

Figure 2.

Simplified outline of Insulin and AA/TOR signaling pathways. ILPs bind membrane Insulin receptor and activate Insulin pathway by series of protein phosphorylations to activate Akt/PKB. Akt phosphorylates FOXO, sequestrates it from the nucleus and allows cell cycle progression. Akt also activates TOR signaling pathway by inhibition of its repressors TSC 1/2 and PRAS40. Nutritional input in a form of AAs regulates TOR pathway through small Rag GTPases. Activated TOR kinase phosphorylates effector proteins such as 4EBP and S6K and thus stimulates protein synthesis, ribosome biogenesis and cell growth. Insulin factors are shown in yellow and TOR-pathway components in blue.

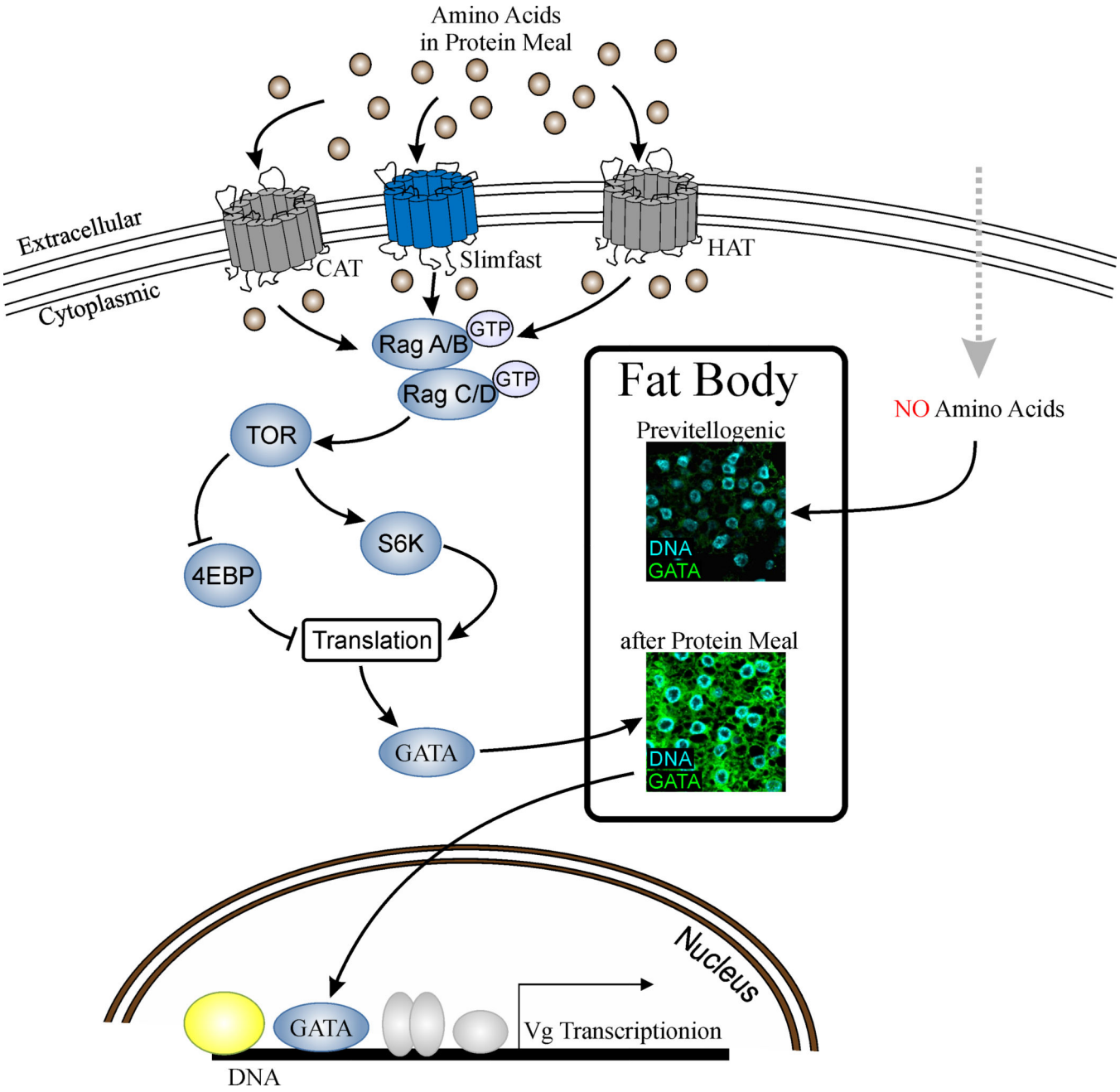

Figure 3.

Nutrient-sensitive activation of translation is a critical step for female reproduction. Amino acids released upon digestion of a protein-rich meal exert TOR-mediated activation of S6K [14, 15*] and lead to a massive translation of GATA transcription factor in the female FB [21**]. GATA protein binds to the *Vg* promoter and activates its transcription. The absence of signaling AAs in previtellogenic females or upon protein-poor meal is not sufficient to trigger GATA translation and consequentially *Vg* gene transcription. CAT = cationic amino acid transporter, HAT = heteromeric amino acid transporter. Inserts: the mosquito fat body

before (upper panel) and after a blood meal (low panel). Immunocytochemistry with anti-GATA antibody and Dapi staining.

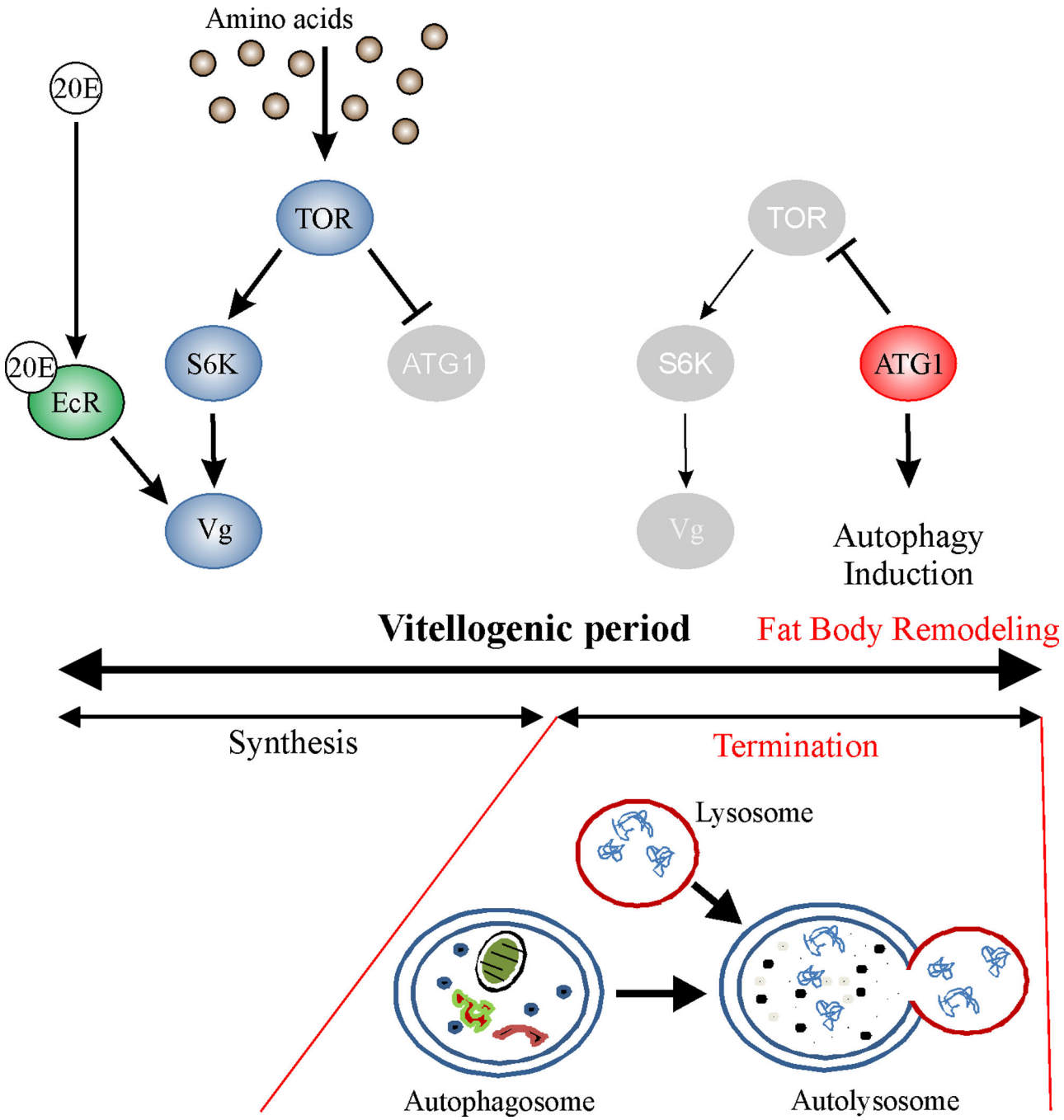

Figure 4.

The role of programmed autophagy in vitellogenesis. Vg synthesis is stimulated by AA/TOR and ecdysone pathways in the female mosquito *A. aegypti* fat body (FB). When active, TOR blocks FB autophagy by repressing the initiator of autophagy, Autophagy related 1 (ATG1) [25*]. Reduced TOR signaling is no more inhibiting ATG1 during the terminal phase of the vitellogenesis; instead, ATG1 is suppressing TOR and initiates autophagy. Fat body remodeling during terminal phase of vitellogenesis is a necessary step for the second gonadotropic cycle to occur. During the fat body remodeling, FB cell cytoplasm is being

enclosed into autophagosomes, which fuse with lysosomes to become autolysosomes that digest their content into basic nutrients. Thus, autophagy allows lowering energetic costs until the next gonadotrophic cycle. 20E = 20-Hydroxyecdysone