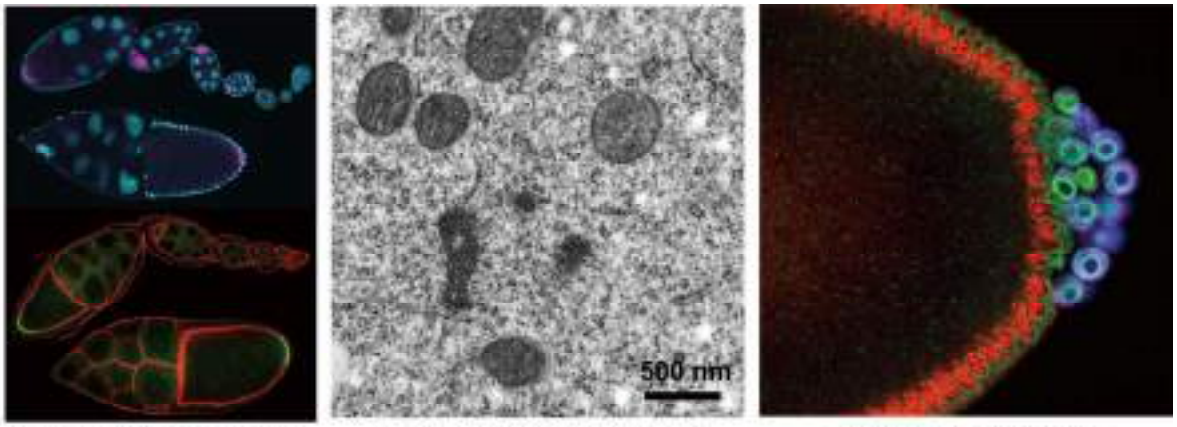
生殖発生分野
Department of Germline Development

私たちの体を構成する細胞は、体細胞と生殖細胞の２種類に大別される。体細胞は、個体の体をつくり生存に必須であるが、個体の寿命とともにその役割を終えて死滅する運命にある。一方、生殖細胞は、個体の生存には必要ないが、親から子へと遺伝情報を伝達し、次世代の生命を生み出し得る唯一の細胞系列である。また、体を構成する全ての細胞は、卵と精子の受精により生じるたった１つの受精卵から作りだされる。そのため、生殖細胞は究極の幹細胞ともいえる。

多くの動物では、卵の一部に生殖質とよばれる特殊な領域が存在しており、この生殖質を取り込んだ細胞が生殖細胞として決定される。生殖質には特定の RNA やタンパク質が局在化している。しかし、生殖質がどのようにして形成・維持されているのか、さらには、生殖質がどのように生殖細胞の決定を制御しているのかはまだよく分かっていない。私たちの研究室では、ショウジョウバエをモデル系として、生殖細胞研究を進めている。

ショウジョウバエは進化上、ヒトと遠い関係にある生物である。一方、ゲノムや遺伝子のレベルから見ると，ハエとヒトとはそれほど大きな違いがないことが分かっている。したがって、実験生物として優れたショウジョウバエを用いて得られた知識は、人間の健康科学・医科学研究にも貢献できると考えている。

Germ cell is the only cell type that transmits genetic information to the next generation. Germ cells are therefore crucial for species preservation as well as evolution. In many animal species, germ cells are formed by the inheritance of a set of specialized germ cell determinants localized in the specific cytoplasmic region within the egg, called the germ plasm. The germ plasm consists of a series of maternal RNAs and proteins, forming large ribonucleoprotein complex, called germ granules. We are seeking to understand the mechanism by which (1) the germ plasm is assembled during oogenesis, and (2) how germ plasm factors induce the germ cell fate. Although fly and human are divergent in an evolutionarily viewpoint, similar cellular mechanisms are known to operate during development and organisms' homeostatic maintenance. Therefore, we believe that knowledge obtained from fly research will provide important information that should deepen our understanding on human physiology and medicine.



生殖質のアッセンブリーにおける
mRNA 局在（上段）と翻訳制御（下段）

生殖顆粒（mRNP 複合体）の動態制御

生殖細胞（青）の特質の理解

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研究概略　Projects

生殖細胞は遺伝情報を次世代へ受け渡す唯一の細胞種であり、生物種の維持・進化に極めて重要な役割を担っている。多くの動物において、生殖細胞は胚発生の早い段階で確立され、体細胞とは異なった発生プログラムに従って分化・維持される。私たちの研究室では、このような特徴を持つ生殖細胞がどのようにして確立するのか、また、生殖細胞の特質とは何かに興味を持って研究を進めている。

私たちが研究材料としているショウジョウバエを初めとして、線虫、カエル、ゼブラフィッシュなどモデル生物として扱われている動物の多くでは、生殖細胞の形成・分化に必要な因子は卵形成の過程で合成される母性因子として、卵内の特定の領域に集積していることがわかっており、この特殊な細胞質領域は生殖質（germ plasm）と呼ばれる。すなわち、胚発生過程において、生殖質を取り込んだ細胞が生殖細胞へと分化する（図１）。生殖質は卵に偏在して形成されることから、生殖質の研究を通して、細胞の極性、細胞内の物質輸送、そして、分化決定因子の機能といった多様な研究に展開することが可能である。また、ショウジョウバエ生殖細胞は、幹細胞システムにより維持され多数の配偶子産生を可能としていることから、生殖細胞研究を通して。幹細胞システムの分子基盤についても重要な知見を提供すると期待される。

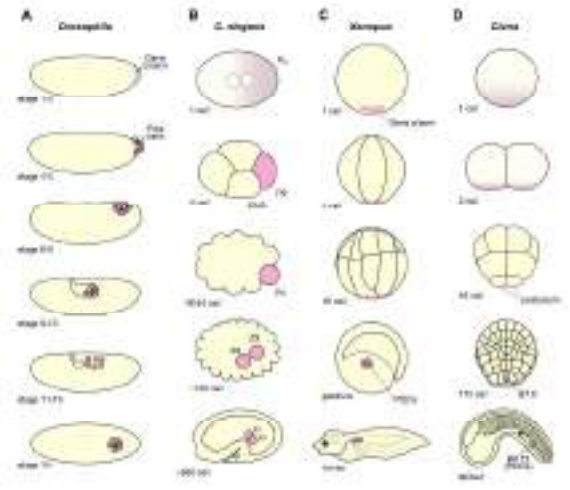

図１：様々な動物胚における生殖細胞形成過程。
生殖質、及び生殖細胞をマゼンタで示す。(A) ショウジョウバエ，(B) 線虫，(C) ツメガエル，(D) カタユウレイボヤ

1.　生殖細胞形成における転写抑制とその意義.

生殖質の持つ機能の１つとして、生殖細胞形成時における転写抑制が知られている。このような生殖細胞形成過程における一過的な転写抑制は、ショウジョウバエばかりでなく、線虫、ツメガエル、ホヤなどでも観察され、進化的に保存されたメカニズムである。これは、体細胞分化のプログラムを抑制することにより、生殖質因子で制御される生殖細胞形成を保証するために必要なメカニズムであると考えられている。興味深いことに、私たちも含めた国内外の研究から、この転写抑制に関わる生殖質因子が動物種によって多様化していることが明らかとなった (Nakamura Curr. Opin Cell Biol. 2010; Shirae-Kurabayashi et al. Development 2011)。このような多様性は、生殖質の獲得が動物の進化の過程で系統ごとに独立して生じたとする説を支持するものであり、収斂進化の好例である。さらに、生殖質を持たず、細胞間シグナルによって生殖細胞形成が制御されるマウスにおいても、生殖細胞分化の過程で体細胞を規定する遺伝子の発現が積極的に抑制されることがわかっている。すなわち、生殖細胞形成過程における、体細胞への分化経路の遮断が、きわめて重要であることを示している (Nakamura and Seydoux Development 2008; Nakamura et al., Curr. Opin Cell Biol. 2010)。

ショウジョウバエにおける転写抑制では、*polar granule component* (*pgc*) が機能する。母性 *pgc* mRNA は生殖質因子であり (Nakamura et al. Science 1996）、生殖細胞形成直後にタンパク質へと翻訳される。興味深いことに、Pgc はわずか 71 アミノ酸残基からなる小タンパク質であり、RNA ポリメラーゼ II 依存的転写の基本因子の１つである P-TEFb (positive transcription elongation factor b) と結合して、その転写活性化領域へのリクルートメントを阻害する。このようなメカニズムにより、形成直後の生殖細胞では、mRNA 転写が一過的、かつゲノムワイドに抑制される

(Hanyu-Nakamura et al. Nature 2008)。一方、*pgc* を欠く生殖細胞や P-TEFb を強制発現させて転写抑制を解除した生殖細胞は、胚発生中期以降に消失する。私たちは、これら転写抑制が破綻した生殖細胞がアポトーシスによって失われていることを見出している。現在、転写抑制が破綻した生殖細胞が細胞死を迎える過程における分子イベントを明らかにしつつある（投稿中）。

2. 生殖質アッセンブリーにおける mRNA 局在と翻訳制御.

ショウジョウバエ生殖質には、生殖細胞形成因子をコードする各種 RNA とタンパク質とが集積した RNP 顆粒（生殖顆粒）が存在し、RNP 複合体の機能と動態を解析する上での良いモデル系である。生殖質は、生殖質形成因子（Oskar）をコードする mRNA の輸送と翻訳、Oskar に依存した生殖質下流因子のリクルートと F アクチン再編のプロセスを経て、卵母細胞後極に形成され安定に繋留される (Kato and Nakamura Develop. Growth Differ. 2012)。私たちは、ショウジョウバエの生殖質形成に関わる mRNA の局在と翻訳とを連携制御する RNP 顆粒の実体を世界に先駆けて報告すると共に (Nakamura et al. Development 2001, Boag et al. Development 2005; Barbee et al. Neuron 2006; Pradhan et al. J. Cell Sci. 2012; Kato and Nakamura Develop. Growth Differ. 2012)、生殖質形成因子である *oskar* の翻訳抑制機構の一端を明らかにした (Nakamura et al. Dev. Cell 2004; Kim et al. PLOS Genet. 2015)。興味深いことに、細胞質 RNP 顆粒は、生殖細胞ばかりでなく、神経細胞樹状突起や体細胞にも存在し、

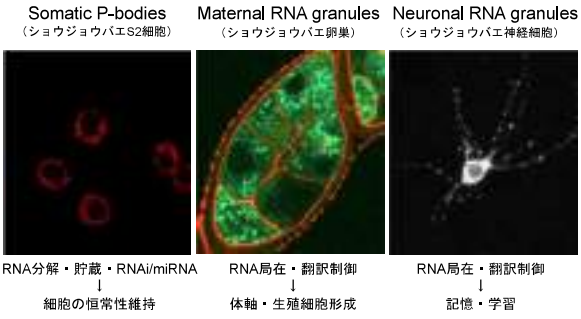

図２：細胞質 RNP 顆粒の共通性
細胞質 RNP 顆粒は、体細胞、生殖細胞、神経性某樹状突起など様々な細胞種、領域で観察される。興味深いことに、その構成タンパク質もまた、共通していることがわかってきた。

その構成タンパク質や生理機能が高度に保存されていることがわかってきた（図２）。

私たちは、RNP 顆粒の新規因子を複数同定しており、その機能解析を進めることにより母性 RNA の輸送局在と翻訳を時空間的に制御するメカニズムや、細胞 RNP 顆粒による転写制御の普遍的や機能の解明に繋がると期待している。

3. 生殖細胞形成・分化に関わる新規母性因子の探索と機能解析.

私たちは、ショウジョウバエ胚発生における生殖細胞の形成・生殖巣への移動過程に関わる新規因子を同定することを目的とした遺伝学的スクリーン、ならびに分子スクリーンを行った。現在までに、生殖細胞の形成に異常を示す新規突然変異 (*tiny pole plasm*; *tpp*) を始めとして、ノックダウンあるいはノックアウトにより生殖細胞の振る舞いが異常となる複数の因子を同定し、その機能解析を進めている（図３）。興味深いことに、*tpp* はわずか 93 アミノ酸残基の小タンパク質をコードし、そのホモログは哺乳類にも保存されている。現在、ショウジョウバエにおける *tpp* の分子機能解析を精力的に進めているところであるが、進捗によっては哺乳類における分子機能解析にも着手したいと考えている。

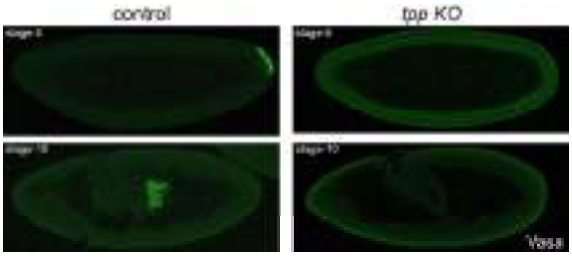

図３：母性 *tpp* 欠失胚の表現型
新たに同定した遺伝子 *tiny pole plasm* (*tpp*) 突然変異体のメス由来の胚では、抗 Vasa 抗体で可視化される生殖細胞（緑色）の形成が不全となる。一方、体細胞のボディプランは正常に形成される。

4. エンドサイトーシスによる極性形成と生殖質形成の制御機構.

私たちは以前に、生殖質形成機構に関する新たな知見を得る目的とした遺伝学的スクリーニングを行った。その結果、エンドサイトーシスや小胞輸送に関与する因子をコードする遺伝子の変異体が複数得られた。このことに端を発して、生殖質形成におけるエンドサイトーシスの

役割と制御機構に注目した研究を行っている。これまでに、卵母細胞でのエンドサイトーシスが微小管の配向性とアクチンの再構築の制御を介して、極性形成と生殖質形成に必須の役割を果たすことを明らかとし、エンドサイトーシスによる生殖質の形成制御機構の存在を世界に先駆けて報告した (Tanaka and Nakamura Development 2008; Tanaka et al Development 2011; Reviewed in Tanaka and Nakamura BioArchitecture 2011)。さらに、卵黄タンパク受容体のエンドサイトーシスが細胞骨格制御を介した極性形成・生殖質形成に重要であることを見い出している（投稿準備中）。これは、卵黄タンパクの取り込みには、「胚発生に必要な栄養素の蓄積」という従来から知られている役割に加えて、「細胞極性と生殖質形成の制御」という新たな役割を持つことを初めて示すものである。このような知見は卵黄タンパクを利用する幅広い動物種に適応できると予想している。

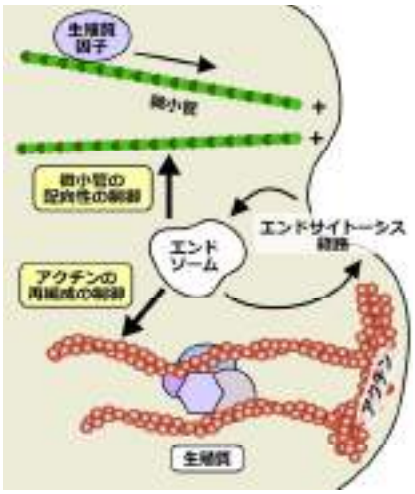

図４：エンドサイトーシスによる生殖質の形成制御
エンドサイトーシスは微小管の配向性の制御を介して生殖質因子の輸送に必要であるとともに、アクチンの再編成の制御を介して生殖質因子の係留にも必要である。

5. エンドサイトーシスを介した RNAi 誘導の制御機構.

二本鎖 RNA により遺伝子の発現が抑制される現象（RNA 干渉; RNAi）は、発生・分化の制御やウイルス感染防御など多彩な生命現象を支えているだけでなく、がんなどの難治性疾患の新しい治療法（RNAi 医薬）としての応用が期待されている。dsRNA のエンドサイトーシスを介した RNAi 誘導経路において、エンドサイトーシスされた dsRNA は小胞輸送と小胞膜の通過を経て、RNAi 誘導の場である細胞質に到達する。RNAi 誘導における細胞質での過程については多くの知見が蓄積しつつあるが、dsRNA がエンドサイトーシスされてから細胞質に至るまでの過程の制御機構はほとんど明らかになっていない。私たちは、リソソームに局在するイオン交換輸送複合体が dsRNA のエンドサイトーシスを介した RNAi 誘導に必要であることを見い出しており、その役割の詳細について解析を進めている。本研究では、このイオン交換輸送複合体の解析を足がかりとして、エンドサイトーシスを介した RNAi 誘導の制御機構の解明を目指す。

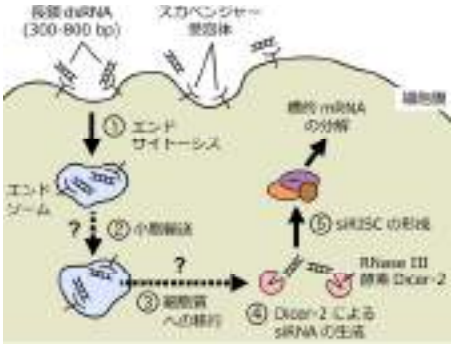

図 5：長鎖 dsRNA を介した RNAi の誘導経路
エンドサイトーシスを介した RNAi 誘導経路において、エンドサイトーシスされた dsRNA は小胞輸送と小胞膜の通過を経て、細胞質に到達する（経路①～③）。細胞質では、dsRNA は siRNA 生成と RISC 形成を経て、標的 RNA を分解する（経路④、⑤）。

In many animal species, germ cell formation and differentiation are controlled by maternally inherited RNAs and proteins. These maternal factors often localize in a particular region within the egg to assemble a specialized cytoplasm, called the germ plasm. Thus, the germ plasm represents a fascinating and experimentally tractable example of a localized determinant and cell polarity. To gain better insights into the mechanisms by which the germ plasm is assembled and controls germline development, our lab uses *Drosophila* as a model system.

1. Roles of germ plasm components in germ cell development.

One of the key activities in the germ plasm is to

repress mRNA transcription during germ cell formation. This is thought to be a mechanism that ensures germ cell fate by preventing induction of the somatic transcriptional program In many animal embryos including *Drosophila*, *C. elegans* and *Xenopus*, the repression of mRNA transcription during germ cell specification correlates with an absence of phosphorylation of Ser 2 residues in the carboxyl-terminal domain (CTD) of RNA polymerase II (RNAPII), a critical modification for transcriptional elongation. We found that *polar granule component* (*pgc*) RNA, a germ plasm component we identified, encodes a small 71-amino acid protein that interferes with P-TEFb (positive transcription elongation factor-b), the RNAPII CTD Ser 2 kinase complex (Hanyu-Nakamura et al., Nature 2008). We conducted genetic and biochemical analyses to elucidate the mechanisms of *pgc*-mediated transcriptional repression, and its impact on germ cell development. Germ cells lacking *pgc* fail to maintain germ plasm mRNAs, including an evolutionarily conserved germ cell survival factor, *nanos*, and eventually die through apoptosis. We have revealed the mechanism by which transcriptional derepression in *pgc*$^{-}$ germ cells causes destabilization of germ plasm RNAs (MS submitted for publication).

2. Spatio-temporal regulation of RNA localization and translation during germ plasm assembly.

In *Drosophila*, the germ plasm is assembled during oogenesis, and is located at the posterior pole of the egg. The assembly of the germ plasm is directed by the localization and translation of *oskar* (*osk*) RNA at the posterior pole of the oocycte. Translation of *osk* RNA is tightly coupled with RNA localization, but the underlying mechanisms have not yet been elucidated. Furthermore, the mechanisms by which Osk assembles and anchors the germ plasm at the posterior cortex remain unknown.

RNA localization and translation are regulated by the interplay between the target mRNA and *trans*-acting proteins that form a ribonucleoprotein (RNP) complex. The DEAD-box RNA-binding protein, Me31B, has been identified as a component of *osk* mRNA-containing maternal RNP granules and is involved in the repression of *osk* translation during early oogenesis. Consistent with the roles of maternal RNP granules in *osk* regulation, the granules also enrich in the eIF4E-binding translational repressor, Cup, and the *osk* 3′ UTR-binding protein, Bruno (Nakamura et al., Dev. Cell 2004). Intriguingly, many proteins in the maternal RNP granules, including Me31B, are known to accumulate in processing (P) bodies in somatic cells and in neuronal RNA granules in dendrites, suggesting a conserved architecture and function among cytoplasmic RNPs (Nakamura et al. Development 2001, Boag et al. Development 2005; Barbee et al. Neuron 2006; Pradhan et al. J. Cell Sci. 2012; Kato and Nakamura Develop. Growth Differ. 2012).

We have identified additional proteins in Me31B-containing RNP granules. Elucidating their molecular functions will deepen our understanding the mechanisms by which RNA localization is coupled with translational control.

3. New maternal factors involved in germ cell formation and migration.

To identify additional maternal factors that are involved in germ cell formation as well as germ cell migration to the gonads in embryos, we conducted genetic screen by inducing RNAi-mediated gene knockdown during oogenesis. We also conducted CRISPR-Cas9 mediated knockout screening for candidate genes that might act in this process. We chose uncharacterized genes whose transcripts are reported to enrich in the germ plasm, and genes whose transcriptome profiles are similar to those of known germ plasm components. From these approaches, we have identified several candidate genes, of which absence causes defects in germ cell formation and migration. We are currently characterizing their functions in detail. We particularly focus on a new gene, named *tiny pole plasm* (*tpp*), which encodes a small 93-amino acid protein. As *tpp* homologs exist in mammals, we expect that its function in germ cell development may be evolutionary conserved.

4. Roles of endocytic regulation in the oocyte polarization and germ plasm assembly.

We performed a genetic screen to isolate mutants defective in pole plasm assembly. The screen recovered several genes that encode factors involved in the endocytosis or vesicle trafficking. We have focused on these factors and found that the endocytic regulation in oocyte is required for the polarization of microtubule arrays and F-actin remodeling in the germ plasm assembly (Tanaka and Nakamura Development 2008; Tanaka et al Development 2011; Reviewed in Tanaka and Nakamura BioArchitecture 2011). We further found that the endocytosis of yolk protein receptor is crucial for the cytoskeletal regulation to polarize the oocyte and assemble the germ plasm. Our data provide a paradigm by which yolk uptake is not merely a process of nutrient deposition for the embryonic development, but also plays instructive roles in the oocyte polarization and

the germ plasm assembly. We expect to adapt our *Drosophila* model to other species with the egg yolk.

5. The mechanism of endocytosis-mediated RNAi. Double-stranded RNA (dsRNA)-mediated gene silencing, termed RNA interference (RNAi), is a fundamental mechanism of gene regulation in most eukaryotes. This mechanism has great potential for use in targeted cancer therapy or antiviral therapy. In the RNAi induction mediated by the endocytosis of dsRNA, endocytosed dsRNA is trafficked via vesicle-mediated pathways, and ultimately released into the cytoplasm where dsRNA leads to degradation of targeted RNA. Although the mechanisms by which RNAi is induced in the cytoplasm have been well studied, less is known about steps of dsRNA processing within vesicles, such as dsRNA uptake, vesicular trafficking of dsRNA, and dsRNA release from vesicles into the cytoplasm. We found that a lysosomal ion-exchanger complex is required for the endocytosis-mediated RNAi induction but its functional roles remain to be determined. Our goal in this project is to understand the molecular mechanisms underlying the endocytosis-mediated RNAi induction.

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RESEARCH ARTICLE Open Access

The evolutionally-conserved function of group B1 Sox family members confers the unique role of Sox2 in mouse ES cells

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Abstract

Background: In mouse ES cells, the function of Sox2 is essential for the maintenance of pluripotency. Since the Sox-family of transcription factors are well conserved in the animal kingdom, addressing the evolutionary origin of *Sox2* function in pluripotent stem cells is intriguing from the perspective of understanding the origin of pluripotency.

Results: Here we approach this question using a functional complementation assay in inducible *Sox2*-null ES cells. Assaying mouse Sox proteins from different Groups, we found that only Group B1 and Group G proteins were able to support pluripotency. Interestingly, invertebrate homologs of mammalian Group B1 Sox proteins were able to replace the pluripotency-associated function of mouse *Sox2*. Moreover, the mouse ES cells rescued by the *Drosophila SoxNeuro* protein are able to contribute to chimeric embryos.

Conclusions: These data indicate that the function of mouse *Sox2* supporting pluripotency is based on an evolutionally conserved activity of the Group B1 Sox family. Since pluripotent stem cell population in developmental process could be regarded as the evolutional novelty in vertebrates, it could be regarded as a co-optional use of their evolutionally conserved function.

Keywords: Pluripotent stem cells, Sox2, Evolution, Co-option

Background

Pluripotency is a unique feature of the cells found in early vertebrate embryos. Pluripotent stem cells give rise to all cell types of the organism, including germ cells, but, unlike zygotes, they do not have the ability to give rise organisms autonomously [1]. The pluripotent phenotype is primarily determined by the expression of a set of pluripotency-associated transcription factors, as demonstrated by the induction of pluripotency in somatic cells transfected with four transcription factors to give rise to induced pluripotent stem (iPS) cells [2]. Of these four transcription factors, Oct3/4 (encoded by *Pou5f1*) and Sox2 are known to be essential for maintaining pluripotency in mouse embryonic stem (ES) cells [3, 4]. In contrast, the functions of Klf4 and Myc are dispensable for pluripotency, but primarily support self-renewal in the absence of the cytokine leukemia inhibitory factor (LIF) [5–8].

Pluripotent stem cell populations have been definitively identified in mammalian embryos, but their presence in other vertebrate embryos remains unclear, with no pluripotent stem cell lines yet isolated from other taxa. Frog animal cap cells behave similarly to pluripotent cells, but have never been shown to yield stem cells capable of propagating in vitro [9]. The absence of pluripotent stem cells is evident in ascidian embryos, since blastomeres exhibit mosaic behavior upon artificial separation [10]. Likewise there is no evidence of pluripotent stem cells in well studied invertebrates,

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Efficient Endocytic Uptake and Maturation in *Drosophila* Oocytes Requires Dynamitin/p50

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ABSTRACT Dynactin is a multi-subunit complex that functions as a regulator of the Dynein motor. A central component of this complex is Dynamitin/p50 (Dmn). Dmn is required for endosome motility in mammalian cell lines. However, the extent to which Dmn participates in the sorting of cargo via the endosomal system is unknown. In this study, we examined the endocytic role of Dmn using the *Drosophila melanogaster* oocyte as a model. Yolk proteins are internalized into the oocyte via clathrin-mediated endocytosis, trafficked through the endocytic pathway, and stored in condensed yolk granules. Oocytes that were depleted of Dmn contained fewer yolk granules than controls. In addition, these oocytes accumulated numerous endocytic intermediate structures. Particularly prominent were enlarged endosomes that were relatively devoid of Yolk proteins. Ultrastructural and genetic analyses indicate that the endocytic intermediates are produced downstream of Rab5. Similar phenotypes were observed upon depleting Dynein heavy chain (Dhc) or Lis1. Dhc is the motor subunit of the Dynein complex and Lis1 is a regulator of Dynein activity. We therefore propose that Dmn performs its function in endocytosis via the Dynein motor. Consistent with a role for Dynein in endocytosis, the motor colocalized with the endocytic machinery at the oocyte cortex in an endocytosis-dependent manner. Our results suggest a model whereby endocytic activity recruits Dynein to the oocyte cortex. The motor along with its regulators, Dynactin and Lis1, functions to ensure efficient endocytic uptake and maturation.

KEYWORDS microtubule motors; endocytosis; cell polarity; kinesin; dynactin

MICROTUBULE motors such as cytoplasmic Dynein (hereafter referred to as Dynein) and proteins of the Kinesin superfamily play essential roles in cargo transport. Dynein is a minus-end motor and is responsible for the majority of minus-end transport within the cell (Kardon and Vale 2009). Mammalian genomes encode >40 different Kinesins, and most of these move cargo toward the plus-end of microtubules (Hirokawa *et al.* 2009). One type of cargo that is known to be transported by microtubule motors are vesicles of the endolysosomal system.

Cargoes that enter the cell via endocytosis follow numerous sorting pathways that ultimately determine their fate. For example, nutrient receptors such as the Transferrin receptor, are recycled back to the plasma membrane (Mayor *et al.* 1993; Huotari and Helenius 2011). Growth factor receptors and signaling molecules are often targeted for degradation (Beguinot *et al.* 1984; Huotari and Helenius 2011). This is necessary to attenuate growth-promoting signals. Persistent and uncontrolled growth-promoting signals are associated with cancer (Normanno *et al.* 2006). Cargoes that are destined for degradation transit through vesicles that undergo maturation from early endosome to late endosome. Late endosomes eventually fuse with acidic, degradative organelles known as lysosomes. Endocytic maturation involves the progressive and ordered association of specific factors with sorting vesicles (Huotari and Helenius 2011). Consequently, early endosomes are associated with a distinct set of proteins in comparison to late endosomes and lysosomes. However, endocytic maturation represents a continuum. Thus, vesicles of mixed identity can also be observed (Rink *et al.* 2005; Vonderheit and Helenius 2005).

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RESEARCH ARTICLE

The Nutrient-Responsive Hormone CCHamide-2 Controls Growth by Regulating Insulin-like Peptides in the Brain of *Drosophila melanogaster*

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Abstract

The coordination of growth with nutritional status is essential for proper development and physiology. Nutritional information is mostly perceived by peripheral organs before being relayed to the brain, which modulates physiological responses. Hormonal signaling ensures this organ-to-organ communication, and the failure of endocrine regulation in humans can cause diseases including obesity and diabetes. In *Drosophila melanogaster*, the fat body (adipose tissue) has been suggested to play an important role in coupling growth with nutritional status. Here, we show that the peripheral tissue-derived peptide hormone CCHamide-2 (CCHa2) acts as a nutrient-dependent regulator of *Drosophila* insulin-like peptides (Dilps). A BAC-based transgenic reporter revealed strong expression of CCHa2 receptor (CCHa2-R) in insulin-producing cells (IPCs) in the brain. Calcium imaging of brain explants and IPC-specific *CCHa2-R* knockdown demonstrated that peripheral-tissue derived CCHa2 directly activates IPCs. Interestingly, genetic disruption of either *CCHa2* or *CCHa2-R* caused almost identical defects in larval growth and developmental timing. Consistent with these phenotypes, the expression of *dilp5*, and the release of both Dilp2 and Dilp5, were severely reduced. Furthermore, transcription of *CCHa2* is altered in response to nutritional levels, particularly of glucose. These findings demonstrate that CCHa2 and CCHa2-R form a direct link between peripheral tissues and the brain, and that this pathway is essential for the coordination of systemic growth with nutritional availability. A mammalian homologue of CCHa2-R, Bombesin receptor subtype-3 (Brs3), is an orphan receptor that is expressed in the islet β-cells; however, the role of Brs3 in insulin regulation remains elusive. Our genetic

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RESEARCH ARTICLE

Region-Specific Activation of *oskar* mRNA Translation by Inhibition of Bruno-Mediated Repression

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Abstract

A complex program of translational repression, mRNA localization, and translational activation ensures that Oskar (Osk) protein accumulates only at the posterior pole of the *Drosophila* oocyte. Inappropriate expression of Osk disrupts embryonic axial patterning, and is lethal. A key factor in translational repression is Bruno (Bru), which binds to regulatory elements in the *osk* mRNA 3′ UTR. After posterior localization of *osk* mRNA, repression by Bru must be alleviated. Here we describe an *in vivo* assay system to monitor the spatial pattern of Bru-dependent repression, separate from the full complexity of *osk* regulation. This assay reveals a form of translational activation—region-specific activation—which acts regionally in the oocyte, is not mechanistically coupled to mRNA localization, and functions by inhibiting repression by Bru. We also show that Bru dimerizes and identify mutations that disrupt this interaction to test its role *in vivo*. Loss of dimerization does not disrupt repression, as might have been expected from an existing model for the mechanism of repression. However, loss of dimerization does impair regional activation of translation, suggesting that dimerization may constrain, not promote, repression. Our work provides new insight into the question of how localized mRNAs become translationally active, showing that repression of *osk* mRNA is locally inactivated by a mechanism acting independent of mRNA localization.

Author Summary

Proteins are often enriched to specific regions within cells via localization of mRNAs. This phenomenon serves a variety of roles, both bringing together factors involved in particular cellular processes to enhance their efficiency, and in restricting proteins that could do harm if deployed at inappropriate positions. In the latter situation, translational repression prevents expression before mRNA localization, and there must be activation mechanisms

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RESEARCH ARTICLE

Endophilin B is required for the *Drosophila* oocyte to endocytose yolk downstream of Oskar

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ABSTRACT

The nutritional environment is crucial for *Drosophila* oogenesis in terms of controlling hormonal conditions that regulate yolk production and the progress of vitellogenesis. Here, we discovered that *Drosophila* Endophilin B (D-EndoB), a member of the endophilin family, is required for yolk endocytosis as it regulates membrane dynamics in developing egg chambers. Loss of D-EndoB leads to yolk content reduction, similar to that seen in *yolkless* mutants, and also causes poor fecundity. In addition, mutant egg chambers exhibit an arrest at the previtellogenic stage. D-EndoB displayed a crescent localization at the oocyte posterior pole in an Oskar-dependent manner; however, it did not contribute to pole plasm assembly. D-EndoB was found to partially colocalize with Long Oskar and Yolkless at the endocytic membranes in ultrastructure analysis. Using an FM4-64 dye incorporation assay, D-EndoB was also found to promote endocytosis in the oocyte. When expressing the full-length *D-endoB*^{FL} or *D-endoB*$^{\Delta SH3}$ mutant transgenes in oocytes, the blockage of vitellogenesis and the defect in fecundity in *D-endoB* mutants was restored. By contrast, a truncated N-BAR domain of the D-EndoB only partially rescued these defects. Taken together, these results allow us to conclude that D-EndoB contributes to the endocytic activity downstream of Oskar by facilitating membrane dynamics through its N-BAR domain in the yolk uptake process, thereby leading to normal progression of vitellogenesis.

KEY WORDS: Endophilin B, Oskar, Vitellogenesis, Yolk protein, Fecundity, *Drosophila*

INTRODUCTION

In *Drosophila melanogaster*, a nutritional environment modulates hormonal conditions to regulate oogenesis through yolk protein synthesis and the progression of vitellogenesis (Bownes et al., 1988; Compagnon et al., 2009; Drummond-Barbosa and Spradling, 2001; Tufail and Takeda, 2009). Yolk proteins are the sole nutritional source for *Drosophila* embryogenesis. They are synthesized in follicle cells and fat bodies, and are taken up by the oocytes through endocytosis during *Drosophila* oogenesis (Compagnon et al., 2009; Morrison et al., 2008; Richard et al., 2001). *Drosophila* oogenesis can be divided into 14 stages involving a process of egg chamber growth and maturation. The germline of 15 nurse cells and one oocyte are surrounded by a single-layered follicle cell epithelium. Yolk proteins are synthesized in the somatic follicle cells from stage 8 onwards, which is the beginning of vitellogenesis (Brennan et al., 1982). The yolk protein receptor Yolkless, which belongs to the low density lipoprotein receptor (LDLR) family, transports the yolk protein into oocytes through clathrin-dependent endocytosis and is recycled back to the membrane through tubular membrane structures (Schonbaum et al., 1995; Schonbaum et al., 2000; Sommer et al., 2005). Even though several endocytic components are known to be required for endocytosis in the oocyte, the process of yolk endocytosis is not completely understood.

Oskar protein is spatially restricted to the posterior pole of the oocyte by transport, asymmetric anchorage, and local translation of *oskar* mRNA (Riechmann and Ephrussi, 2001). *oskar* mRNA encodes two protein isoforms, which perform different functions in pole plasm assembly and are localized to different subcellular structures. Short Oskar is concentrated in the polar granules where it recruits *vasa*, *tudor* and *nanos* for assembling the pole plasm (Breitwieser et al., 1996; Markussen et al., 1995; Vanzo et al., 2007; Vanzo and Ephrussi, 2002). Long Oskar is localized at the endocytic membrane and is required for anchoring the pole plasm (Vanzo et al., 2007; Vanzo and Ephrussi, 2002). Recently, the role of Long Oskar in the yolk endocytosis and the F-actin projection at the posterior pole were found (Vanzo et al., 2007). Rab5 and its effector protein, Rbsn5, are also involved in yolk endocytosis (Compagnon et al., 2009; Morrison et al., 2008). Rbsn5 acts downstream of Long Oskar for the pole plasm and endosomal protein anchorage (Tanaka and Nakamura, 2008). Furthermore, downstream of the Long Oskar regulated endocytic pathway, a Golgi-endosomal protein, Mon2, regulates the Capu-Spir-Rho1 complex to promote F-actin projections for pole plasm anchoring (Tanaka et al., 2011). Although Oskar-regulated pole plasm assembly has been well studied, it is still unclear how Oskar regulates yolk content.

The endophilin family proteins were first identified in the search for SH3 domain-containing proteins (Micheva et al., 1997). All endophilins contain the N-BAR (Bin-Amphiphysin-Rvs) and SH3 (Src-homology 3) domains. The N-BAR domain contributes to membrane binding and bending, and the SH3 domain interacts with proteins containing a proline-rich domain (Ringstad et al., 1997; Simpson et al., 1999; Sundborger et al., 2011). Endophilin family proteins bind to the membrane to drive the membrane curvature in two distinct ways (Dawson et al., 2006; Masuda et al., 2006; Peter et al., 2004). First, the N-BAR domain senses and binds to already bent membranes. Second, the N-BAR domain can directly induce the flat membrane curvature for its own association. Insertion of two amphipathic helices in the N-BAR, H0 and H1I, promote membrane curvature, and the BAR main body dimerization stabilizes the endophilin protein on the membrane. Finally, two SH3 domains are exposed and allow other

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Rab11 facilitates cross-talk between autophagy and endosomal pathway through regulation of Hook localization

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ABSTRACT During autophagy, double-membrane autophagosomes deliver sequestered cytoplasmic content to late endosomes and lysosomes for degradation. The molecular mechanism of autophagosome maturation is still poorly characterized. The small GTPase Rab11 regulates endosomal traffic and is thought to function at the level of recycling endosomes. We show that loss of Rab11 leads to accumulation of autophagosomes and late endosomes in *Drosophila melanogaster*. Rab11 translocates from recycling endosomes to autophagosomes in response to autophagy induction and physically interacts with Hook, a negative regulator of endosome maturation. Hook anchors endosomes to microtubules, and we show that Rab11 facilitates the fusion of endosomes and autophagosomes by removing Hook from mature late endosomes and inhibiting its homodimerization. Thus induction of autophagy appears to promote autophagic flux by increased convergence with the endosomal pathway.

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INTRODUCTION

Macroautophagy (hereafter autophagy) is an evolutionarily conserved bulk degradation process of eukaryotic cells. The regulation of this process was originally described in yeast (*Saccharomyces cerevisiae*) but even has a key role in multicellular organisms as a cytoprotective response to stress and pathological conditions (Levine and Kroemer, 2008; Mizushima *et al.*, 2008). Autophagy has the capacity to engulf large portions of the cytoplasm through the formation of double-membrane vesicles, called *autophagosomes*. These vesicles arise from preautophagosomal structures (PAS), which are defined sites of cytoplasm, marked by a subset of autophagy-related (*Atg*) proteins (Mizushima *et al.*, 2011). Closed autophagosomes undergo a maturation process, as they subsequently fuse with endosomes and lysosomes.

On autophagy induction, the Atg1 kinase complex (ULK1/2 in mammals) localizes to the PAS (Chan and Tooze, 2009; Mizushima, 2010) and together with the class III phosphatidylinositol-3-kinase (Vps34) complex initiates the phagophore nucleation and expansion (Funderburk *et al.*, 2010). After these events, the members of two ubiquitin-like conjugation systems are recruited to the phagophore membrane: the Atg5-12-16 complex and the phosphatidylethanolamine-conjugated Atg8a (LC3 in mammals; Geng and Klionsky, 2008). The lipid-conjugated form of Atg8a (Atg8a-II) is located on both sides of the membrane of the phagophore and autophagosomes as well. While the Atg8a located on the outer membrane is routed for recyclization, the other portion of Atg8a becomes trapped in the autolysosomal lumen and is degraded by lysosomal hydrolases. Thus Atg8a is a widely used marker of autophagic structures (Klionsky *et al.*, 2012).

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Abbreviations used: *Atg*, autophagy-related gene; BDSC, Bloomington Drosophila Stock Center; BSA, bovine serum albumin; CQ, chloroquine; DAPI, 4′,6-diamidino-2-phenylindole; DGRC, Drosophila Genomics Resource Center; GFP, green fluorescent protein; HA, hemagglutinin; LTR, LysoTracker Red; PAS, preautophagosomal structures; PBS, phosphate-buffered saline; pMT, metallothionein promoter; RE, recycling endosome; RNAi, RNA interference; TBST, Tween-20/TBS; TfR, transferrin receptor; TRA, Texas Red Avidin; VDRC, Vienna Drosophila RNAi Center; YFP, yellow fluorescent protein.

The conserved P body component HPat/Pat1 negatively regulates synaptic terminal growth at the larval *Drosophila* neuromuscular junction

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Summary

The temporal and spatial regulation of protein synthesis plays an important role in the control of neural physiology. In axons and dendrites, translationally repressed mRNAs are actively transported to their destinations in a variety of ribonucleoprotein particles (RNPs). A subset of these neuronal RNPs has been shown to contain proteins associated with mRNA processing bodies (P bodies). P bodies are a class of highly conserved cytoplasmic granules that have been linked to both mRNA decay and translational repression via general and miRNA-mediated pathways. Here, we characterize functions for HPat/Pat1 (also known as Patr-1), a core component of P bodies, at the glutamatergic larval *Drosophila* neuromuscular junction (NMJ). We show that *hpat* mutants exhibit a strong synaptic hyperplasia at the NMJ. The synaptic defects observed in *hpat* mutants are associated with rearrangement of the axonal microtubule cytoskeleton suggesting that HPat negatively regulates presynaptic microtubule-based growth during NMJ development. Consistent with this, overexpression of HPat also blocks the rapid growth of presynaptic boutons induced by spaced depolarization. Finally, we demonstrate that HPat interacts genetically with the catalytic subunit of the deadenylase complex (twin/CCR4) and the miRNA pathway (Argonaute 1) to control bouton formation. We propose that HPat is required to target mRNAs involved in the control of microtubule architecture and synaptic terminal growth for repression, presumably in P bodies, via both general and miRNA-mediated mechanisms.

Key words: P bodies, Ribonucleoprotein particles, Pat1, Synaptogenesis, Neuromuscular junction

Introduction

In neurons, the local regulation of mRNA translation is required to control processes ranging from axon guidance and synaptogenesis to the persistent modifications in synaptic efficacy associated with long-term synaptic plasticity (Jung et al., 2012; Kindler and Kreienkamp, 2012). The spatial and temporal control of mRNA translation is regulated by cis-acting elements, most often found in their untranslated regions (UTRs), acting in concert with trans-acting factors such as RNA-binding proteins (RBPs) and microRNAs (miRNAs). In axons and dendrites, these translationally silent mRNAs are actively transported to their destination associated with a heterogeneous group of RNPs that include RNA transport granules, stress granules (SGs), and neuronal P bodies (Sossin and DesGroseillers, 2006).

P bodies are highly conserved cytoplasmic RNPs linked to both mRNA decay and translational repression pathways. In the former, mRNAs are targeted for deadenylation followed by decapping and then 5′-to-3′ exonucleolytic degradation (Cougot et al., 2004; Sheth and Parker, 2003). In the latter, mRNAs are deadenylated and targeted for translational repression and storage within larger P body aggregates (Teixeira et al., 2005). Orthologous RNPs can be found in *Drosophila melanogaster* that contain the fly decapping enzyme (Dcp2), enhancers of decapping (Dhh1, Dcp1, Edc3, and Pat1), the 5′-to-3′ exoribonuclease (Xrn1), and the miRNA RNA induced silencing complex (miRISC) proteins (Ago1, Ago2 and GW182; Eulalio et al., 2007a). Neurons in *Drosophila* and mammals contain populations of specialized P bodies with largely unknown functions (Barbee et al., 2006; Cougot et al., 2008; Zeitelhofer et al., 2008a). Interestingly, neuronal P bodies exhibit a significant amount of overlap with components of RNA transport granules including the Fragile X Mental Retardation Protein (FMRP). P bodies in dendrites of cultured hippocampal neurons exhibit motorized movements and re-localize towards distal sites in response to synaptic activation (Cougot et al., 2008). Additionally, acute synaptic stimulation results in a significant decrease in the number of dendritic P bodies suggesting that they can disassemble after neural activity (Zeitelhofer et al., 2008a). Most dendritic P bodies lack Xrn1, a catalytic component in the 5′-to-3′ decay pathway (Cougot et al., 2008). Together, these data support a model where neuronal P bodies accumulate translationally repressed mRNAs that are released from storage, and potentially repression, following synaptic activity.

The assembly of P bodies is influenced by a balance between translational activation and repression (Franks and Lykke-Andersen, 2008). P body assembly is a stepwise process where key P body components, notably Pat1 and a complex of Lsm proteins, are first recruited to an mRNA to form a P body monomer. Under certain cellular conditions these monomers can

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Review Article

Roles of cytoplasmic RNP granules in intracellular RNA localization and translational control in the *Drosophila* oocyte

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Intracellular mRNA localization and translation are ways to achieve asymmetric protein sorting in polarized cells, and they play fundamental roles in cell-fate decisions and body patterning during animal development. These processes are regulated by the interplay between *cis*-acting elements and *trans*-acting RNA-binding proteins that form and occur within a ribonucleoprotein (RNP) complex. Recent studies in the *Drosophila* oocyte have revealed that RNP complex assembly in the nucleus is critical for the regulation of cytoplasmic mRNA localization and translation. Furthermore, several *trans*-acting factors promote the reorganization of target mRNAs in the cytoplasm into higher-order RNP granules, which are often visible by light microscopy. Therefore, RNA localization and translation are likely to be coupled within these RNP granules. Notably, diverse cytoplasmic RNP granules observed in different cell types share conserved sets of proteins, suggesting they have fundamental and common cellular functions.

Key words: *Drosophila*, mRNA localization, P body, ribonucleoprotein, translational control.

Introduction

Intracellular mRNA localization is a conserved posttranscriptional mechanism for achieving spatially restricted protein production in polarized cells. This mode of protein sorting occurs in various biological processes, including asymmetric cell division, cell motility, embryonic axis determination, germ-cell formation, and neuronal plasticity (Johnstone & Lasko 2001; Palacios & St Johnston 2001; Kloc *et al.* 2002; Holt & Bullock 2009; Martin & Ephrussi 2009; Percipalle *et al.* 2009). However, mRNA localization alone is insufficient to achieve the precise restriction of protein expression within a specific site; it must be coupled with translational control to prevent premature and ectopic protein production (Johnstone & Lasko 2001; Palacios & St Johnston 2001; St Johnston 2005; Besse & Ephrussi 2008).

Proper mRNA localization and translation are governed by the interactions between *cis*-acting RNA elements and *trans*-acting RNA-binding proteins. These *cis*-acting elements are often found in, albeit not absolutely restricted to, the 3′ untranslated region (UTR) of the mRNA. Specific *trans*-acting factors recognize and bind these elements, forming a ribonucleoprotein (RNP) complex. In many cases, RNP complexes congregate to form large granules that are often visible by light microscopy. Therefore, mRNA localization and translation are probably coupled, at least in part, through interactions between *trans*-acting factors within the RNP granules.

Drosophila oogenesis has been used as a tractable model system for studying the mechanisms of mRNA localization and translational control. The localizations of several maternal mRNAs in the *Drosophila* oocyte are key events for formation of the embryonic body axes and germ cells (Johnstone & Lasko 2001; Kuersten & Goodwin 2003). Taking advantage of the powerful genetics available in *Drosophila*, combined with biochemistry and live-cell imaging techniques, the *cis*-acting elements and their *trans*-acting factors have been functionally analyzed in detail for a number of localized mRNAs, including *bicoid* (*bcd*), *gurken* (*grk*), *nanos* (*nos*), and *oskar* (*osk*). In this review, we summarize recent advances in the mechanisms of mRNA localization and translational control during *Drosophila* oogenesis. We focus

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