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逆命にある。一方、生殖細胞は、個体の生存には必要がないが、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たす1つの受精卵から作りだされる。そのため、生殖細胞は完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充分明らかにされていない。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.
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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、体細胞とは異なる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、緑虫、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・分化に必要なノンに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細胞（germ plasm）と呼ばれる。すなわち、胚発生過程において、生殖細胞を取り込んだ細胞が生殖細胞へと分化する（図1）。生殖細胞は卵に偏在して形成されることから、生殖細胞の研究を通じて、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る多数の配偶子産生を可能としていることから、生殖細胞研究を通じて、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。

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過剰な転写抑制は、ショウジョウバエばかりではなく、縄虫、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。


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は生殖細胞で、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転写が過剰、かつノリノウハクワに抑制される。

図1：様々な動物種における生殖細胞形成過程。生殖質、及び生殖細胞をマーカーで示す。（A）ショウジョウバエ、（B）緑虫、（C）ツメガエル、（D）カタクイウアイボヤ
(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、これ転写抑制が破壊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、転写抑制が破壊した生殖細胞が死亡を迎えてい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. 生殖質アッセスプリーにおける mRNA 局在と翻訳制御。


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・生殖質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ニング、ならびに分子スクリーニングを行った。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）始めて、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行っているところであるが、進歩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 欠失胎の発現型
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 Vasal 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て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。このことにより、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にし、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応用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を制御して生殖質因子の輸送に必要であることを示し、アクチンの再編成の制御を介して生殖質因子の保存にも必要である。

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（RNAi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誘導の場である生殖質に到達する。RNAi誘導における生殖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生殖質に至るまでの過程の制御機構はほとんど明らかにされていない。私たちは、リソゾ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、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掛かりとして、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、生殖質に到達する（経路①～⑤）；細胞質中では、dsRNAはsiRNA生成とRISC形成を経て、標的RNAを分解する（経路⑥、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).


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 oocyte. 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 assemblies 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 of 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 evolutionarily 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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著書・総説目録  Publications


2. 中村 輝 (2013) 生殖細胞と体細胞、動物学の百科事典 (丸善), 刷り上がり 2 ページ

2. 中村 輝 (2013) 生殖細胞と体細胞、動物学の百科事典 (丸善), 刷り上がり 2 ページ

3. 中村 輝 (2013) 生殖細胞と体細胞、動物学の百科事典 (丸善), 刷り上がり 2 ページ

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授賞 Award

1. 中村 輝、自然科学研究助成、三菱財団、2016年

2. 中村 輝、生命科学研究助成、武田科学振興財団、2013年

3. 中村 輝、ノバルティス研究奨励金、ノバルティス科学振興財団、2012年
The evolutionarily-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 Sox2 family of transcription factors are well conserved in the animal kingdom, addressing the evolutionary origin of Sox function in pluripotent stem cells is important from the perspective of understanding the origin of pluripotency.

Results: Here we approach this question using a functional complementation assay in inducible Sox2-A1 ES cells. Assaying mouse Sox proteins from different groups, we found that only Group B1 and Group C proteins were able to support pluripotency. Interestingly, intermolecular homodimers of mammalian Group B1 Sox proteins were able to rescue the pluripotency-associated function of mouse Sox2. Moreover, the mouse ES cells rescued by the Drosophila Salventino protein are able to contribute to chimeric embryos.

Conclusions: These data indicate that the function of mouse Sox supporting pluripotency is based on an evolutionarily conserved activity of the Group B1 Sox family. Since pluripotent stem cell populations in development could be regarded as the evolutionarily novelties in vertebrates, it could be regarded as a co-optation of their evolutionarily conserved function.

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

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 to embryos 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 that give rise to induced pluripotent stem (iPS) cells [2-3].

These four transcription factors, Oct4/6 (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 frog. Many animal 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 cell lines 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, these exceptions aside, the evolutionarily conserved function of Sox2 in mouse ES cells suggests that Sox2 is essential for maintaining pluripotency in higher eukaryotes. Sox2 is also essential for early mammalian development, with mutations in the Sox2 gene leading to early embryonic lethality [11].

Efficient Endocytic Uptake and Maturation in Drosophila Oocytes Requires Dynamin/p50

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Abstract

Dynamin is a multifunctional GTPase that functions as a regulator of the dynamin motor. A central component of this complex is Dynamin/p50 (Dm). Dynm is required for endosome motility and mammalian cells. However, the extent to which Dm is a distinct organelle is still not clear. This study examines the endosomal role of Dynm using the Drosophila melanogaster oocyte as a model. Whole proteins are internalized into the oocyte via defects [12]. Membrane-bound endosomes, tethered through the dynamin pathway, are detected in confined condense granules. Occludin proteins that are depleted in Dm are observed to form fewer endosomes than controls. In addition, these endocytosed accumulated numerous endocytic intermediates that are not observed in controls. Ultrastructural and genetic analyses indicate that the endocytic intermediates are processed through Rab7, similar phenotypes were observed upon depletion Dynm [13]. Dm is also a GTPase activator of the Dynm complex which is independent of Dm1 activity. We therefore propose that Dm1 functions in endosomes via its Rho GTPase activity. Consistent with this role for Dynain in endosomes, the model calculated with the endocytic machinery at the oocyte cortex in an endosome-dependent manner. Our results suggest a model whereby endocytic activity results in Dynain to the oocyte cortex. The model allows for these studies to be extended to other systems.

Keyword: endocytosis, endosomes, cell polarity, kinetics, dynamin

Microtubule minus end motors, endocytosis, cell polarity, kinetics, dynamin

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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 (gut-tissue) has a role in regulating systemic growth and development. Here, we show that the CCHamide-2 (CCHA2) peptide in the fat body acts as a nutrient-dependent regulator of Drosophila insulin-like peptides (DILPs). A BAC-based transgenic reporter reveals 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 tissues-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 dilp1 and dilp2, the release of both DILP1 and DILP2, 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, Bmp18 receptor subunit-3 (Bms3), is an orphan receptor that is expressed in the kidney; however, the role of Bms3 in insulin regulation remains elusive. Our genetic

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 (Bri), which binds to regulatory elements in the osk mRNA 3'-UTR. After posterior localization of osk mRNA repression by Bri must be alleviated. Here we describe an in vivo assay system to monitor the spatial pattern of Bri-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 mechanically coupled to mRNA localization, and functions by inhibiting repression by Bri. We also show that Bri dimers and identify mutations that disrupt this interaction to limit its role in vivo. Loss of dimerization does not disrupt repression, which 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 our 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 mRNA. 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 in inappropriate positions. In the latter situation, translational repression prevents expression before mRNA localization, and there must be activation mechanisms...
Endophilin B is required for the Drosophila oocyte to endocytose yolk downstream of Oskar
Yi-Cheng Tse1*, Wei Chang1, Williás Licó, Wei-Hao Liao, Yu-Wei Chang, Pei-Yu Wang, Yi-Chen Lin, Tsuiho Tanaka*, Akira Nakamur* and Li-Mei Pai1,2

ABSTRACT

The maternal environment is crucial for Drosophila oogenesis in terms of controlling hormonal conditions that regulate yolk production and the progress of yolk vitellogenesis. Here, we discovered that Drosophila Oskari and BP (D-rab11) is a member of the endophilin family, is required for yolk endocytosis as it regulates membrane dynamics in developing egg chambers. Loss of D-rab11 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 previtelligenic stage. D-rab11 is required for yolk vitellogenesis and the core vitellogenic arrest in D-rab11 mutants but was restored, by contrast, to a normal D-rab11 domain in D-rab11, only partially rescued these defects. Taken together, these results allow us to conclude that D-rab11 contributes to the systemic activity downstream of Oskar by facilitating membrane dynamics through an N-BAR domain in the yolk uptake process, thereby leading to normal progression of stembogenesis.

KEY WORDS: Endophilin B, Oskari, Vitellogenesis, Yolk protein, Feeding, Drosophila

INTRODUCTION

In Drosophila melanogaster, a maternal environment modulates hormonal conditions that regulate oogenesis through yolk protein synthesis and the progress of vitellogenesis (Bremers et al., 1988; Compagnon et al., 2009; Drummond-Davis and Spradling, 2001; Tutfil and Takeda, 2009). Yolk proteins are the sole nutritional sources for Drosophila embryos. They are synthesized in follicle cells and body fat, and are taken up by the oocytes through endocytosis during Drosophila oogenesis (Compagnon et al., 2009; Morisone et al., 2008; Richard et al., 2003). 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 (Bremers et al., 1988). The yolk protein receptor Yolkless, which belongs to the low density lipoprotein receptor (LDLR) family, transports the yolk proteins into oocytes through clathrin-dependent endocytosis and is recycled back to the membrane through tubulovesicular membrane carriers (Schubmann et al., 1995; Schubmann et al., 2008; Sommer et al., 2005). Even though several clathrin components are known to be required for endocytosis in the oocyte, the process of yolk endocytosis is not completely understood.

Oskari is specifically restricted to the posterior pole of the oocyte by transport, asymmetric gradients, and localized activation of oskar mRNA (Rothman and Ephrussi, 2001). Oskari mRNA encodes two protein isoforms, which perform different functions in the yolk protein assembly and are localized to different tubulovesicular structures. Short Oskari is concentrated on the granule where it remains in a active and secreted form for the yolk granule (Brezowski et al., 1990; Martiusen et al., 1995; Vervoort et al., 2007; Vervoort and Ephrussi, 2003). Long Oskari is localized at the oocyte membrane and is required for anchoring the yolk protein (Vervoort et al., 2007; Vervoort and Ephrussi, 2003). Recently, the role of Long Oskari in the yolk uptake and the Fasun protein at the posterior pole were found (Vervoort et al., 2007; Vervoort et al., 2003). Rab and its effectors, Rab9, are also involved in yolk endocytosis (Compagnon et al., 2009; Morisone et al., 2008). Rab9 acts downstream of Long Oskari for the polar vesicle correction and receptor-mediated uptake in the follicle cell (Tanaka and Nakamura, 2008). Furthermore, downstream of the Long Oskari regulated endocytic pathway, a Gippas-endosomal protein, Meri, regulates the Cepac-Spo-11 complex to promote Fasun projections for polar protein anchoring (Tanaka et al., 2011). Although Oskari-regulated polar vesicle assembly has been well studied, it is unclear how Oskari regulates yolk content.

The endophilin family proteins were first identified in the search for SH3 domain-containing proteins (Michov et al., 1997). All endophilins contain the N-BAR (Bin/ amphiphysin/Rvs) and SH3 (Src homology-3) domains. The N-BAR domain mediates membrane binding and bending, and the SH3 domain interacts with proteins containing a proline-rich domain (Rassoulzadegan et al., 1997; Simpson et al., 1999; Schindler et al., 2001). Endophilin family proteins localize to the membrane to drive the membrane curvature in two distinct ways (Dworniczak et al., 2004; Maeda et al., 2003; Peter et al., 2004). First, the N-BAR domain sequesters and binds to tubular membranes. Second, the N-BAR domain can directly induce the flat membrane curvature for its own association. Insertion of two amphiphatic α-helices into the membrane leads to the formation of two SH3 domains that are capable of oligomerization (18 and 111, promote membrane curvature, and the BAR protein body domain stimulates the endophilin proteins on the membrane. Finally, two SH3 domains are exposed and allow other

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 translates from recycling endosomes to autophagosome 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.

INTRODUCTION

Macroautophagy (hereafter autophagy) is an evolutionarily conserved bulk degradation process of cytoplasmic contents (Tanaka and Nakamura, 2008). The regulation of the process of autophagy (the vesicle formation—invagination—exonucleation) has been studied but even has a key role in multicellular organisms as a cytoprotective mechanism against stress and pathological conditions (Lewin and Kreisler, 2008; Mizushima et al., 2008). Rab11 has the capacity to engulf large portions of the cytoplasm through the formation of double-membrane vesicles, called autophagosomes. These vesicles arise from pre-autophagosomal structures (PAS), which are defined sites of autophagosome, marked by a subunit of autophagosome (Atg proteins) (Mizushima et al., 2008). Class VI- and V-type autophagosomes undergo a maturation process, as they subsequently fuse with endosomes and lysosomes.

On autophagy induction, the Atg1 kinase complex (Atg1/12 in mammalian) localizes to the PALS (Chen and Tasset, 2009; Mizushima, 2010) and together with the class III phosphatidylinositol 3-kinase (PI3K) complex initiates the phagophore nucleation and expansion (Pandolfi et al., 2010). After these events, the members of two ubiquitination-conjugation systems are recruited to the phagophore membrane: the Atg13/16 complex and the phosphatidylserine-conjugating Atg33 (Lin and Anderson, 2008). The class III-conjugated form of Atg33 (Atg33-Glu) is located on both sides of the membrane of the phagophore and autophagosomes as well. While the Atg33 located on the outer membrane is required for recycling, the other portion of Atg33 becomes trapped in the autophagosome lumen and is degraded by lysosomal hydrolases. Thus Atg33 is a widely used marker of autophagic structures (Donohoe et al., 2017).
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 regulated mRNAs are actively transported to their destinations in a variety of eukaryotic 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 mRNA-mediated pathways. Here, we characterize functions for 18P bodies (also known as Pat1, a core component of P bodies, at the glutamatergic larval Drosophila neuromuscular junction (NMJ). We show that Pat1 mutants exhibit a strong synaptic hypoplasia at the NMJ. The synaptic defects observed in Pat1 mutants are associated with rearrangement of the axonal motoric cytoskeleton, suggesting that Pat1 negatively regulates presynaptic microtubule growth during NMJ development. Consistent with this, overexpression of Pat1 also blocks the rapid growth of presynaptic boutons induced by spaced depolarization. Finally, we show that Pat1 interacts genetically with the catalytic subunit of the dynactin complex (twinC/twinB) and the mRNA pathway (Argonaute 1) to control bouton formation. We propose that Pat1 is required to target mRNAs involved in the control of microtubule architecture and synaptic terminal growth for repression, presumably in P bodies, both via general and mRNA-mediated mechanisms.

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

Development. Growth & Differentiation

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 are now considered to be 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, gametogenesis, and neuronal plasticity (Johstone and Lasko 2001; Palacios and St Johnston 2001; Pao et al. 2002; Holt and Robock 2006; Martin and Ephrussi 2008; Pocius et al. 2008). 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 (Johstone and Lasko 2001; Palacios and St Johnston 2001; St Johnston 2000; Besse and 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 (3’UTR) of the mRNA. Specific trans-acting factors recognize and bind these elements, forming a ribonucleo- protein (RNP) complex. In many cases, RNP complexes aggregate 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 axis and germ cells (Johstone and Lasko 2001; Kuehnert and Goodwin 2003). Taking advantage of the powerful genetic tools available in Drosophila, combined with biochemistry and freeze-etching imaging techniques, the cis-acting elements and their trans-acting factors can be functionally analyzed in detail for a number of localized mRNAs, including blood (bcd), eggshell (gey), 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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