The Other Lives of Ribosomal Proteins

Metrah M. Nader
Portland State University

THE OTHER LIVES OF RIBOSOMAL PROTEINS

By

Metrah Mohammad Nader
**Faculty Mentor: Sean Speese, PhD**

Abstract

Cellular mRNAs are produced in the nucleus and exported to the cytoplasm through nuclear pore complexes (NPC) that are found in the nuclear membrane. The correctly processed mRNAs are exported in the form of messenger ribonucleoproteins (mRNPs). Since the NPC allows the passage of only one mRNA molecule at a time, export of large ribonucleoproteins (megaRNP) found in the nucleus cannot be transported through the NPC. However, a recent study has discovered an alternative export mechanism known as nuclear envelope budding, which allows megaRNP granules to be exported. This study particularly focuses on identifying ribosomal proteins (RPs) found within large RNA granules within the salivary gland, and neuromuscular junction (NMJ) of *Drosophila melanogaster* larvae. Although, RPs are known mainly for playing an essential role in ribosome assembly and protein translation that occurs in the cytoplasm, recent discoveries have shown that ribosomal proteins possess ribosome-independent functions, which are essential for a cell survival and proper animal development. This study finds that Rps3 is possibly localized within large RNA granules within NMJ of *Drosophila* larvae; however, the future in-depth studies are needed to verify this finding and identify the specific function of Rps3 in the megaRNP granules.

Keywords: Biology, Ribosomes, Ribosomal Proteins

Introduction

Inside every eukaryotic cell the transcription of mRNA occurs in the nucleus, which makes the translocation of mRNA across the nuclear envelope (NE) through nuclear pore complex (NPC) a critical determinant of proper gene expression and cell survival. Historically, the translocation of mRNA to the cytoplasm has been thought to occur only through nuclear pore complexes (NPC) that are found in the nuclear membrane. This transport is mediated by transport receptors that shuttle between the nucleus and cytoplasm, which provide the means for RNAs to enter and exit an otherwise impermeable capsule (Nakielny et al., 1997; Conti et al., 2001). Recently, however, significant advances in our understanding of the mechanism of mature RNA export between the nucleus and the cytoplasm have been made. Our understanding of mRNP, export from the nucleus into the cytoplasm has been advanced by the discovery of nuclear envelope budding which permits large ribonucleoprotein particles (RNPs)/megaRNPs to be exported from the nucleus. In this pathway, the granules exit the nucleus by passing through the nuclear membranes. Granules are enveloped by the inner nuclear membrane, which pinches off into the perinuclear space, and then fuses with the outer nuclear membrane and finally exit the nucleus (figure 1) (Speese et al., 2012). MegaRNPa are transport granules that contain many mRNAs assembled with RNA-binding proteins, RPs for small and large subunits of ribosomes and translation initiation factors that are transported to sites of translation (Anderson et al., 2006; Khandjian et al., 2009).
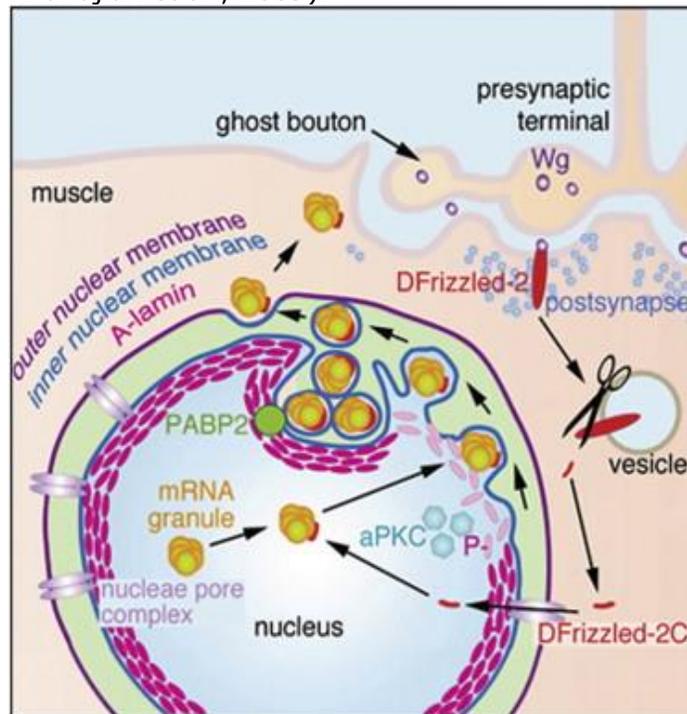
Figure 1. Nuclear Envelope Budding in Drosophila Muscle Cells

Another critical determinant of proper gene expression and cell survival is the presence and proper function of all 79 RPs that are found within eukaryotic ribosome (Zhou et al., 2015). Ribosome is a complex molecular machine that is primarily responsible for

translation and protein synthesis from messenger RNA (mRNA). Ribosomal proteins (RPs) are major components of the ribosome and play a significant role in the initiation, elongation, or termination phase of protein translation (Wang et al., 2014; Nissen et al., 2000). Therefore, this indicates that ribosomal proteins are essential for both ribosome formation and protein translation. However, growing evidence has suggested that some ribosomal proteins have functions distinct from their role in the ribosome and protein synthesis (Wool, 1996; Warner and McIntosh, 2009). In some instances, their regulatory functions are so significant that mutations in specific ribosomal protein genes (RPGs) can lead to specific physiological defects in eukaryotic organisms. For example, one study showed that mutations of the ribosomal protein L38 (Rpl38) gene in mice exhibit tissues specific patterning defects, particularly, homeotic transformations of the axial skeleton in Rpl38 mutant embryos (Kondrashov et al., 2012). This study also demonstrates that Rpl38 plays a critical role for translational control of Hox gene expression, which is required to establish the mammalian body plan. Similarly, deletion and mutation of multiple RPs in zebrafish has demonstrated a variety of physiological defects as well as increase in the incidence of peripheral nervous system tumors (Amsterdam et al., 2004). In addition, functional studies of some mutant mammalian RPs, have linked mutations in a number of RPs to congenital birth defects such as Diamond–Blackfan anemia (Lipton and Ellis, 2010). Therefore, the existence of tissue specific physiological defects suggests that some RPs indeed have functions independent of the ribosome. In the context of the present study, the screening for potential RPs found within large RNA granules reveals enrichment of Rps3 within mega RNPs in postsynaptic muscle nuclei.

Materials and Methods

Fly Strains

Fly cultures and crosses were grown on standard fly medium at 25°C. w+/-; w;t; HS-UAS-GAL4 strains for salivary glands and w+/-; ;;Mhc UAS-GAL4 strains for NMJ were used. In this case, HS-gal4 and Mh-gal4 were used to drive UAS controlled transgenes encoding ribosomal proteins fused to HA and GFP tags.

Dissection

For the NMJ analysis, w+/-; ;;Mhc UAS-Gal4 larvae grown at 25°C were used. Drosophila larvae were carefully dissected using Jan's Saline without $CaCl_2$. A longitudinal mid-dorsal incision was made and the edges of the tissues were pinned so that the preparation was spread out on a glass slide in the preparation dish. All the internal organs were carefully removed while leaving the body wall intact, particularly the ventral longitudinal muscles. Consequently, the dissected larva was fixed in 4% paraformaldehyde for 20 minutes. The dissected larva was then washed three times with 0.2% TritonX-100 in 1X (PBS-TX). The larva was then transferred into a glass dish containing 1x PBS, 0.2% Triton X.

For salivary glands analysis, w+/-; w;t; HS UAS-Gal4 larvae grown at 25°C were used. The tip of the mouth hooks were grasped with one pair of forceps, while holding the body about 2/3 of the way down with the other pair. The mouth hooks were pulled and the salivary glands were exposed. The salivary glands were dissected using Jan's Saline in 0.5 $CaCl_2$. Next, the salivary glands were fixed in 4% formaldehyde for 25 minutes. The dissected larvae were then washed three times in 1x PBS, 0.2% Triton X. The larvae were then transferred into a glass dish containing 1x PBS, 0.2% Triton X.

Immunohistochemistry

Primary antibody master mixes were prepared at the following concentrations, diluting the antibody in 1x PBS, 0.2% Triton X:

LamDm0- stained with α-LamDm0 primary (1:100)
FlyORF - 3xHA tagged stained with α-LamDm0 (1:100) and α-HA (1:250) primaries

Secondary antibody master mixes were prepared at the following concentration, diluting the antibody in 1x PBS, 0.2% Triton X:

α-Ms 647 secondary (1:400); Con A 594 (1:200) to NMJ only
FlyORF-3xHA tagged: α-Ms min-x Rat 488 (1:400); α-Rt min-x Ms 549 (1:400)

400 μL of primary antibody solution was dispensed to each well. Then, the dissected larvae were incubated in primary antibody at 4ºC overnight. The dissected NMJ were then washed three times for 15 minutes in 1x PBS, 0.2% Triton X at room temperature. Similarly, the dissected salivary glands were washed three times for 30 minutes in 1x PBS, 0.2% Triton X.

400 μL of secondary antibody solution was dispensed to each well. Then, the dissected larvae were incubated at room temperature for 2.5-3 hours. Finally, they were washed 3 x 30 minutes in 1x PBS, 0.2% Triton X.

Mounting

The head and tail of dissected NMJ were removed with a fresh razor blade on the processing glass slide. The dissected NMJ and salivary glands were transferred to the glass well of a dissection dish and the wash solution was carefully removed with a pipette. A drop of Vectashield was then dispensed onto the salivary glands and NMJ. They were then carefully transferred from the dissection dish to microscope slides. Each microscope slide contained 3-4 larva for NMJ dissection and 10-12 for the salivary glands. The samples were aligned and oriented so that ventral longitudinal muscles were facing up. Small drops of Vectashield were added once all the samples were oriented and covered with coverslip. The coverslip was sealed around the border with a layer of CoverGrip and dried at room temperature overnight.

Confocal Microscopy

The images of dissected NMJ and salivary glands were taken on a Zeiss confocal LSM 7.0 microscope using a 40X oil immersion lens. For the NMJ and salivary glands staining, 20 slices through the Z plane were taken per NMJ and salivary at an interval of 1 um. For each samples appropriate settings were chosen for laser power, PMT gain, and offset. Regions of interest were identified from confocal tiles and the composite images of the Z-series were collected. The qualification analysis was completed through ZEN software.
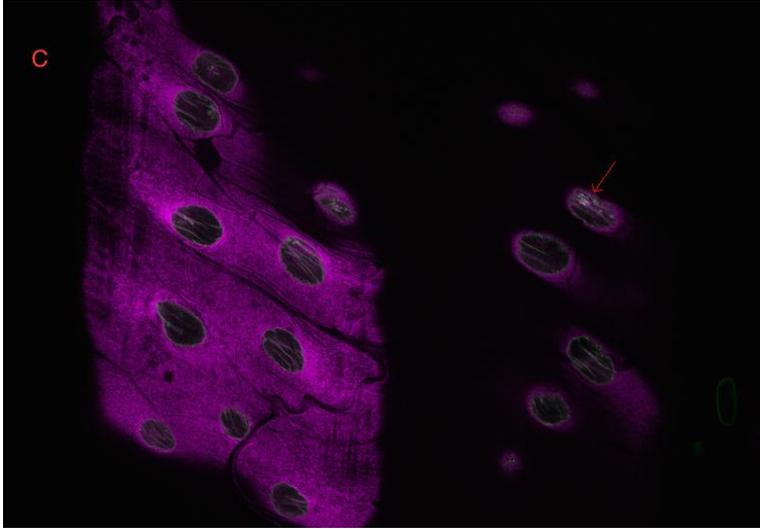
Results:
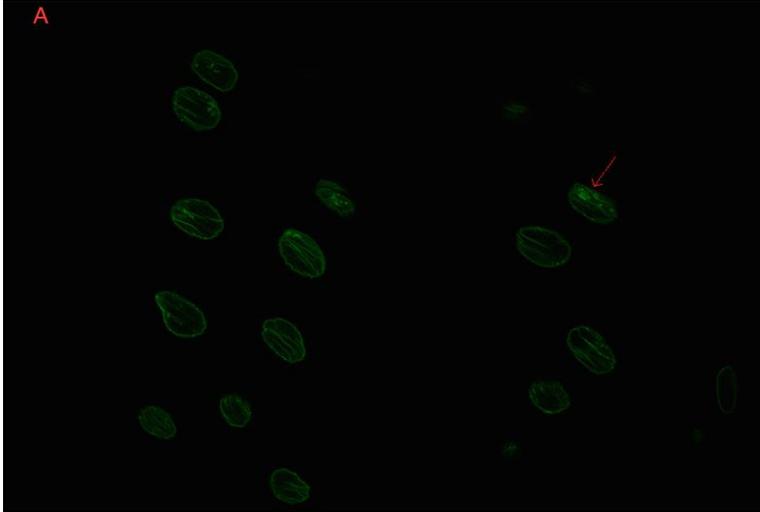
(A) Larval NMJ labeled with LamDm0 antibody to mark sites where megaRNP granules are in the nucleus.
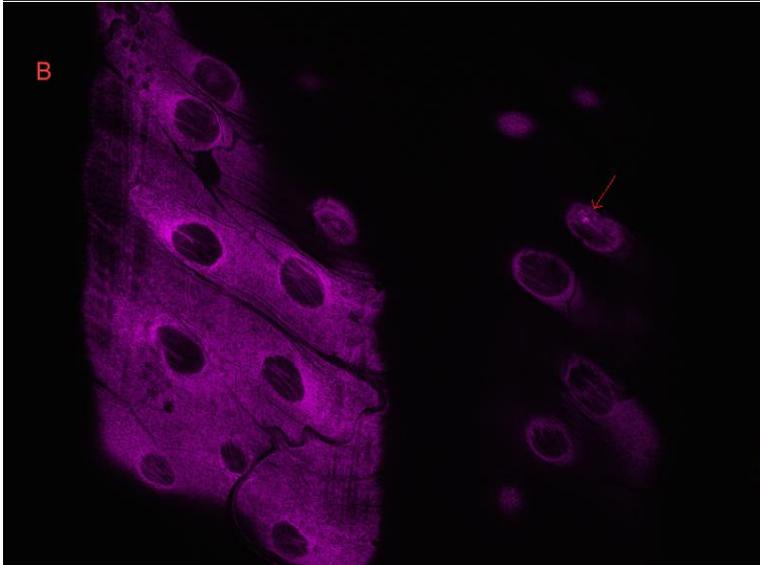
(B) Larval NMJ expressing Rps3-HA labeled with an anti-HA antibody

(C) Overlay of images A and B showing enrichment of Rps3 at sites where megaRNP granules have formed, suggesting that Rps3 is a component of the granules (see red arrow).

Figure 2. Localization of Rps3 in Larval Muscle Nuclei

GFP tagged RPs	NMJ (x ;;MhcGal4)	Salivary Gland (x ;;hsGal4)
RpS9	no enrichment at granules	no enrichment at granules
RpS5a	no enrichment at granules	no enrichment at granules
RpS5a	no enrichment at granules	no enrichment at granules
RpS15	no enrichment at granules	no enrichment at granules
RpS15	no enrichment at granules	no enrichment at granules
RpS13	no enrichment at granules	no enrichment at granules
RpS13	no enrichment at granules	no enrichment at granules
RpS11	no enrichment at granules	no enrichment at granules
RpS11	no enrichment at granules	no enrichment at granules
RpS2	no enrichment at granules	no enrichment at granules
RpS2	no enrichment at granules	no enrichment at granules
FlyORF - 3xHA tagged		
RpS15	no enrichment at granules	no enrichment at granules
RpS5b	no enrichment at granules	no enrichment at granules
RpS15Ab	no enrichment at granules	no enrichment at granules
RpS15Aa	no enrichment at granules	no enrichment at granules
RpS3	enrichment at RNA granules	no enrichment at granules
RpS19a	no enrichment at granules	no enrichment at granules
RpS19b	no enrichment at granules	no enrichment at granules
RpL11	no enrichment at granules	no enrichment at granules
RpS18	no enrichment at granules	no enrichment at granules
RpS25	no enrichment at granules	no enrichment at granules

Table 1: Fluorescent tagged RPs within large RNA granules within the salivary gland, and neuromuscular junction (NMJ) of *Drosophila* larvae*.

Discussion

     During the past few decades our understanding of mRNA export, the ribosome, and RPs has considerably improved. RNAs and ribonucleoprotein particles (RNPs) were initially thought to access the cytoplasm solely through the NPC, but are now considered to access the cytoplasm through nuclear envelope budding (NEB). Similarly, RPs were initially thought to be involved only in ribosome assembly, but some RPs are now considered to have regulatory functions outside their function in the ribosome. Their roles in cellular functions range from regulation of mammalian body plan to cellular metabolism. Significantly, deregulation in any of these processes may lead to an abnormal phenotype. Unexpectedly, this study reveals that RNA granules within NMJ are enriched with Rps3. Further investigations will be required to reveal the regulatory network associated with Rps3. For instance, the exact mechanisms that control Rps3 and its specific role in the cell are unknown. Therefore, the future in-depth studies are needed to identify the novel functions of Rps3 in megaRNP granules.

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