Developmental Biology 326 (2009) 68–74

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Glorund interactions in the regulation of *gurken* and *oskar* mRNAs

Yossi Kalifa, Stephen T. Armenti, Elizabeth R. Gavis *

Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

ARTICLE INFO

Article history:
Received for publication 2 June 2008
Revised 17 October 2008
Accepted 22 October 2008
Available online 5 November 2008

Keywords:
Glorund
gurken
oskar
hnRNP
mRNA localization
Splicing
Otu
Hpf
Hrp48

ABSTRACT

Precise temporal and spatial regulation of gene expression during *Drosophila* oogenesis is essential for patterning the anterior–posterior and dorsal–ventral body axes. Establishment of the anterior–posterior axis requires posterior localization and translational control of both *oskar* and *nanos* mRNAs. Establishment of the dorsal–ventral axis depends on the precise restriction of *gurken* mRNA and protein to the dorsal–anterior corner of the oocyte. We have previously shown that Glorund, the *Drosophila* hnRNP F/H homolog, contributes to anterior–posterior axis patterning by regulating translation of *nanos* mRNA, through a direct interaction with its 3′ untranslated region. To investigate the pleiotropy of the *glorund* mutant phenotype, which includes dorsal–ventral and nuclear morphology defects, we searched for proteins that interact with Glorund. Here we show that Glorund is part of a complex containing the hnRNP protein Hrp48 and the splicing factor Half-pint and plays a role both in mRNA localization and nurse cell chromosome organization, probably by regulating alternative splicing of *ovarian tumor*. We propose that Glorund is a component of multiple protein complexes and functions both as a translational repressor and splicing regulator for anterior–posterior and dorsal–ventral patterning.

© 2008 Elsevier Inc. All rights reserved.

Introduction

Asymmetric mRNA localization is essential to establish and maintain polarity of the *Drosophila* oocyte. Protein asymmetries arising from localized mRNA translation also govern the patterning of the embryonic body axes and the segregation of the somatic and germline lineages. Localization of *gurken* (*grk*) mRNA to the posterior pole of the early oocyte results in local production of the Grk TGFα ligand, which signals to the *Drosophila* EGF receptor (EGF-R) on adjacent somatic follicle cells (González-Reyes et al., 1995; and Roth et al., 1995). The follicle cells respond by inducing a reorientation of the oocyte microtubule cytoskeleton that promotes mRNA transport along the anterior–posterior axis of the oocyte (González-Reyes et al., 1995; and Theurkauf et al., 1992). Consequently, *grk* mRNA is transported to the anterior margin of the oocyte and then to the future anterodorsal corner (MacDougall et al., 2003; and Neuman-Silberberg and Schüpbach, 1993). Synthesis of Grk at this site results in the localized activation of EGF-R in the overlying follicle cells and the specification of dorsal fates, thereby defining the dorsal–ventral axis of the egg and, ultimately, the embryo (Nilson and Schüpbach, 1999; and van Eeden and St Johnston, 1999). Concomitant with *grk* localization to the future dorsal–anterior region of the oocyte, *oskar* (*osk*) mRNA accumulates at the posterior pole. Osk protein synthesized from localized *osk* mRNA nucleates the assembly of the germ plasm, which determines germ cell fate in the embryo. In addition, Osk-dependent assembly of germ plasm is essential for the posterior localization and translation of *nanos* (*nos*) mRNA, which is in turn required for abdomen formation in the embryo (Gavis and Lehmann, 1994; and Wang et al., 1994).

Localization of *grk* and *osk* mRNAs is essential for their function, as mutations that abolish localization of either produce polarity defects. Furthermore, localization must be tightly coupled to translation, since precocious or ectopic translation of these mRNAs also produces deleterious defects in dorsal–ventral and anterior–posterior polarities. Genetic and biochemical studies have identified various proteins that participate in localization and translational regulation of *grk* and *osk* mRNAs. Among these, Squid (Sqd), Hrb27C/Hrp48 (referred to hereafter as Hrp48), and Ovarian tumor (Otu) are required both for anterodorsal localization and translational repression of *grk* mRNA. In mutants for these proteins, *grk* is mislocalized around the entire anterior cortex and this mislocalized *grk* is translated, producing dorsalized embryos (Goodrich et al., 2004; and Norvell et al., 1999). Hrp48 and Sqd are both members of the heterogeneous ribonucleoprotein (hnRNP) A/B family and both bind to the *grk* 3'untranslated region (3'UTR). Hrp48 interacts with Sqd and Otu, suggesting that these three proteins are components of a *grk* ribonucleoprotein (RNP) complex (Goodrich et al., 2004; and Norvell et al., 1999). Intriguingly, Sqd, Hrp48, and Otu also participate in *osk* mRNA localization and/or translation (Huynh et al., 2004; Norvell et al., 2005; Tirronen et al., 1995; and Yano et al., 2004) and Sqd and Hrp48 interact with *osk* mRNA in vitro (Huynh et al., 2004; Norvell et al., 2005; and Yano et al., 2004).

Mutations in *half-pint (hfp)* also cause defects in both *grk* and *osk* localization (Van Buskirk and Schüpbach, 2002). *hfp* encodes the

* Corresponding author. Fax: +1 609 258 1343.
E-mail address: gavis@princeton.edu (E.R. Gavis).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.10.032

Drosophila homolog of the human RNA binding protein PUF60 and regulates alternative splicing of several ovarian transcripts including *otu*. Since the *grk* localization defect of *hfp* mutants can be rescued by expression of the Otu isoform (Otu-104) that is missing in *hfp* mutants, Hfp's primary contribution to *grk* regulation appears to be the generation of Otu-104 (Van Buskirk and Schüpbach, 2002). Mutation of *hfp*, as well as mutation of *sqd*, *hrp48*, and *otu* produces defects in nurse cell chromatin organization and, similarly to the *grk* localization defect, the chromatin defect of *hfp* mutants is rescued by expression of Otu-104 (Goodrich et al., 2004; and Van Buskirk and Schüpbach, 2002). Together, these results suggest that Sqd, Hrp48, and Otu act together to regulate multiple mRNAs involved in different developmental processes during oogenesis and that Hfp plays a role in supplying Otu to this complex.

We have previously identified and characterized an hnRNP F/H family member, Glorund (Glo), that is required for translational repression of unlocalized *nos* mRNA in late oocytes. In addition to defects in *nos* regulation, a small proportion of *glo* mutant embryos show *osk* mRNA localization defects (Kalifa et al., 2006). Here we show that ovaries derived from *glo* mutant germline clones exhibit defects in dorsal–ventral polarity of the oocyte as well as defects in nurse cell chromosome organization. To better understand these different roles for Glo in oogenesis, we searched for proteins that interact with Glo. We provide evidence that Glo participates in a complex with Hrp48 and Hfp that functions in both *grk* mRNA localization and nurse cell chromosome dispersion by regulating *otu*.

Materials and methods

Fly stocks

The following mutants and transgenic lines were used: glo^{162x} and *g-gloS* (Kalifa et al., 2006), *khc:lacZ* (Clark et al., 1994), and *potu-104* (Sass et al., 1995). glo^{162x} germline clones were induced by the dominant female sterile method (Chou et al., 1993) using the P{neoFRT}82B, P{ovoD1-18}3R chromosome. The yw^{67c23} and Oregon R strains (Lindsley and Zimm, 1992) were used as wild-type controls.

In situ hybridization and immunostaining

In situ hybridization with digoxigenin-labeled RNA probes for *grk* and *osk* was performed as described previously (Gavis and Lehmann, 1992). Anti-Grk and anti-Br-C immunostaining was performed according to Goodrich et al. (2004) using 1:10 monoclonal anti-Grk (1D12; Queenan et al., 1999) or 1:100 monoclonal anti-Br core (25E9.D7; Yakoby et al., 2008), followed by 1:1000 AlexaFluor 568 anti-mouse (Invitrogen/Molecular Probes). Anti-β-galactosidase immunostaining was performed according to Kalifa et al. (2006) using 1:1000 rabbit anti-βgal (ICN Cappel) and 1:1000 AlexaFluor 568 anti-rabbit antibodies. DNA and actin were visualized with DAPI and Oregon Green 488 phalloidin (Invitrogen/Molecular Probes), respectively.

Immunoprecipitation and immunoblot analysis

Ovaries of well fed females were dissected in PBS, washed twice with IP buffer [25 mM Hepes (Na^+) pH 7.4, 150 mM NaCl, 2. 5 mM $MgCl_2$, 0. 5 mM EDTA, 0.01% Triton X-100, 1× complete protease inhibitor cocktail (Roche), and 10 µg/ml pepstatin], homogenized, and cleared by centrifugation at 13,000 rpm for 10 min at 4 °C. The pellet was rehomogenized and centrifuged, and the cleared extracts were pooled. Aliquots of the extract were supplemented with either RNase [100 µg/ml RNase A and 100 units/ml RNase One (Promega)] or 1 unit/µl RNasin (Promega) and incubated with Dynabeads Protein G (Invitrogen) for 1 hr at 4 °C. The pre-absorbed extract was then incubated overnight at 4 °C with Dynabeads Protein G (Invitrogen) coated with one of the following antibodies: anti-Glo (monoclonals 5B7, 1H2 or polyclonal mouse anti-Glo; Kalifa et al., 2006), rabbit anti-Hrp48 (Siebel et al., 1994), rabbit anti-βgal (Invitrogen/Molecular Probes), monoclonal anti-Sqd (8F3; Goodrich et al., 2004), monoclonal anti-Hfp (6G10; Van Buskirk and Schüpbach, 2002), monoclonal anti-Sxl (m104; Penn and Schedl, 2007), rabbit anti-GFP (Abcam). Beads were washed five times with IP buffer and bound protein was eluted by boiling in SDS-PAGE sample buffer. For co-immunoprecipitation of Sxl and Snf, nuclear extract (Deshpande et al., 1996) kindly provided by P. Graham was treated with RNase or RNasin as above and complexes were recovered using Protein A/G Plus-Agarose (Santa Cruz Biotechnology) pre-coated with anti-Sxl (m104) antibody.

Eluted proteins were resolved by SDS-PAGE and transferred to nitrocellulose membrane. Immunoblotting was carried out in 10 mM Tris–HCl pH 7.5/150 mM NaCl/5% nonfat dry milk with the following primary antibodies: 1:400 anti-Glo (5B7); 1:100 anti-Sqd (8F3); 1:20 anti-Hfp (6G10); 1:5000 anti-Hrp48; 1:2000 rabbit anti-Osk (Vanzo and Ephrussi, 2002); 1:10,000 monoclonal anti-Snf (4G3; gift of P. Schedl). Proteins were visualized by ECL (Roche).

GST pull-down assay

Recombinant full length GST-Hrp48 and GST-Hfp were expressed in *E. coli* from plasmids generously provided by T. Schüpbach and bound to glutathione-agarose resin (Sigma) according the manufacturer. Resin was equilibrated in IP buffer and incubated with purified MBP-Glo (Kalifa et al., 2006) for 1 h at 4 °C. After extensive washing with IP buffer, bound proteins were eluted with glutathione (Sigma) as specified by the manufacturer, resolved by SDS-PAGE, and immunoblotted with either anti-Glo or anti-GST (Santa Cruz Biotechnology) antibodies.

Results

Multiple functions for glo during oogenesis

Since animals that are homozygous mutant for a *glo* null allele (glo^{162x}) do not survive to adulthood (Kalifa et al., 2006), we investigated requirements for *glo* during oogenesis by generating homozygous glo^{162x} germline clones in females heterozygous for glo^{162x} using the dominant female-sterile method (Chou et al., 1993). Approximately 30% of eggs laid by females with glo^{162x} germline clones have abnormal dorsal appendages, ranging from short, wide appendages to fused appendages that extend laterally around the anterior of the egg (Figs. 1B–D). Analysis of ovaries dissected from these females (referred to as glo^{162x} ovaries) showed a higher frequency (55%) of dorsal appendage defects among late oocytes, suggesting that some of these oocytes never mature as eggs. In the wild-type ovary, the 15 germline derived nurse cells supply maternal mRNAs and other metabolites to the oocyte. As they complete their role, the nurse cells initiate apoptosis and rapidly transfer or “dump” their contents into the oocyte. In glo^{162x} ovaries, we observed egg chambers that failed to undergo nurse cell dumping. Finally, DAPI staining of nuclei revealed a developmental defect in chromatin organization in nurse cells from glo^{162x} ovaries. In wild-type ovaries, nurse cell chromosomes are initially polytene but disperse toward the middle stages of oogenesis. In nurse cells from glo^{162x} ovaries, chromosomes fail to disperse during mid-oogenesis and maintain a polytene morphology (Figs. 1G, H; also see Figs. 5A, B). All of the observed phenotypes are rescued by a single copy of a genomic *glo* transgene (Kalifa et al., 2006), confirming that they result from loss of *glo* function.

Mislocalization and ectopic translation of grk mRNA in glo mutant ovaries

The dorsal appendage defects exhibited by *glo* mutant eggs suggest a defect in specification of dorsal follicle cell fates and, consequently, that *glo* may be required for proper regulation of *grk*

Download English Version:

https://daneshyari.com/en/article/10933532

Download Persian Version:

https://daneshyari.com/article/10933532

Daneshyari.com