Published in final edited form as:

Gene. 2013 September 10; 526(2): 187–194. doi:10.1016/j.gene.2013.05.018.

Poly(ADP-ribose) Glycohydrolase and Poly(ADP-ribose)-interacting Protein Hrp38 Regulate Pattern Formation during *Drosophila* Eye Development

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Abstract

Drosophila Hrp38, a homolog of human hnRNP A1, has been shown to regulate splicing, but its function can be modified by poly(ADP-ribosylation). Notwithstanding such findings, our understanding of the roles of poly(ADP-ribosylated) Hrp38 on development is limited. Here, we have demonstrated that Hrp38 is essential for fly eye development based on a rough-eye phenotype with disorganized ommatidia observed in adult escapers of the *hrp38* mutant. We also observed that Poly(ADP-ribose) Glycohydrolase (*Parg*) loss-of-function, which caused increased Hrp38 poly(ADP-ribosylation), also resulted in the rough-eye phenotype with disrupted ommatidial lattice and reduced number of photoreceptor cells. In addition, ectopic expression of DE-cadherin, which is required for retinal morphogenesis, fully rescued the rough-eye phenotype of the *hrp38* mutant. Similarly, *Parg* mutant eye clones had decreased expression level of DE-cadherin with orientation defects, which is reminiscent of DE-cadherin mutant eye phenotype. Therefore, our results suggest that Hrp38 poly(ADP-ribosylation) controls eye pattern formation via regulation of DE-cadherin expression, a finding which has implications for understanding the pathogenic mechanisms of Hrp38-related Fragile X syndrome and PARP1-related retinal degeneration diseases.

Keywords

Drosophila; Hrp38; poly(ADP-ribosylation); pattern formation; eye development

1. Introduction

Drosophila Hrb98DE/Hrp38 gene, a member of the hnRNP family of proteins, has two RNA-binding motifs and one glycine-rich region, having highest identity with human hnRNP A1 (Haynes *et al.*, 1990). The *hrp38* gene has been shown to regulate alternative splicing, both *in vivo* and *in vitro*, using the S2 cell line (Shen *et al.*, 1995; Blanchette *et al.*, 2009; Borah *et al.*, 2009). Alternative splicing is used extensively to produce the different mRNA isoforms of a gene to increase the complexity of the transcriptome in higher eukaryotic genomes (Nilsen and Graveley, 2010). It is generally believed that two groups of

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RNA-binding proteins, hnRNPs and serine-arginine-rich splicing factor (SR protein), regulate alternative splicing by binding with exonic and intronic splicing silencers (ESEs and ISEs) and enhancers (ESSs and ISSs), respectively (Matlin *et al.*, 2005). However, emerging evidence suggests that hnRNPs can also bind with ESSs and ISSs to enhance splicing (Kiesler *et al.*, 2005; Blanchette *et al.*, 2009; Borah *et al.*, 2009). In addition, the genome-wide analysis of the mRNA bound by hnRNP and SR proteins revealed that *Drosophila* hnRNPs regulate quite different sets of genes compared with SR proteins in *Drosophila* cell lines, challenging the current model that hnRNP and SR proteins have an antagonistic effect on splicing regulation (Blanchette *et al.*, 2005).

As an alternative to changing the concentration of splicing factors in different tissues or developmental stages (Matlin *et al.*, 2005), regulating the splicing activities of hnRNPs and SR proteins may be accomplished by post-translational modification via poly(ADP-ribosylation) (Gagne *et al.*, 2003; Malanga *et al.*, 2008; Ji and Tulin, 2009; Ji and Tulin, 2010). Specifically, Hrp38 is modified at the post-translational level by poly(ADP-ribosylation) through the activity of Poly(ADP-ribose) Polymerase 1 (PARP1) in *Drosophila* (Ji and Tulin, 2009). In addition, protein poly(ADP-ribosylation) can be reversed by Poly(ADP-ribose) Glycohydrolase (PARG), which degrades poly(ADP-ribose) polymer (Hanai *et al.*, 2004; Tulin *et al.*, 2006). Consequently, Hrp38 is highly poly(ADP-ribosylated) in the *Parg* mutant (Ji and Tulin, 2009). Furthermore, it appears that poly(ADP-ribosylation) inhibits the RNA-binding ability of hnRNPs and can modulate the alternative splicing pathways (Ji and Tulin, 2009). Our recent study suggested that poly(ADP-ribosylation) regulates Hrp38-dependent translation of DE-cadherin by the inhibition of Hrp38 binding to 5' UTR of DE-cadherin mRNA (Ji and Tulin, 2012). Based on this evidence, it could be reasonably concluded that post-translational modification of hnRNPs by poly(ADP-ribose) is a novel mechanism that regulates such hnRNP-dependent pathways as splicing and translation.

Therefore, we have been further assessing whether hnRNP poly(ADP-ribosylation) regulates gene expression during development. In our previous study, we have demonstrated that Hrp38 poly(ADP-ribosylation) controls germline stem cell (GSC) self-renewal and oocyte localization during *Drosophila* oogenesis by regulating DE-cadherin translation (Ji and Tulin 2012). Importantly, we note that DE-cadherin-mediated adherens junctions are required for retinal morphogenesis by organizing photoreceptor cell patterns and regulating ommatidial rotation (Tepass and Harris, 2007). Accordingly, in the present study, we further found that both Hrp38 loss-of-function and its poly(ADP-ribosylation) cause a rough-eye phenotype displaying disorganized ommatidia. As expected, the rough-eye phenotype in the *hrp38* mutant was rescued by overexpression of DE-cadherin in the eye, while the *Parg* mutant eye clones showed decreased expression of DE-cadherin. These results suggest that Hrp38 poly(ADP-ribosylation) plays a role during eye pattern formation by regulating DE-cadherin expression.

2. Material and Methods

2.1 *Drosophila* strains

Flies were cultured on standard cornmeal-molasses-agar media at 22°C. *Hrb98DE/hrp38* GFP trap line (ZCL588) (Morin *et al.*, 2001; Ji and Tulin, 2009), *P{w[+mC]=GAL4-ninaE.GMR}12* (stock number: 1104) and *P{w[+mC]=longGMR-GAL4}2* (stock number: 8605) were from the Bloomington *Drosophila* Stock Center. A P-element insertion of the *hrp38* gene (*w**, *P{XP}^{d05172}/TM6B, Tb¹*), a *hrp38* region deficiency line (*w*¹¹¹⁸; *Df(3R)Exel6209, P{XP-U}Exel6209/TM6B, Tb¹*) and the UAS-Hrp38:RFP transgenic line were previously described (Ji and Tulin, 2012). The UAS-DE-cadherin:GFP (UAS-DEFL) transgenic line is a gift from the laboratory of Dr. Yamashita (Inaba *et al.*, 2010). *Tubulin-*

DE-cadherin transgenic line is a gift from Dr. Mark Van Doren (Mathews *et al.*, 2006). Two *Hrp38* RNAi lines ($w^{1118}; P\{GD14939\}v29523$ and $w^{1118}; P\{GD14939\}v29524/CyO$) were from the Vienna *Drosophila* RNAi Centre.

2.2 FRT/FLP Clonal Analysis

The *Parg* female heterozygotes ($Parg^{27.1}/FM7a, w^a$) (Hanai *et al.*, 2004; Tulin *et al.*, 2006) were crossed with $w^{1118}, sn^3, P\{neoFRT\}19A/Y$ (Xu and Rubin, 1993) to generate the FRT-bearing *Parg* mutations ($Parg^{27.1}, P\{neoFRT\}19A/FM7a, w^a$) by genetic recombination. To induce the adult *Parg* mutant and wild-type eye clones, $Parg^{27.1}, P\{neoFRT\}19A/FM7a, w^a$ or $w^{1118}, sn^3, P\{neoFRT\}19A/Y$ was crossed with $P\{GMR-hid\}SS1, y^l w^*$ $P\{neoFRT\}19A; P\{GAL4-ey.H\}SS5, P\{UAS-FLP1.D\}JD2$ using the ey-Gal4/UAS-FLP/GMR-hid method (Stowers and Schwarz, 1999). To induce the *Parg* mutant eye disc clones in the third-instar larvae stage, $Parg^{27.1}, P\{FRT(w^{hs})101/FM7a, w^a$ (Ji and Tulin, 2012) was crossed with Ubi-GFP, $P\{FRT(w^{hs})101/Y; P\{GAL4-ey.H\}SS5, P\{UAS-FLP1.D\}JD2$ to select the GFP mosaic eye imaginal disc.

2.3 Western Blotting

Total protein (50 ug) from the wild-type fly, mutants (non-GFP homozygotes) at the wandering third-instar larvae, and the head and body of the *Parg* mutant mosaic adult was isolated and measured as described previously (Ji and Tulin, 2009). The proteins were then resolved in SDS-PAGE and transferred to nitrocellulose membrane (0.45 μ m, Bio-Rad). The blot was incubated with rabbit anti-pADPr antibody (Calbiochem) at 1:1000 dilution. The signals were detected with horseradish peroxidase-conjugated secondary antiserum and ECLTM reagents (GE Healthcare). The blots were stripped and detected with mouse anti α -tubulin antibody (DM1A, Sigma) at 1:1000 dilution.

2.4 Immunohistochemistry

The eye imaginal discs of the third-instar larvae were dissected in Grace's insect medium and fixed in 4% paraformaldehyde + 0.1% Triton X-100 in PBS for 20 min. Afterwards, the discs were incubated with mouse anti-Elav (1:10; DSHB) and Alexa Fluor 488 goat anti-mouse antibody (1:400; Invitrogen), respectively. The nuclear DNA was stained with DRAQ5 dye (Biostatus). The pupal retinae at 44 hours after pupation were dissected and stained with Alexa Fluor 633 phalloidin (1:40; Invitrogen) for 30 minutes. The adult eyes were dissected and stained with anti-rhodopsin (4C5) antibody (1:10, DSHB) based on the published protocol (Williamson and Hiesinger, 2010). All the images were visualized using the Leica TCS-NT confocal microscope.

2.5 Electron Microscopy

For scanning EM, the dissected heads were fixed as for TEM, postfixed in 1% OsO₄ for 3 hours, dehydrated in ethanol and critical point dried as described (Anderson 1951). The samples were viewed on an Autoscan scanning electron microscope (ETEC, Hayward, CA). For ultrastructural analysis by transmission EM, the heads were dissected, fixed with 2% formaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) in 0.1% Triton X-100 overnight, postfixed for 1 hour with osmium tetroxide, dehydrated in ethanol and propyleneoxide, and embedded in EMbed-812 (EMS, Fort Washington, PA) in flat molds. After polymerization for 60 hours at 65°C, 70 nm sections were cut on a Leica UC6 microtome (Leica, Austria), placed on formvar/carbon-coated grids, and stained with 2% uranyl acetate/lead citrate. Sections were viewed on a Tecnai 12 transmission electron microscope (FEI, Hillsboro, OR).

3. Results

3.1 Loss-of-function of the *hrp38* gene causes the rough-eye phenotypes

Our previous study suggested that the *hrp38* gene is important for fly development because a full 75% of the *hrp38* hemizygotes (*hrp38^{d05172}/Df*) died before the pupa stage (Ji and Tulin 2012). Interestingly, we observed that the adult escapers of the *hrp38* hemizygotes showed rough-eye phenotype with disorganized ommatidia and bristles (Figure 1D and 1D'), while the wild-type fly had no defects (Figure 1A). Occasionally, loss of one photoreceptor cell was observed in the ommatidia (Figure 1E) compared to the wild type (Figure 1B). In addition, Hrp38 expression was observed in the photoreceptor cells in the ommatidia in a Hrp38:GFP protein trap line (Figure 1C). We further examined the larval eye disc stained with anti-Elav (a neuron marker) antibody in the wild-type and *hrp38* mutant. It appears that both *hrp38* mutant cells (Figure 1H) and wild type (Figure 1F) had normal specification of the photoreceptor fate. However, the pattern of photoreceptor cells in the *hrp38* mutant cells was irregular (Figure 1H') compared to the wild-type cells (Figure 1F'). Moreover, the *hrp38* mutant pupal eye showed a disrupted ommatidial lattice (Figure 1I) compared with the wild-type eye (Figure 1G). Based on this evidence, we concluded that the rough-eye phenotype caused by Hrp38 loss-of-function results from disrupted pattern formation shown as early as at the larval stage.

3.2 Expression of Hrp38:RFP transgene in the eye rescued the rough-eye phenotype of the *Hrp38* mutant

In addition, we used RNAi to knock down *hrp38* expression in the eye using two eye-specific GAL4 drivers (*ninaE.GMR-Gal4* and *longGMR-Gal4*). While *ninaE.GMR-Gal4* heterozygotes, as the control, had the wild-type eye phenotype (Figure 2A and 2A'), *hrp38* RNAi induced by *ninaE.GMR-Gal4* driver also caused the rough-eye phenotype with disrupted ommatidia and bristle organizations (Figure 2B and 2B'). However, *hrp38* knockdown by *longGMR-Gal4* driver did not show any mutated phenotype. This observation may reflect the different Gal4 expression level of two GMR-Gal4 drivers because the promoter of *ninaE-GMR* transgene, which is composed of a pentamerized 29bp glass binding site named as "short GMR", has a stronger ability of inducing GAL expression than the *longGMR-Gal4* driver whose promoter has a pentamerized 38 bp glass binding site termed as "long GMR" (Freeman, 1996; Wernet, *et al.* 2003). Indeed, the expression of a *UAS-hrp38-RFP* transgene induced by the *longGMR-Gal4* driver rescued the observed rough-eye phenotypes of the hemizygotes (*hrp38^{d05172}/Df*) (Figure 2C and 2C'), which further demonstrates that loss-of-function of the *hrp38* gene in the eye results in the observed phenotypes in the *hrp38* hemizygotes. In contrast, overexpression of the *UAS-hrp38-RFP* transgene induced by the *ninaE.GMR-Gal4* (short-GMR) driver could not rescue the rough-eye phenotype of the *hrp38* mutant (Figure 2D and 2D'), suggesting that the expression level of the *hrp38* gene in the eye is critical for normal eye development. Therefore, Hrp38 protein regulates eye pattern formation.

3.3 Hrp38 Poly(ADP-ribosyl)ation causes rough-eye phenotypes

We have shown that Hrp38 is highly poly(ADP-ribosyl)ated in the *Parg* mutant which affects hnRNP-dependent pathways, such as splicing and translation, by changing the RNA-binding ability of hnRNP proteins (Ji and Tulin, 2009; Ji and Tulin, 2012). In order to determine if Hrp38 poly(ADP-ribosyl)ation also plays a role during eye development, we examined eye development in the *Parg* mutant. Since *Parg* mutants are completely lethal and died at the pharate stages (Hanai *et al.* 2004), we used the *ey-Gal4/UAS-FLP/GMR-hid* method (Stowers and Schwarz, 1999) to generate the *Parg* mutant eye. Western blotting also showed that the *Parg* mutant eye does indeed accumulate a large amount of poly(ADP-ribose) (pADPr) (Figure 3A). The homozygous FRT19A eyes generated by Flippase

induction showed normal eye structure (Figure 3B, 3B' and 3C). However, knocking out the *Parg* gene in the eye resulted in the rough-eye phenotype with the disrupted pattern of ommatidia and bristles (Figure 3D and D'). Furthermore, we found that around half of the ommatidia in the *Parg* mutant eye were missing two or three photoreceptor cells (Figure 3E), which suggests that the *Parg* gene is required for the growth and differentiation of photoreceptor cells. Taken together, these results suggest that *Parg* genes are also required for eye development. The phenotype similarity between the *hrp38* and *Parg* mutations implies that the *Parg* gene may regulate the same pathway as Hrp38 through Hrp38 poly(ADP-ribosyl)ation during eye development.

3.4 Hrp38 Poly(ADP-ribosyl)ation regulates DE-cadherin expression in the eye imaginal disc

Our previous study has demonstrated that both *hrp38* and *Parg* mutants had a lower expression level of DE-cadherin compared with the wild-type fly (Ji and Tulin, 2012), suggesting that both *hrp38* and *Parg* genes are required for normal DE-cadherin expression during fly development. Since DE-cadherin-mediated adherens junctions are very important for retinal morphogenesis (Grzeschik and Knust, 2005; Mirkovic and Mlodzik, 2006; Tepass and Harris, 2007), we also examined DE-cadherin expression in the *Parg* mutant eye imaginal disc clones generated by the eye-FLP in the 3rd instar larvae stage. As shown in Figure 4A, 4A' and 4A'', DE-cadherin was enriched between the members of the photoreceptor precursor cells behind the morphogenic furrow in the wild-type eye imaginal disc, as reported before (Grzeschik and Knust, 2005). However, it appeared that DE-cadherin expression was lost in the *Parg* mutant photoreceptor precursor cell clones (Figure 4B, 4B', 4B''). These *Parg* eye imaginal disc clones also showed rotation defects (Figure 4B'''), which is reminiscent of the phenotype shown in DE-cadherin mutant eye clones (Mirkovic and Mlodzik, 2006).

Because Hrp38 has been shown to be required for DE-cadherin translation by binding to 5' UTR of DE-cadherin (Ji and Tulin, 2012), decreased DE-cadherin expression may also be responsible for the rough-eye phenotype in the *hrp38* mutant. Indeed, expression of a UAS-DE-cadherin:GFP transgene (Oda *et al.*, 1994) induced by GMR-Gal4 driver fully rescued the rough-eye phenotypes in the *hrp38* mutants in both the third-instar larvae stage (Figure 4C) and adult stage (Figure 4D and 4E). However, even expression of DE-cadherin transgene with ubiquitous *tubulin* promoter (*Tub-DE-cad*) (Mathews *et al.* 2006) could not restore normal eye pattern in the adult *Parg* mutant eye (Figure 4F and 4G), suggesting that decreased DE-cadherin expression is not the sole reason for eye development defects in the *Parg* mutant.

4. Discussion

4. 1 The overlapping functions between Hrp38 and Hrp36

It has been reported that *hrp36* deficiency also results in the rough-eye phenotype (Sengupta and Lakhotia, 2006). Therefore, it appears that both *hrp36* and *hrp38* genes are required during eye development. In addition, the genome-wide analysis of the mRNA bound by Hrp36 and Hrp38 proteins revealed that *hrp36* and *hrp38* regulate overlapping sets of genes in *Drosophila* cell lines (Blanchette *et al.*, 2005; Blanchette *et al.*, 2009). Hrp38 and Hrp36 are highly conserved with 76% identity in the RNA-binding motifs and 67% identity in the glycine-rich region (Haynes *et al.*, 1991). A recent study also suggested that Hrp36 is required for normal development, especially under stress conditions (Singh and Lakhotia, 2012). Therefore, this accumulated evidence suggests that the *hrp38* gene has overlapping, but not redundant, functions with the *hrp36* gene, despite high similarity between the two genes.

4.2 Decreased DE-cadherin in a *Drosophila* model of Fragile X syndrome may provide clues for understanding its pathogenesis in human

Our data showed that decreased DE-cadherin expression is an underlying cause of the rough-eye phenotype in the *hrp38* mutant. Interestingly, the rough-eye phenotype caused by *hrp38* loss-of-function was also shown in a *Drosophila* model of Fragile X syndrome as a result of the titration of RNA-binding proteins, including Hrp38, by binding to the CGG repeats (Sofola *et al.*, 2007). It was also shown that Hrp38 overexpression can suppress the rCGG-mediated rough-eye phenotype (Sofola *et al.*, 2007). If decreased DE-cadherin does result in the rCGG-mediated rough-eye phenotype in a *Drosophila* model of Fragile X syndrome, it would provide additional clues for understanding the pathogenesis of Fragile X syndrome.

4.3 PARG activity regulates cellular pADPr level

PARG is responsible for the cleavage of pADPr into the single ADP-ribose unit by hydrolyzing the ribose-ribose bonds within the polymer chain (Miwa and Sugimura 1971; Lin *et al.*, 1997). Therefore, *Parg* knockout in model animals, such as *Drosophila* and mice, resulted in the accumulation of pADPr in the whole animals (Koh *et al.*, 2004; Hanai *et al.*, 2004; Tulin *et al.*, 2006), as well as in the specific tissues shown in this study (Fig. 3A), suggesting that PARG activity controls the level of cellular pADPr. However, a recent study showed that knockdown of PARG expression by siRNA reduced PARP1 expression at the transcriptional level, thereby decreasing pADPr level in treated HeLa S3 cells (Uchiumi *et al.*, 2013). The observed difference in pADPr level between the whole animals and the cultured cells most likely results from the fact that *Drosophila* PARP1 gene has strong maternal expression (Hanai *et al.*, 1998; Tulin *et al.*, 2002). Thus, even if PARG knockout had such effect on PARP1 expression in the fly, maternal PARP1 activity could continue to generate pADPr in the PARG mutants. Furthermore, PARG loss-of-function on PARP1 activity could also result in the failure of PARP1 automodification. This could also inhibit PARP1 activity in the PARG knockout animals because PARP automodification disables its enzymatic ability to synthesize new pADPr (Sato and Lindahl, 1992). However, two recent studies on the enzymes which cleave the terminal ADP-ribose unit from auto(ADP-ribosyl)ated PARP1 (Sharifi *et al.*, 2013; Jankevicius *et al.*, 2013) have shed light on this long-standing puzzle. It is well known that PARG cannot hydrolyze the bond between terminal ADP-ribose and glutamate residues of automodified PARP1 (Slade *et al.*, 2011). It has been reported that two macrodomain proteins, including human MacroD2 and TARG1/C6orf130, have the ability to remove the terminal modification of PARP1 (Sharifi *et al.*, 2013; Jankevicius *et al.*, 2013). It is therefore possible that these two enzymes could directly cleave the whole pADPr chain from the terminal bonds of weakly automodified PARP, thus partially restoring PARP1 activity in the *Parg* mutant background. Consequently, the residual activities of MacroD2 and TARG1 may contribute to the accumulation of pADPr in the *Parg* knockout animals.

4.4 The new insight into the pathogenesis of PARP1-related retinal degeneration diseases

Previous studies have found that excessive pADPr from PARP1 activation contributes to degeneration of photoreceptor cells in the retinal degeneration 1 (*rd1*) mouse model of retinitis pigmentosa (RP) (Paquet-Durand *et al.*, 2007). Our present study further confirms this phenomenon by demonstrating that accumulation of pADPr exclusively in the fly eye by conditional *Parg* knockout also causes eye developmental defects with the rough-eye phenotype and reduced photoreceptor cell numbers. Our finding also implies that decreased *Parg* expression or activation may also play a role in the *rd1* mouse model or human RP disease (Sahaboglu *et al.*, 2010; McLaughlin *et al.*, 1995). In addition, since most human RP shows progressive severity from night blindness in the early stage to total vision loss with aging (Hartong *et al.*, 2006), it is likely that cell death is not the only causal factor

underlying retina developmental defects, even in *RP* patients. Therefore, based on the findings of the present study, other pathways involving PARP1 and PARG genes should be investigated in the future. We found that decreased expression of DE-cadherin in the *Parg* mutant is associated with the rough-eye phenotypes, and this discovery gives additional clues for understanding *RP*. However, because overexpression of DE-cadherin cannot rescue the developmental defects of *Parg* mutant eye, it is very likely that poly(ADP-ribose)ylation affects other Hrp38-dependent pathways during eye pattern formation (Figure 5).

4.5 Conclusion

In summary, this study has further demonstrated that post-translational modification of hnRNPs, such as poly(ADP-ribose)ylation, is an important mechanism in the regulation of gene expression during development, such as eye pattern formation.

Acknowledgments

We thank Drs. Van Doren and Yamashita for providing materials. Drs. M. Robinson, A. O'Reilly, D. Martin and A. Liubimova provided comments on the manuscript. The research was supported by grants from the National Institutes of Health (R01 GM077452) to A.V.T.

ABBREVIATIONS for GENE38629

PARG	Poly(ADP-ribose) Glycohydrolase
PARP1	Poly(ADP-ribose) Polymerase 1
pADPr	poly(ADP-ribose)
hnRNP/Hrp	Heterogeneous nuclear ribonucleoproteins
SR protein	serine-arginine-rich protein
ESSs	exonic splicing silencers
ISSs	intronic splicing silencers
ESEs	exonic splicing enhancers
ISEs	intronic splicing enhancers
GSC	germline stem cell
5'UTR	5' untranslated region
DE-cadherin	Drosophila Epithelial cadherin
Df	deficiency
UAS	upstream activation sequence
rd1	retinal degeneration 1
RP	retinitis pigmentosa

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Highlights

- *Hrp38* knockout caused a rough-eye phenotype with disorganized ommatidia.
- *Parg* knockout fly also exhibited a rough-eye phenotype with reduced photoreceptor cells.
- Ectopic expression of DE-cadherin rescued the rough-eye phenotype of the *hrp38* mutant.
- *Parg* mutant eye clones showed decreased DE-cadherin expression with orientation defects.
- Hrp38 poly(ADP-ribosyl)ation controls eye pattern formation via regulation of DE-cadherin expression.

Figure 1. The *hrp38* gene is required for normal pattern formation of the fly eye

(A) Scanning electron microscopy (SEM) image of wild-type eye. Insert shows magnified view of the regular organization of ommatidia and bristles. (B) Tangential sections of wild-type adult retinae showing seven photoreceptor cells in ommatidia. (C) Confocal section of the pupal eye (44h AP) of the *Hrp38:GFP* strain. F-actin is enriched in rhabdomere. (D,D') SEM image of the *hrp38* hemizygous eye (*hrp38^{d05172}/Df*) showing the disrupted pattern of ommatidia and bristles. (D') shows magnified view of boxed area in (D). (E) Tangential sections of the *hrp38* hemizygous retinae showing that one photoreceptor cell is missing in an ommatidium. (F) and (F') The eye imaginal disc of the wild-type fly stained with Elav showing the regular array of the specified photoreceptor cells. The nuclear DNA was stained with Draq5 (red) in (F). (F') shows magnified view of boxed area in (F). (G) Confocal section of the pupal eye (44h AP) of the wild-type strain stained for F-actin. (H,H') The eye imaginal disc of the *hrp38* hemizygous fly stained with Elav showing the organization of the specified photoreceptor cells. The nuclear DNA in (H) was stained with Draq5 (blue). (H') shows a disrupted pattern of the photoreceptor cells magnified in the boxed area in (H). (I) Confocal section of the pupal eye (44h AP) of the *hrp38* hemizygous fly stained for F-actin, showing the disrupted ommatidial lattice. Scale bars: 100 μ m (A, D); 10 μ m (A insert, C, D', F, F', G, H, H' and I); 2 μ m (B, E).

Figure 2. The rescue of the rough-eye phenotype of the *Hrp38* mutant by the expression of *Hrp38:RFP* transgene

(A,A') SEM eye image of RNAi control line (*ninaE.GMR-Gal4/+*), showing the regular array of ommatidia and bristles. (A') shows magnified view of boxed area in (A). (B,B') SEM image of the *Hrp38* knockdown eye by RNAi (*ninaE.GMR-Gal4/UAS-Hrp38^{RNAi}*), showing the disrupted pattern of ommatidia and bristles. (C,C') Confocal image of the adult eye structure (C) and the third-instar eye imaginal disc (C') of the *hrp38* mutant expressing *Hrp38:RFP* transgene driven by longGMR:Gal4 line (*UAS>Hrp38:RFP/Long-GMR:Gal4;hrp38^{d05172}/Df*). (D,D') Confocal image of the adult eye structure (D) and the third-instar eye imaginal disc (D') of the *hrp38* mutant expressing *Hrp38:RFP* transgene driven by the *ninaE.GMR:Gal4* line (*UAS>Hrp38:RFP/ninaE-GMR:Gal4;hrp38^{d05172}/Df*). In (C',D'), the photoreceptor cells are labeled with anti-ELAV antibody (green). Scale bars: 100 μ m (A, B, C, D); 10 μ m (A', B', C', D').

Figure 3. The *Parg* gene is required for normal pattern formation of the fly eye

(A) Western blotting analysis of pADPr in the wild-type and *Parg* mutant eye. Accumulation of pADPr was shown in the adult head, including the *Parg* mutant eye (*Parg*^{-/-} head), but not in the separated body (*Parg*^{+/+} body) dissected from the adult fly (*Parg*^{27.1}, *FRT19A/FRT19A*, *GMR-hid*; *ey-Gal4*, *UAS-FLP*). *Parg*^{-/-} and *Parg*^{+/+} larvae were used as the positive and negative controls, respectively. (B, B') SEM image of the control eye clones (*FRT19A/FRT19A*, *GMR-hid*; *ey-Gal4*, *UAS-FLP*), showing the regular array of ommatidia and bristles. (B') shows magnified view of boxed area in (B). (C) Tangential sections of the adult retinae of the control fly (*FRT19A/FRT19A*, *GMR-hid*; *ey-Gal4*, *UAS-FLP*), showing seven photoreceptor cells in an ommatidium. (D, D') SEM image of the *Parg*^{-/-} eye clones (*Parg*^{27.1}, *FRT19A/FRT19A*, *GMR-hid*; *ey-Gal4*, *UAS-FLP*), showing the disrupted organization of ommatidia and bristles. (D') shows magnified view of boxed area in (D). (E) Tangential sections of the adult retinae of the *Parg*^{-/-} eye clones, showing missing photoreceptor cells in an ommatidium. Scale bars; 100 μ m (B, D); 10 μ m (B', D'); 2 μ m (C, E).

Figure 4. Hrp38 poly(ADP-ribosyl)ation controls DE-cadherin expression in the eye
 (A,A',A'') DE-cadherin expression pattern in the third-instar eye discs of the wild-type fly (y,w). (B,B',B'',B''') Decreased DE-cadherin expression in the *Parg* mutant clones in the third-instar eye imaginal discs [*Parg*^{27.1}, *P{FRT(w^{hs})101/Ubi-GFP,P{FRT(w^{hs})101;P{GAL4-ey.H}SS5, P{UAS-FLP1.D}JD2/+}*]. The *Parg* mutant clones are GFP-negative and circled in (B,B'',B'''). The black lines indicate the orientation of the wild-type ommatidia in (B'''). The dashed white lines indicate the orientation of the mutant ommatidia in (B'''). The photoreceptor precursor cells were labeled with anti-ELAV antibody in (A) and (B''). (C,D,E) Rescue of the rough-eye phenotype by expression of DE-cadherin:GFP transgene in the *Hrp38* mutant background (*UAS:DEF/longGMR:Gal4; hrp38^{do5172}/Df*). (C) The regular array of the photoreceptor cells labeled with anti-ELAV antibody with DE-cadherin:GFP expression induced by longGMR:Gal4 driver in the third-instar eye disc. (D) The regular ommatidia structure in the adult eye of the rescued fly visualized by confocal imaging. (E) Confocal image of an ommatidium from (D) with the normal seven photoreceptor cells. (F,G) Failed rescue of the *Parg* mutant phenotype with ubiquitous expression of DE-cadherin (*Parg*^{27.1}, *FRT19A/FRT19A,GMR-hid; Tub-DE-cadherin/eye-Gal4,UAS-FLP*). (F) The irregular ommatidia structure in the *Parg* mutant eye. (G) Reduced photoreceptor cell numbers in the *Parg* mutant eye. Adult photoreceptor cells in (D,E,F,G) were labeled with anti-Rhodopsin antibody and F-actin. Scale bars: 10 μ m (A, A', A'', B, B', B'', B''', C); 100 μ m (D, F); 5 μ m (E, G).

Figure 5. Model explaining the regulatory role of hnRNP poly(ADP-ribosylation) in eye pattern development

(A) In a temporal- or spatial-specific way, protein poly(ADP-ribosylation) is reversed by PARG activity. Therefore, HRP proteins are not poly(ADP-ribosylated) and will bind to their target pre-mRNAs for RNA processing or translational control, which further regulates developmental patterns. (B) Once PARG activity is downregulated in different tissues or developmental stages, HRP proteins are poly(ADP-ribosylated), which inhibits HRP proteins from binding to pre-mRNA. Therefore, the pre-mRNA bound by HRP proteins are not processed or translated, and developmental patterns are changed. (C) Mutating HRP proteins blocks pre-mRNA processing or translation.