Overexpression of Poly(ADP-ribose) Polymerase Disrupts Organization of Cytoskeletal F-actin and Tissue Polarity in Drosophila*

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Poly(ADP-ribose) polymerase (PARP) may play important roles in nuclear events such as cell cycle, cell proliferation, and maintenance of chromosomal stability. However, the exact biological role played by PARP or how PARP is involved in these cellular functions is still unclear. To elucidate the biological functions of PARP in vivo, we have constructed transgenic flies that over-express Drosophila PARP in the developing eye primordia. These flies showed mild roughening of the normally smooth ommatidial lattice and tissue polarity disruption caused by improper rotation and chirality of the ommatidia. To clarify how this phenotypical change was induced, here we analyzed transgenic flies overexpressing PARP in the developing eye, embryo, and adult in detail. PARP mRNA level and the phenotype were enhanced in flies carrying more copies of the transgene. Developing eyes from third instar larvae were analyzed by using the neural cell marker to examine the involvement of PARP in cell fate. Morphological disorder of non-neuronal accessory cells was observed in PARP transgenic flies. Interestingly, overexpression of PARP did not interfere with the cell cycle or apoptosis, but it did disrupt the organization of cytoskeletal F-actin, resulting in aberrant cell and tissue morphology. Furthermore, heat-induced PARP expression disrupted organization of cytoskeletal F-actin in embryos and tissue polarity in adult flies. Because these phenotypes closely resembled mutants or transgenic flies of the tissue polarity genes, genetic interaction of PARP with known polarity genes, genetic interaction of PARP with known tissue polarity genes was examined. Transgenic flies expressing either PARP or RhoA GTPase in the eye were crossed, and co-expression of PARP suppressed the effect of RhoA GTPase. Our results indicate that PARP may play a role in cytoskeletal or cytoplasmic events in developmental processes of Drosophila.

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The enzyme poly(ADP-ribose) polymerase, PARP, is found in eukaryotic cells and catalyzes poly(ADP-ribosylation) of protein substrates. In this reaction, poly(ADP-ribose) is transferred to substrates such as histone and non-histone proteins. PARP-1 has nuclear localization signals and zinc finger motifs, and it is activated by binding to single- or double-stranded breaks in DNA (1, 2). In mammalian cells undergoing apoptosis, PARP-1 is cleaved proteolytically at the 214th aspartic acid by caspase-3 prior to initiation of DNA fragmentation (3, 4). Thus, cleavage of PARP-1 is a marker for apoptosis. The results of several studies suggest that PARP-1 takes part mainly in nuclear events such as DNA repair (5, 6), cell cycle (7, 8), and apoptosis (3, 4, 9, 10). PARP-1 is structurally and biochemically conserved among eukaryotic species (4, 11, 12), which is consistent with its playing an important role in fundamental biological events. Recent studies with PARP-1 knockout mice suggest that PARP-1 plays a role in maintaining genomic and chromosomal stability (13, 14). Deficiency of the PARP-1 gene did not lead to any alterations in developmental processes. Recently, novel proteins having poly(ADP-ribosyl)ation activity (PARP-2) have been identified in mammals (15, 16). PARP-1 and PARP-2 have similar biochemical characteristics such as being activated by binding to broken DNA ends (15). Weak poly(ADP-ribosyl)ation activity is detected in PARP-1−/− cell. Thus, involvement of PARP and developmental events including cell cycle and programmed cell death is not obvious.

Our recent study showed that Drosophila PARP (D.PARP), corresponding to PARP-1 in mammals, is expressed abundantly in embryos and at a low level in larvae, pupae, and adults (17). PARP may play a role in developmental processes such as differentiation, cell proliferation, and programmed cell death. However, the biological role of PARP in Drosophila has not yet been determined. The Drosophila eye provides an excellent system, approachable with genetic and molecular tools, for understanding cell differentiation, proliferation, and programmed cell death at the single-cell level (18–20). Differentiation commences in mid-third instar larvae with a wave of development marked by morphogenetic furrow (MF), which progresses from posterior to anterior across the epidermal field of progenitor cells. Anterior to the MF, cells are unpatterned, undifferentiated, and asynchronous (21). All cells are synchro-

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1 The abbreviations used are: PARP, poly(ADP-ribose) polymerase; F-actin, filamentous actin; MF, morphogenetic furrow; D.PARP, Drosophila poly(ADP-ribose) polymerase; TUNEL, terminal deoxy transferase-mediated dUTP nick-end labeling.

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nized in G1 just anterior to the furrow, resulting in a signal to initiate differentiation (22). Posterior to the furrow, clusters of cells, which are destined to become the adult ommatidial units, undergo cell selection events during differentiation.

As we recently reported (23), targeted expression of D.PARP in the developing eye of Drosophila induced rough-eye phenotype. In this study, we analyzed transgenic flies overexpressing D.PARP in the developing eye and also in embryos, clarifying how this phenotypical change was induced. Our results indicate that overexpression of PARP interferes with the organization of cytoskeletal filamentous actin (F-actin) and disrupts tissue polarity. This study may lead to further insight into the role played by PARP-1 in differentiation, proliferation, and programmed cell death during development.

EXPERIMENTAL PROCEDURES

Fly Stocks and Genetics—Flies were cultured at 25 °C. Oregon-R and w; 126A were used as wild-type unless otherwise stated. Mutant and transgenic flies including GMR-p35 were from G. M. Rubin, f2, f2 , dsh and GMR- were from P. N. Adler; GMR-RhoA (Rho1) was from 1. K. Harrower; and 9903 Rhod/CO from M. Dlodz. Genetic crosses were performed at 25 °C.

Vector Construction and Transformation—A full-length D.PARP cDNA was inserted into the multi-cloning site of pGMR (24), pCaSpeR-hs (25), and pUAST (26) to establish GMR-PARP, hs-PARP, and UAS-PARP, respectively. Transgenic flies were generated by P element-mediated germ line transformation as previously described (27). Transgenic lines were isolated with a single P element insertion on the second or third chromosome. To obtain transgenic flies harboring four copies of GMR-PARP (w, GMR-PARP, GMR-PARP), standard genetic crosses were conducted.

Heat Shock Treatment—To analyze the effect of PARP overexpression in the embryo, stage 12–13 embryos were incubated at 37 °C for 2 h, incubated at 25 °C for 2 h, and then fixed. To analyze the effect of PARP overexpression in the adult fly, a 1-h heat pulse was given at 37 °C every 12 h from the early third instar to the end of the pupal stage.

Northern and Western Blotting—Total RNA was isolated from eye-antennal discs of wild-type and GMR-PARP third instar larvae as described (28). Northern blotting and hybridization, carried out as described (17), were analyzed with a Fujix BAS 1500 Imaging Analyzer described (28). Northern blotting and hybridization, carried out as described (27). Transgenic lines were isolated with a single P element insertion on the second or third chromosome. To obtain transgenic flies harboring four copies of GMR-PARP (w, GMR-PARP, GMR-PARP), standard genetic crosses were conducted.

RESULTS

PARP Overexpression Induced Mild Rough-eye Phenotype with Incorrect Chirality—for targeted expression of D.PARP in the developing eye of Drosophila, D.PARP cDNA was placed under the control of GMR such that PARP was expressed only in and posterior to the MF (23, 24). Five transgenic lines have been generated by P element-mediated germ line transformation, each of which has a single transgene integrated on the second or third chromosome (23). Using two independent lines harboring four transgenes each (w; GMR-PARP, GMR-PARP), PARP expression was analyzed in third instar larval eye discs of these transgenic flies by Northern blotting, Western blotting, and immunohistochemistry (Fig. 1). PARP expression was compared in wild-type flies and in flies harboring 2 (1 line) or 4 (2 lines) copies of the transgene. As shown in Fig. 1A, PARP mRNA level increased 5- or 10-fold in flies with two or four copies of the transgene, respectively. Because the transgene has two poly(A) additional signals, one from the PARP cDNA and another from the pGMR vector, the longer sizes of transcript were detected in the GMR-PARP. PARP protein was also increased in flies with two or four copies of GMR-PARP (Fig. 1). However, PARP protein does not increase in a transgene copy number-dependent manner. This result may indicate that the Western blot signal is not in the linear range of detection or is caused by poor titer of anti-PARP antiserum. Immunohistochemistry using anti-PARP antiserum showed weak PARP expression in whole eye antennal discs of wild-type flies. In GMR-PARP flies, PARP expression was elevated only

![Fig. 1. Overexpression of PARP in eye disc.](http://www.jbc.org/)

PARP Overexpression Induced Mild Rough-eye Phenotype with Incorrect Chirality—for targeted expression of D.PARP in the developing eye of Drosophila, D.PARP cDNA was placed under the control of GMR such that PARP was expressed only in and posterior to the MF (23, 24). Five transgenic lines have been generated by P element-mediated germ line transformation, each of which has a single transgene integrated on the second or third chromosome (23). Using two independent lines harboring four transgenes each (w; GMR-PARP, GMR-PARP), PARP expression was analyzed in third instar larval eye discs of these transgenic flies by Northern blotting, Western blotting, and immunohistochemistry (Fig. 1). PARP expression was compared in wild-type flies and in flies harboring 2 (1 line) or 4 (2 lines) copies of the transgene. As shown in Fig. 1A, PARP mRNA level increased 5- or 10-fold in flies with two or four copies of the transgene, respectively. Because the transgene has two poly(A) additional signals, one from the PARP cDNA and another from the pGMR vector, the longer sizes of transcript were detected in the GMR-PARP. PARP protein was also increased in flies with two or four copies of GMR-PARP (Fig. 1). However, PARP protein does not increase in a transgene copy number-dependent manner. This result may indicate that the Western blot signal is not in the linear range of detection or is caused by poor titer of anti-PARP antiserum. Immunohistochemistry using anti-PARP antiserum showed weak PARP expression in whole eye antennal discs of wild-type flies. In GMR-PARP flies, PARP expression was elevated only
in and posterior to the MF, as was the expected pattern of GMR-induced overexpression.

The adult compound eye comprises 800 unit eyes, or ommatidia, consisting of an ordered array of eight photoreceptor neurons and an invariant array of non-neuronal accessory cells (31). In GMR-PARP, the arrangement of ommatidia was disordered because of ommatidia with abnormal shape and size or because of fusion of the ommatidia (Fig. 2). This phenotype was more or less severe in flies with more or fewer transgenes (Fig. 2, B and C). Tangential sections of adult eyes (Fig. 2, D-F) showed that the orientation of the ommatidia is disrupted in GMR-PARP. Polarity in the Drosophila eye is manifested as a dorso-ventral reflection of two chiral forms of the individual unit ommatidia. There is a dorso-ventral midline of mirror symmetry known as the equator, and the two ommatidial forms fall on opposite sides of this line (Fig. 2D, red line). In the wild type, there is a highly ordered array of an asymmetric trapezoidal pattern of seven rhabdomeres in the photoreceptors, but in GMR-PARP, it was slightly disordered (Fig. 2, E and F). Some ommatidia were rotated incorrectly and exhibited inappropriate chiral forms. In some GMR-PARP flies, a rectangular pattern of rhabdomeres was observed; this arrangement was also observed in flies with a tissue polarity phenotype and is caused by bilaterally symmetrical arrangement of R3 and R4 (Fig. 2E, red).

The number and morphology of non-neuronal accessory cells was analyzed by staining retinas from 40-h pupae with cobalt sulfide (Fig. 2, G–I). This analysis clearly showed an incorrect arrangement of non-neuronal ommatidial cells and improper polarity of ommatidia in retinas from GMR-PARP. In the wild type, there is an invariant array of non-neuronal accessory cells at the apical surface of the retina (Fig. 2G). The number of non-neuronal cells was almost normal in GMR-PARP; however, the morphology and symmetrical arrangement were disordered and abnormal (Fig. 2H and I).

**Fig. 2. Targeted expression of PARP in the developing eye causes rough-eye phenotype.** A–C, scanning electron micrographs of adult eyes from wild-type (A), 2 × GMR-PARP (B), and 4 × GMR-PARP (C). D–F, tangential sections of compound eyes from wild-type (D), 2 × GMR-PARP (E), and 4 × GMR-PARP (F). The red line indicates the equator. The right-hand panels of D–F show a schematic drawing of the same area. Numbers 1–7 schematically indicate the arrangement of rhabdomeres in seven photoreceptors in an ommatidium. A rectangular pattern of rhabdomeres is shown in red. G–I, apical surface of cobalt sulfide-stained midpupal retinas from wild-type (G), 2 × GMR-PARP (H), and 4 × GMR-PARP (I). c indicates cone cells; the numbers 1°, 2°, and 3° indicate primary, secondary, and tertiary pigment cells respectively; and b indicates bristle cell.
from GMR-PARP (Fig. 3B) third instar larvae was almost same as in wild type (Fig. 3A), whereas the arrangement of photoreceptor clusters was slightly disordered in GMR-PARP. To investigate the ommatidial polarity in the eye disc of third instar larvae, the pattern of photoreceptors 3 and 4 (R3 and R4) was detected by immunostaining with antibody against the R3/R4 marker, Spalt (36). In the wild-type eye disc, the orientation of Spalt-immunostained R3/R4 cells is identical in all ommatidia, and a highly ordered array of R3/R4 is observed (Fig. 3C). In GMR-PARP, R3/R4 cells were arranged with random orientation in each ommatidia, indicating that the polarity of ommatidia was disordered. These data indicate that overexpression of PARP affects tissue polarity, not cell number, at the initial stage of neuronal differentiation.

Programmed Cell Death and Cell Cycle in Third Instar Larval Eye Discs—During normal development, programmed cell death occurs in 10–20% of all cells posterior to the MF in the eye discs (18). Because PARP is proteolytically degraded by caspase-3 during apoptosis, it is possible that overexpression of PARP inhibits caspase 3-dependent proteolysis, which could alter apoptosis in the developing eye and result in a rough-eye phenotype. To examine the effect of overexpression of PARP on programmed cell death in the developing eye, TUNEL assays were carried out using third instar larval eye discs. As shown in Fig. 4, the number of TUNEL-positive cells posterior to the MF was slightly higher in GMR-PARP (Fig. 4B) than in wild type (Fig. 4A). If the increased frequency of apoptosis disrupts ommatidial polarity of GMR-PARP, then inhibition of apoptosis should normalize polarity. This idea was tested by crossing GMR-PARP with GMR-p35 transgenic flies. GMR-p35 expresses baculovirus p35, which inhibits caspase protease activity and apoptosis in various species including Drosophila (24). 2 × GMR-p35 shows slightly distorted but almost normal ommatidial polarity (Fig. 4G). Misrotation and incorrect chirality of ommatidia induced in 2 × GMR-PARP (Fig. 2, E and H) were not neutralized by coexpression of p35 (Fig. 4H). In addition, the number of ommatidia and neuronal/non-neuronal cells was almost the same as wild-type (Fig. 2), suggesting that the extra unnecessary cell proliferation followed by apoptosis might occur and cause an increase in the number of apoptotic cells. BrdUrd incorporation and immunolabeling using antibody against phosphorylated histon H3 in the eye disc from wild-type (E) and 4 × GMR-PARP (F), G and H, tangential sections of 2 × GMR-p35 (G, w1118; +/+) and 2 × GMR-PARP/2 × GMR-p35 (H, w; GMR-PARP, GMR-p35).

Fig. 3. Immunohistochemistry of developing eye with neuronal cell type-specific antibodies. The anterior view is to the left and dorsal is above. A and B, immunostaining of neuronal cells with anti-Elav antibody in third instar larval eye from wild-type (A) and 4 × GMR-PARP (B). C and D, anti-Spalt immunostaining highlighting R3 and R4 photoreceptor cells in the third instar larval eye from wild-type (C) and 4 × GMR-PARP (D). The white arrows indicate examples of correctly oriented clusters, and the green arrows depict misoriented clusters. The primary antibody was detected by Cy3-conjugated secondary antibody and analyzed by confocal laser microscope.

Fig. 4. Effect of overexpression of PARP on programmed cell death and cell cycle in eye discs. An arrowhead indicates the position of MF. A and B, apoptotic cells were detected by TUNEL in the eye disc of third instar larvae from wild-type (A) and 4 × GMR-PARP (B). C and D, detection of S phase cells by BrdUrd incorporation in the eye disc from wild-type (C) and 4 × GMR-PARP (D). E and F, M phase cells were detected by immunohistochemistry using antibody against phosphorylated histon H3 in the eye disc from wild-type (E) and 4 × GMR-PARP (F). G and H, tangential sections of 2 × GMR-p35 (G, w1118; +/+) and 2 × GMR-PARP/2 × GMR-p35 (H, w; GMR-PARP, GMR-p35).
abnormality of the cytoskeleton. This idea was consistent with the observation of the fact that the organization of cytoskeletal F-actin was disrupted in retinas from GMR-PARP. In wild-type retinas, the lattice of the ommatidia is regular, and the outlines of cone cells, pigment cells, and bristles are clear. F-actin is organized into neat bundles and forms a close spokewise pattern at the apical surface (Fig. 5A). F-actin forms a star-like pattern in the middle plane at the center of the ommatidium (Fig. 5B). There is an ordered petal pattern formed by the secondary and tertiary pigment cells and bristle complexes with the circular center of the photoreceptor axon bundles in the basal floor (Fig. 5C). In contrast, these distinct patterns were not observed in retinas from GMR-PARP. In retinas from GMR-PARP, F-actin was disorganized in every cell. F-actin was sporadically organized at the apical surface, and a spoke-wise pattern of F-actin was almost completely absent (Fig. 5D). In the middle plane, some ommatidia lacked the normal accumulation of F-actin at the center, which is the precursor of the rhabdomere (Fig. 5E). Cell morphology and arrangement of photoreceptors in each ommatidium were also distorted. Morphology was also disrupted at the basal floor with abnormal morphology and arrangement of secondary and tertiary pigment cells and bristle complexes (Fig. 5F).

**Tissue Polarity Disruption and Cytoskeletal Changes by Heat-induced PARP Overexpression**—If overexpression of PARP disrupts the tissue polarity and cytoskeleton, these effects should be evident in all tissues in the fly. This idea was tested by overexpression of PARP from the heat shock promoter in hs-PARP transgenic flies (Fig. 6). Fig. 6, A–C, shows wing hair of adult flies with and without heat shock. Induction of the PARP expression disrupts polarity and the direction of wing hair in hs-PARP (Fig. 6B). Aberrant polarity was also observed in several epidermal tissues of the adult fly including the notum, abdomen, and eye (data not shown). Heat-treated wild-type and nontreated hs-PARP were used as controls, and they showed no change in polarity (Fig. 6, A and C). Moreover, heat treatment during embryonic development disrupted the organization of cytoskeletal F-actin in the epidermis of the embryo, as did it in the midpupal retinas in GMR-PARP (Fig. 6, D–F). These results strongly suggest that the overexpression of PARP disrupts tissue polarity and disrupts the organization of cytoskeletal F-actin. Because similar effects are observed with different transgenic lines expressing PARP and with transgenes expressing PARP from different promoters, it is unlikely that these effects are artificial or are caused by mutations created at the site of P element insertion.

**Suppression of RhoA Overexpression Phenotype by Co-expression of PARP**—The phenotype of GMR-PARP closely resembles the phenotype of mutants and transgenic flies involving the tissue polarity genes frizzled (fz), disheveled (dsh), and RhoA.
GTPase, which are involved in a single signaling pathway (37, 38). PARP was tested for genetic interaction with these genes by genetic crosses between GMR-PARP and mutants or transgenic flies of these genes (Table I). Although, genetic interaction between PARP and fz or dsh were not observed in this experiment, interestingly, GMR-PARP genetically interacted with GMR-RhoA expressing RhoA GTPase under the control of GMR (34). Small GTPase, RhoA, is well known to regulate the organization of cytoskeleton and tissue polarity in developmental process of Drosophila (37, 38). GMR-RhoA flies have a rough-eye phenotype with dramatic disruption of ommatidial architecture (Fig. 7, B and E). The retina is markedly reduced in thickness, and the lattices formed by secondary and tertiary pigment cells are completely absent. Pigment cells are restricted to the apical regions of the retina. As shown in Fig. 7, C and F, this phenotype was partially rescued by co-expression of PARP. In GMR-PARP/GMR-RhoA (w; GMR-PARP/+; GMR-PARP/2 × GMR-RhoA) eyes, ommatidial lattices were observed, and pigment cells were distributed to deeper regions than in flies overexpressing RhoA alone. A distinct rhabdomere structure also appeared. Based on these results, it might be expected that overexpression of PARP would enhance the hemizygosity of RhoA. This idea was tested by crossing GMR-PARP with a deficiency mutant of RhoA, Df 903 RhoA P2. However, genetic interaction was not observed in this experiment (Table I). GMR-RhoA was crossed with GMR-GAL4 as a control experiment. The phenotype of RhoA overexpression was not affected by co-expression of GAL4 (data not shown).

**DISCUSSION**

This study reports the result of overexpression of PARP in transgenic flies and, unexpectedly, this experiment showed that overexpression of PARP causes disorganization of cytoskeletal F-actin and disruption of tissue polarity. These data suggest that PARP may play a role in the developmental process in Drosophila.

Although the rough-eye phenotype was modest for high levels of PARP expression, all of the GMR-PARP transgenic lines

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**TABLE I**

| Genetic interactiona | Genetic interaction |
|----------------------|---------------------|
| fz1/ +               | –                   |
| fz,027z/ +           | –                   |
| GMR-fz/ +            | –                   |
| dsh/ +               | –                   |
| Df903 RhoA P2/ +     | –                   |
| GMR-RhoA/ +          | +                   |

a Heterozygous for GMR-PARP.

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**Fig. 7. Genetic interaction of PARP with small GTPase, RhoA.** A–C, scanning electron micrographs of adult eyes of 2 × GMR-PARP (A; w; GMR-PARP/+; GMR-PARP/2 × GMR-RhoA (B; w; 2 × GMR-RhoA/TM6B Tb; Hu), and 2 × GMR-PARP/2 × GMR-RhoA (C, w; GMR-PARP/+; GMR-PARP/2 × GMR-RhoA). D–F, tangential sections of 2 × GMR-PARP (D), 2 × GMR-RhoA (E), and 2 × GMR-PARP/2 × GMR-RhoA (F). The lower panels are highly magnified views of the upper panels.
showed disruption of tissue polarity characterized by misrotation and incorrect chirality of ommatidia in eye. A rectangular pattern of rhabdomeres was observed in some eyes, which is caused by a bilaterally symmetrical arrangement of R3 and R4. This pattern is also observed in mutants in tissue polarity genes. Furthermore, planar polarity in various epidermal tissues was disrupted in hs-PARP. These phenotypes closely resemble the phenotypes of mutant or transgenic flies with alterations in tissue polarity genes (37).

One possible explanation for the effects of PARP overexpression is that it alters the processes of apoptosis and/or cell proliferation. However, in these experiments, no significant change in the apoptotic pathway or cell cycle was detected in GMR-PARP flies, and the number of neuronal/non-neuronal cells in the adult eyes of GMR-PARP was almost normal. If PARP directly regulates apoptosis, then it is expected that these processes would be altered by overexpression of PARP. Although apoptosis increased posterior to the MF in GMR-PARP, overexpression of p35 to prevent apoptosis did not prevent disruption of tissue polarity. On the contrary, tissue polarity was not rescued by co-expression of p35. Apoptosis might increase in GMR-PARP flies as a secondary effect because of altered cell morphology and/or tissue/cell polarity. These results suggest that PARP may directly affect tissue polarity but is not likely to directly affect apoptosis.

Recent reports show that PARP is a transcriptional co-activator (40–43). It is possible that overexpression of PARP alters the expression of some genes which play a role in determining tissue polarity or cytoskeleton. For example, gene expression of some cytoskeletal molecules was altered in cells from PARP-1 knockout mice. (44). In these studies, the level of F-actin appeared somewhat reduced when GMR-PARP retinas and hs-PARP embryos were stained with phalloidin. However, it is not clear whether overexpression of PARP reduces the level of actin molecule, which might also influence organization of F-actin. It is possible that overexpression of PARP alters the expression of actin molecule or that overproduced mono- or poly(ADP-ribose) interacts(with actin (45). Furthermore, the function of protein involved in cytoskeletal organization might be affected by PARP overexpression. Small GTPase, RhoA, is well known and well studied as a regulator of cytoskeletal organization. In recent reports, RhoA is considered one of the genes regulating tissue polarity in developmental process of Drosophila (37, 38). Because the phenotypes of flies overexpressing PARP closely resemble the phenotypes of mutants or transgenic flies with altered expression of tissue polarity genes, fz, dsh, or RhoA, we tested for genetic interaction with those genes. Interestingly, the effect of RhoA overexpression was partially rescued by co-expression of PARP, indicating genetic interaction of PARP and RhoA. Although the mechanism of interaction between PARP and RhoA is not clear, this observation may be one possible explanation of the mechanism of disruption of tissue polarity and cytoskeleton by PARP overexpression. Although a loss-of-function mutant fly for PARP is not available, PARP transgenic flies may provide genetic evidence for the biological role played by PARP. Further mechanistic insight is required to understand why the cytoskeletal organization is disrupted in PARP transgenic flies. Biochemical studies using cultured Drosophila and mammalian cells are being carried out to address these questions.

Our study suggests the involvement of PARP in cytoskeletal organization and the determination of tissue polarity during Drosophila development. This role may be mediated by an interaction between PARP and the signaling pathway in cytoskeletal organization. These observations were unanticipated, and they may have important implications for signaling processes that involve both the nuclear and cytoplasmic compartments. These findings shed light on the biological role played by PARP and on cellular motility associated with cancer cell metastasis and invasion.

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