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Rescue of Photoreceptor Degeneration in Rhodopsin-Null *Drosophila* Mutants by Activated Rac1

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Rhodopsin is essential for photoreceptor morphogenesis; photoreceptors lacking rhodopsin degenerate in humans, mice, and *Drosophila*. Here we report that transgenic expression of a dominant-active *Drosophila* Rho guanosine triphosphatase, Drac1, rescued photoreceptor morphogenesis in rhodopsin-null mutants; expression of dominant-negative Drac1 resulted in a phenotype similar to that seen in rhodopsin-null mutants. Drac1 was localized in a specialization of the photoreceptor cortical actin cytoskeleton, which was lost in rhodopsin-null mutants. Thus, rhodopsin appears to organize the actin cytoskeleton through Drac1, contributing a structural support essential for photoreceptor morphogenesis.

Sensory neurons present a challenge for morphogenesis: to harness the generic mechanisms of the cytoskeleton to shape a cell to the needs of its specific sensory protein. For photoreceptors, it is clear that morphogenesis and maintenance of the photosensitive organelle, the rhabdomeres of *Drosophila* and the outer segments vertebrate rods and cones, depend on their sensory protein, rhodopsin (*1*–*3*). Rhabdomeres and outer segments are orderly stacks of photosensitive plasma membrane organized from enormously expanded apical cell surfaces. The forces that constrain this expansion and organize it into a dense stack are incompletely understood, but the cortical actin cytoskeleton and its associated proteins are substantial contributors (*4*, *5*). We suggest that in addition to its sensory role, *Drosophila* rhodopsin organizes the cortical actin cytoskeleton into an essential morphogenetic constraint (*6*), the rhabdomere terminal web (RTW). The RTW defines the regular, curving base of the rhabdomere that partitions the rhabdomere from the photoreceptor cytoplasm. In rhodopsin-null mutants, the rhabdomere base fails to organize correctly, and the rhabdomere collapses deep into the photoreceptor cytoplasm in convoluted sheets of apposed membrane (*1*).

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A chimeric protein that decorates F-actin with green fluorescent protein (GFP) (*7*) revealed the RTW as bundled microfilaments extending from the rhabdomere base deep into the photoreceptor (Fig. 1). Before rhodopsin expression, the RTW of developing photoreceptors showed less microfilament bundling, resembling a house painter's brush (Fig. 1C). At about 90% of pupal development (pd), after the onset of rhodopsin expression at 75% pd, RTW microfilaments elongated commensurate with the increasing microvillar length and gathered into bundles (Fig. 1D).

RTW maturation and rhabdomere morphogenesis fail in photoreceptors lacking rhodopsin. Paralleling the normal initiation of microvillar organization observed in rhodopsin-null mutants, the RTW of mutant photoreceptors appeared normal before the time when rhodopsin expression would normally commence (Fig. 1E). The RTW growth and bundling that normally follow rhodopsin expression failed in rhodopsin-null photoreceptors (Fig. 1F). Unlike wild-type rhabdomeres (Fig. 2A), the smaller, flattened rhabdomeres formed in the rhodopsin-null mutant collapsed into the photoreceptor cytoplasm in convoluted sheets of apposed membrane during the first day after eclosion (Fig. 2B). The actin cytoskeleton becomes thoroughly disorganized in the absence of rhodopsin.

Although rhodopsin contributes about 50% of rhabdomere membrane protein (*8*), it is unlikely to support morphogenesis by a simple mass effect. Smaller, but ultrastructurally normal rhabdomeres form in mutants in which Rh1 is reduced by over 99% (*9*). Furthermore, a pulse of rhodopsin expression restricted to a narrow window of development is sufficient to rescue rhabdomere morphogenesis in photoreceptors otherwise lacking rhodopsin (*10*). We propose that an additional role for rhodopsin is to contribute an activity required to organize the RTW into an effective subapical barrier.

Rho family guanosine triphosphatases (GTPases), which include Rho, Rac, and Cdc42, have emerged as key regulators of the actin cytoskeleton (*11*). Like their Ras cousins, they cycle between inactive guanosine diphosphate– and active guanosine triphosphate–bound forms and mediate signals from membrane receptors to downstream effectors that can modify actin-associated proteins and thereby regulate cytoskeletal architecture and dynamics. Profound defects of morphogenesis result from perturbation of Rho family signaling (*12*–*14*). G protein–coupled membrane receptors, including rhodopsin family members, have been shown to regulate the Rho family (*15*), raising the possibility that rhodopsin contributes to rhabdomere morphogenesis through Rho family GTPases.

Drosophila Rac1, Drac1, localized to the rhabdomere base beginning with the onset of microvillar organization during midpupal development (Fig. 3A); it remained subapical in adult eyes (Fig. 3B). To explore potential Drac1 functions in rhabdomere morphogenesis, we expressed dominant-negative N17Drac1 at defined stages of eye development. N17Drac1 expression during rhabdomere morphogenesis led to reduced, disordered rhabdomeres (Fig. 2C). Fewer microvilli were seen in cross section, and a well-defined rhabdomere base was not formed; apposed sheets of rhabdomere membrane involuted into the photoreceptor cytoplasm. Although these defects were reminiscent of those seen in rhodopsin-null mutants, the phenotype was not a consequence of a failure of rhodopsin delivery to the rhabdomeres (Fig. 4C). The actin cytoskeleton, however, appeared diffuse and disordered as a result of transgene expression (Fig. 4C).

The resemblance of the rhabdomere base defects caused by N17Drac1 to those seen in

rhodopsin-null mutants prompted us to speculate that rhodopsin might exert its structural effect through Drac1. If so, we reasoned that expression of constitutively active V12Drac1 might rescue rhabdomere morphogenesis in photoreceptors lacking rhodopsin. To test this idea, we expressed V12Drac1 during rhabdomere morphogenesis in *ninaE*^{I17} mutants that lack rhodopsin in photoreceptors R1 to R6. Substantial rescue of rhabdomere morphogenesis was observed (Fig. 2D). Occasional loops of rhabdomere membrane intruded into the photoreceptor, but most terminated at a well-defined base. The RTW was more tightly organized in V12Drac1-expressing animals (Fig. 4D). Similar to rhodopsin-null rhabdomeres rescued by a pulse of rhodopsin expression (*10*), V12Drac1-expressing animals showed substantial rescue 5 days after eclosion. Thus, V12Drac1 appeared to supply a durable organizing activity lost in rhodopsin-null mutants.

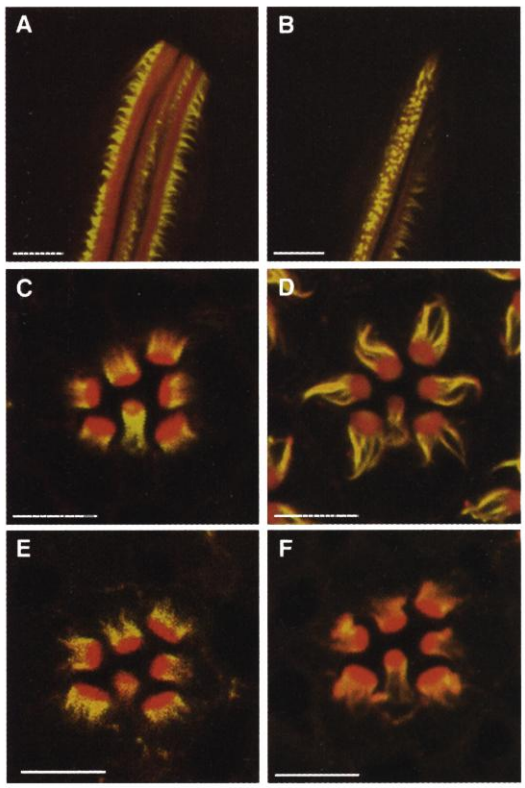

Fig. 1. The RTW defines the rhabdomere base. In these confocal micrographs, the actin cytoskeleton is double-stained with rhodamine phalloidin (red) and GFP-moe (green), a chimeric protein coupling GFP and the actin-binding domain of moesin (*7*, *25*). GFP-moe does not decorate the axial microfilaments of rhabdomere microvilli. (**A**) The quasi-periodic bundling of the RTW (yellow-green) is evident in a longitudinal view of an isolated adult ommatidium. (**B**) In a deeper optical plane of the same ommatidium, RTW bundles meet the rhabdomere base in irregular "feet." (**C**) Before 88% pd, the RTW is unbundled. (**D**) By 94% pd, the RTW is reorganized into microfilament bundles. (**E**) At 88% pd, the RTW of rhodopsin-null photoreceptors appears normal. (**F**) The RTW remains unbundled in 94% pd rhodopsin-null photoreceptors. Scale bar, 5 μm.

Similar to the requirement of small amounts of rhodopsin for normal morphogenesis, rescue appeared quite sensitive to Drac1V12. About 18% of R1 to R6 rhabdomeres were rescued in non–heat-shocked hsGAL/SM1; UAS-Drac1V12 ninaE^{I17} eyes, rising to 90% in animals heat-shocked at 80% pd. Substantial rhabdomeres and tighter organization of the RTW were evident. To examine rescue specificity among Rho small GTPases, we also expressed constitutively active V12Cdc42 and V14Rho in ninaE-null mutants. V12Cdc42 rescued rhodopsin-null morphogenesis, but V14Rho did not. Neither Rho nor Cdc42 immunolocalized to the RTW of normal flies, but Cdc42 has been found to activate Rac in other systems (*16*) and may do so here.

The observations reported here suggest that Drac1 links rhodopsin to photoreceptor

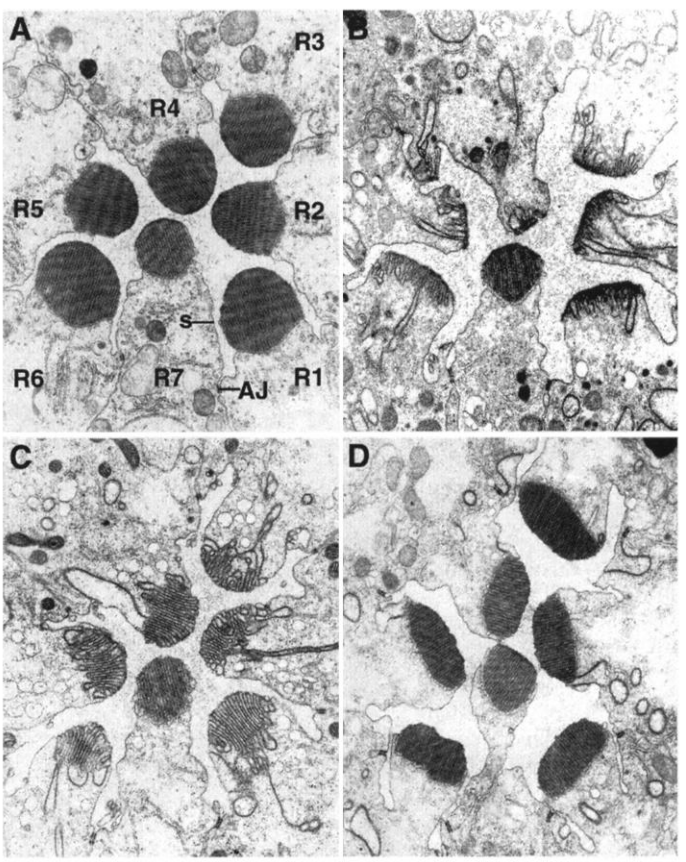

Fig. 2. Rhodopsin and Drac1 regulate rhabdomere morphogenesis. (**A**) In an electron micrograph of a wild-type ommatidium cross section, rhabdomeres of the six outer photoreceptors, R1 to R6, form a trapezoid; photoreceptor R7's rhabdomere occupies the central axis. Rhabdomeres are collared by the stalk (s), which lies between the rhabdomere and the apical junctional complex (AJ). The rhabdomere base describes a catenarylike curve separating the sensory membrane from the photoreceptor cytoplasm. The RTW is not evident in these thin-section electron micrographs. Scale bar, 2 μm [(A), (B), (C), and (D) are the same magnification]. (**B**) Rhabdomeres lacking rhodopsin degenerate. In this ommatidium of a newly eclosed Rh1-null *ninaE*^{I17} mutant, rhabdomere remnants persist in R1, R2, and R6, whereas rhabdomeres of R3, R4, and R5 are nearly completely degenerated. R7 uses a rhodopsin not encoded by *ninaE* and is structurally normal. (**C**) Expression of dominant-negative N17Drac1 at 70 to 80% pd disrupts rhabdomere morphogenesis in R1 to R6; microvilli are reduced in number and are nonuniform in caliber, expanding at their distal ends. The failure to organize a normal rhabdomere base resembles defects seen in *ninaE*^{I17} mutants. Later expression of N17Drac1 disrupts R7 morphogenesis. (**D**) V12Drac1 rescues rhabdomere morphogenesis in rhodopsin-null photoreceptors. Expression of constitutively active V12Drac1 starting at 80% of pupal development prevents catastrophic involution of the rhabdomere membrane in *ninaE*^{I17} photoreceptors. Compare with (B). About 90% of rhodopsin-null photoreceptors show substantial rescue (*26*).

morphogenesis: Targeted delivery of rhodopsin to the developing rhabdomere promotes localized Drac1 activity that, in turn, orchestrates assembly of the RTW (*6*). In rhodopsin-null photoreceptors, failure to correctly organize the RTW, likely including a failure of microfilament cross linking, would allow sheets of self-adhesive rhabdomere membrane (*17*) to intrude unopposed into the photoreceptor cytoplasm. How rhodopsin contributes to Drac1 activity, as well as its downstream effectors, remains to be determined. Two attractive effector candidates are nonmuscle myosin II and moesin (*18*), which localize to the base of the developing rhabdomere and which, in other systems, lie downstream of small GTPases (*19*).

An actin barrier may also shape vertebrate photoreceptors, constraining newly added photosensitive membrane to the outer segment. Actin and actin-associated proteins localize to the site of outer segment disc membrane evagination (*4*, *5*), and nascent outer segment disc membrane intrudes into the cytoplasm of rabbit photoreceptors exposed to cytochalasin D (*20*, *21*). Given the several parallels between vertebrate and *Drosophila* retinal development and the highly conserved mechanisms of the cytoskeleton, it is interesting to speculate that vertebrate rhodopsin may also regulate the photoreceptor cytoskeleton. It is possible that some mutant rhodopsins, including those causing human retinitis pigmentosa, may result in photoreceptor degeneration because of an inability to correctly organize the actin cytoskeleton.

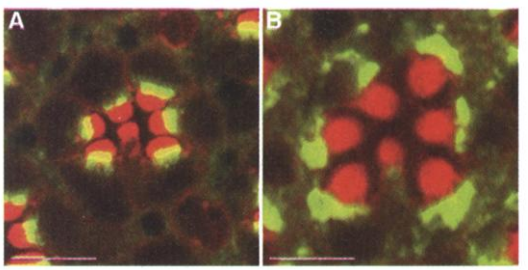

Fig. 3. Drac1 immunolocalizes to the RTW. (**A**) At 75% of pupal development, about the time of Rh1 expression, Drac1 (green) immunolocalizes to the R1 to R6 rhabdomere base. A prominent yellow band marks Drac1 in close proximity with actin (red). Drac1 staining of R7 begins at about 97% pd and is not seen in this section. (**B**) In adult eyes, Drac1 immunolocalizes in subapical patches that overlap deeper RTW filaments; Drac1 appears to be absent directly adjacent to the mature rhabdomere base. Scale bar, 5 μm.

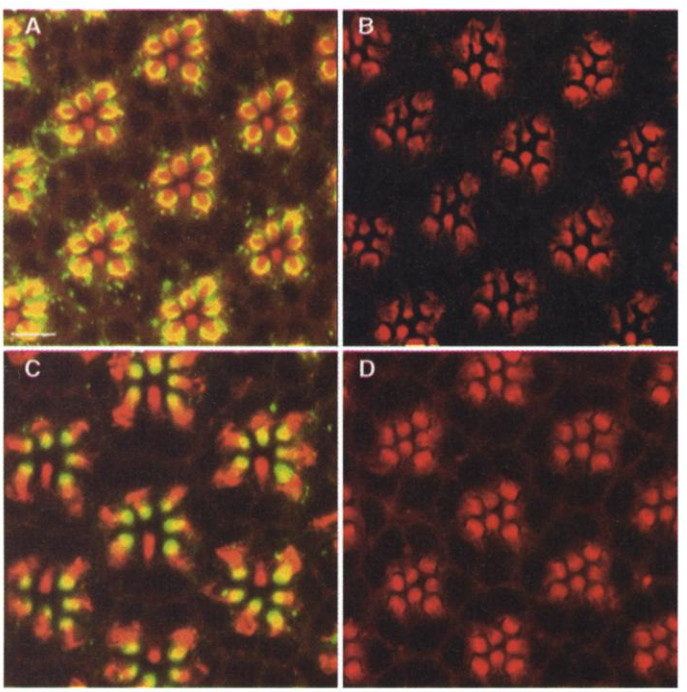

Fig. 4. Drac1 activity is essential for RTW organization. In these confocal images, Rh1 rhodopsin was immunolocalized (green), and the actin cytoskeleton was visualized with rhodamine phalloidin (red). (**A**) In this confocal section through a wild-type eye, Rh1 appears as a brighter crescent of stain at the base of R1 to R6 rhabdomeres. Rh1-bearing vesicles are evident in the cytoplasm. Central R7 rhabdomeres are unstained. Scale bar, 5 μm. (**B**) Rh1 staining is absent in *ninaE*^{I17}, and R1 to R6 rhabdomeres are degenerated. Ragged tails of F-actin, some associated with involuting rhabdomere membrane, fill the cytoplasm adjacent to the degenerating rhabdomere. Intact R7 rhabdomeres mark each ommatidium. (**C**) In eyes expressing N17Drac, Rh1 (green) is present in reduced R1 to R6 rhabdomeres. The RTW appears diffuse and disorganized. (**D**) V12Drac rescues rhabdomeres in *ninaE*^{I17} eyes. As in (B), Rh1 staining is absent. Most R1 to R6 rhabdomeres appear normal.

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25. Phalloidin and immunostaining were performed with whole mount preparation as previously described (*22*). Specimens were examined with a Bio-Rad MRC-1024 confocal microscope.
26. Canton S was used as wild type. Flies were staged by the collection of white prepupae (0% pd) and aged at 20°C. The rhodopsin Rh1-null mutant *ninaE*^{I17} was used as *sr ninaE*^{I17} *e*^{s}, kindly provided by W. Pak. UAS-Drac1N17.1 was a gift from L. Luo (*12*). UAS-Drac1V12.1 and V12.2 transgenic lines were generated with polymerase chain reaction (PCR) with primers CCTCCGCCGCCGCCCCAAAA and GTTTTGTTTTGTTTTTGGTT to amplify Drac1 genomic DNA, which was cloned into pCR2.1 (Invitrogen, San Diego, CA). Constitutive active V12Drac1 DNA was generated by PCR with synthetic mutation primer GTAGCCGTCGGAAAGACCTGCCTG, which mutates Gly^{12} to Val. V12Drac1 sequence was confirmed and subcloned into pUAST (*23*). Two independent UAS-Drac1V12 lines, UAS-Drac1V12.1 and UAS-Drac1V12.2, each on the third chromosome, were generated with P-element–mediated germ line transformation (*24*). A third UAS-Drac1V12 was provided by L. Luo. Crosses were made to establish Hs-Gal4/SM1; UAS-Drac1V12.1 *sr ninaE*^{I17} *e*^{s}. Dominant-negative N17Drac1 and constitutively active V12Drac1 proteins were expressed with Gal4/UAS-targeted expression. Transmission electron microscopy of adult eyes was carried out as described (*22*).

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