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Competing interests statement

The authors declare that they have no competing financial interests.

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Rac function and regulation during *Drosophila* development

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Rac GTPases regulate the actin cytoskeleton to control changes in cell shape[1,2]. To date, the analysis of Rac function during development has relied heavily on the use of dominant mutant isoforms. Here, we use loss-of-function mutations to show that the three *Drosophila Rac* genes, *Rac1*, *Rac2* and *Mtl*, have overlapping functions in the control of epithelial morphogenesis, myoblast fusion, and axon growth and guidance. They are not required for the establishment of planar cell polarity, as had been suggested on the basis of studies using dominant mutant isoforms[3,4]. The guanine nucleotide exchange factor, Trio, is essential for Rac function in axon growth and guidance, but not for epithelial morphogenesis or myoblast fusion. Different Rac activators thus act in different developmental processes. The specific cellular response to Rac activation may be determined more by the upstream activator than the specific Rac protein involved.

In *Drosophila*, studies using constitutively active and dominant negative mutants have implicated Rac1 in closure of the dorsal epidermis[5], myoblast fusion[6], the establishment of planar cell polarity[3,4], and the control of axon growth[6] and guidance[7]. Each of these processes requires dynamic remodelling of the actin cytoskeleton, although the extracellular signals and the cellular responses involved seem to be different in each case. Given the ability of dominant mutant Rac proteins to interfere with cytoskeletal dynamics[1], it is not surprising to find that they perturb each of these processes. But are endogenous Rac proteins actually required for these processes, and if so, which proteins are involved, and how are they regulated? These questions cannot be answered using dominant mutant proteins. They require the phenotypic analysis of loss-of-function mutations in each of the endogenous *Rac* genes.

The *Drosophila* genome contains two highly similar *Rac* genes, *Rac1* and *Rac2* (refs 5, 6, 8, 9). A third gene, *Mtl*, encodes a closely related GTPase that is structurally similar to both Rac and Cdc42 GTPases[10], but functionally (as we now show) behaves like Rac1 and Rac2. We therefore refer to *Rac1*, *Rac2* and *Mtl* collectively as the *Drosophila Rac* genes (Fig. 1a). All three genes are ubiquitously expressed during development[5,6,8,10]. The isolation of loss-of-function *Rac1* and *Rac2* mutations is described in the accompanying paper[11]. A loss-of-function mutation in the *Mtl* gene was generated by imprecise excision of a P-element inserted in the first non-coding exon. We recovered a 2,068-base pair (bp) deletion that removes the entire *Mtl* open reading frame, but no part of any other predicted gene (Fig. 1b). Animals homozygous for this deletion, Mtl^{Δ}, as well as both *Rac1* and *Rac2* single mutants[11], are viable and fertile. The *Rac2 Mtl* double mutant is also viable and fertile. All other combinations are homozygous lethal.

We used these loss-of-function mutations to assess the contribution of each Rac protein to a set of distinct cell-shape changes that occur during *Drosophila* development. We examined embryos lacking both the maternal and zygotic contributions of one or more *Rac* gene, and also pupae and adults that were homozygous mutant either entirely or in large clones of cells. For pupae and adults, we used both the strong hypomorph $Rac1^{J10}$ and the null allele $Rac1^{J11}$, together with the null deletion alleles for *Rac2* and *Mtl* ($Rac2^{\Delta}$ and Mtl^{Δ}). Analyses in the embryo were restricted to the use of the $Rac1^{J10}$ allele, as triple mutant embryos could not be recovered using the null allele $Rac1^{J11}$. Evidently, Rac proteins also have important but still uncharacterized functions during oogenesis and early embryogenesis.

During *Drosophila* embryogenesis, opposing lateral epidermal sheets move towards one another, meeting and fusing seamlessly at the dorsal midline. This process of dorsal closure resembles ventral enclosure in *Caenorhabditis elegans*[12]; and wound healing in vertebrates[13]. It is believed to be driven, at least in part, by an actomyosin contractile ring that assembles at the leading edge[14,15], with lamellipodial and filopodial protrusions facilitating adhesion and alignment as the epidermis is sealed[16]. Expression of dominant negative Rac1 in epidermal cells prevents formation of the actomyosin cable and completion of dorsal closure[5], suggesting that at least one endogenous Rac protein might be involved. We determined that all three Rac proteins contribute to dorsal closure (Fig. 1c–g). Triple mutant *Rac* embryos fail to complete dorsal closure (Fig. 1c, e). Little or no actin accumulation is seen at the leading epidermal edge, and both lamellipodia and filopodia are lacking (Fig. 1g). The underlying amnioserosa cells appear normal. Weaker and less frequent defects are also seen in *Rac1 Rac2* and *Rac1 Mtl* double mutant embryos (Fig. 1c). All remaining single and double mutant embryos successfully complete dorsal closure (Fig. 1c). Dorsal closure thus relies more on Rac1 than either Rac2 or Mtl, although any one of the three is largely sufficient.

Quite different cell-shape changes occur during cell fusion, a striking example of which is the fusion of myoblasts to form multinucleate muscle fibres[17]. The role of the actin cytoskeleton in myoblast fusion remains unclear. Most likely, it is involved in the formation of a vesicular prefusion complex that assembles at the apposed plasma membranes[18]. Expression of either dominant negative or dominant active Rac1 in *Drosophila* myoblasts blocks their fusion[6], but here too the precise roles and contributions of individual *Rac* genes are unknown. We found that little or no myoblast fusion occurs in either *Rac1 Rac2* double mutant (Fig. 1j) or *Rac1 Rac2 Mtl* triple mutant embryos. In contrast, myoblast fusion appears to be complete in *Rac1* and *Mtl* single and double mutants, whereas only a few isolated myoblasts fail to fuse in *Rac2* single mutants and *Rac2 Mtl* double mutants (Fig. 1i). Myoblast fusion thus requires either Rac1 or Rac2, but not Mtl.

Actin rearrangements also underlie the establishment of planar cell polarity (PCP) within an epithelium[19]. In *Drosophila*, PCP has been studied most extensively in the context of eye and wing development. Photoreceptors in the eye are arranged in a trapezoi-

dal fashion, giving each ommatidium a specific chirality and orientation. In the wing, each epithelial cell forms a single distally oriented hair, preceded by the assembly of an actin-based 'pre-hair' at the distal vertex of the cell. The involvement of Rac proteins in PCP has been suggested by the finding that both dominant negative and constitutively active forms of Rac1 disrupt ommatidial orientation in the eye[4], and that dominant negative Rac1 also induces the formation of multiple hairs per cell in the wing[3]. To test more critically a requirement for Rac proteins in PCP, we examined clones of cells in the eye and the wing that were triply mutant for null alleles of *Rac1*, *Rac2* and *Mtl*. No PCP defects could be detected within these clones in either tissue (Fig. 1l, m). Presumably, the PCP defects previously reported[3,4] are due to cross-inhibition or cross-activation of other pathways by the dominant mutant Rac proteins used.

The most complex changes in cell shape that occur during development take place in the nervous system, as differentiating neurons extend axons and dendrites towards their specific target cells. Dominant mutant Rac proteins are crude tools with which to address the complexities and subtleties of neuronal differentiation[2], and it is perhaps not surprising that their use has sometimes led to conflicting results[6,7]. To begin to tease apart the diverse functions of Rac GTPases in nervous system development, we examined the embryonic central nervous system (CNS) and peripheral nervous system (PNS), and the adult visual system, in single, double, and triple mutants for *Rac1*, *Rac2* and *Mtl*.

Embryonic CNS axon pathways were examined using anti-Fasciclin II (FasII) monoclonal antibody 1D4. FasII labels axons in three longitudinal fascicles on each side of the midline, and is thus a sensitive marker to detect any misrouting of longitudinal axons across the midline (Fig. 2a, f). Such midline guidance errors occur in 33% of segments in *Mtl* mutant embryos (Fig. 2b, k), but only at very low frequency (less than 2%) in embryos lacking one or both of *Rac1* and *Rac2* (Fig. 2a, k). Mutations in *Rac1*, and to a lesser extent *Rac2*, enhance the frequency of the midline guidance errors in *Mtl* mutant embryos (to 75% and 42% of segments, respectively; Fig. 2c, k).

Axon guidance defects also occur in the visual system of whole-eye *Rac* mosaics generated using *eyFLP* (ref. 20). In wild-type adults and control mosaics, photoreceptor axons establish precise topographic connections in the lamina and medulla of the optic lobe (Fig. 3a, f). These projection patterns are largely normal in each of the single mutants, and only mild defects occur in *Rac1 Rac2* and *Rac2 Mtl* double mutants (Fig. 3b, d, g). Projection defects are more pronounced in *Rac1 Mtl* double mutants (Fig. 3c, g), and are severe in the triple mutant (Fig. 3e, g). These defects include local disruptions in topographic mapping, and a frequent misrouting of photoreceptor axons around and beyond the medulla (Fig. 3c, e–

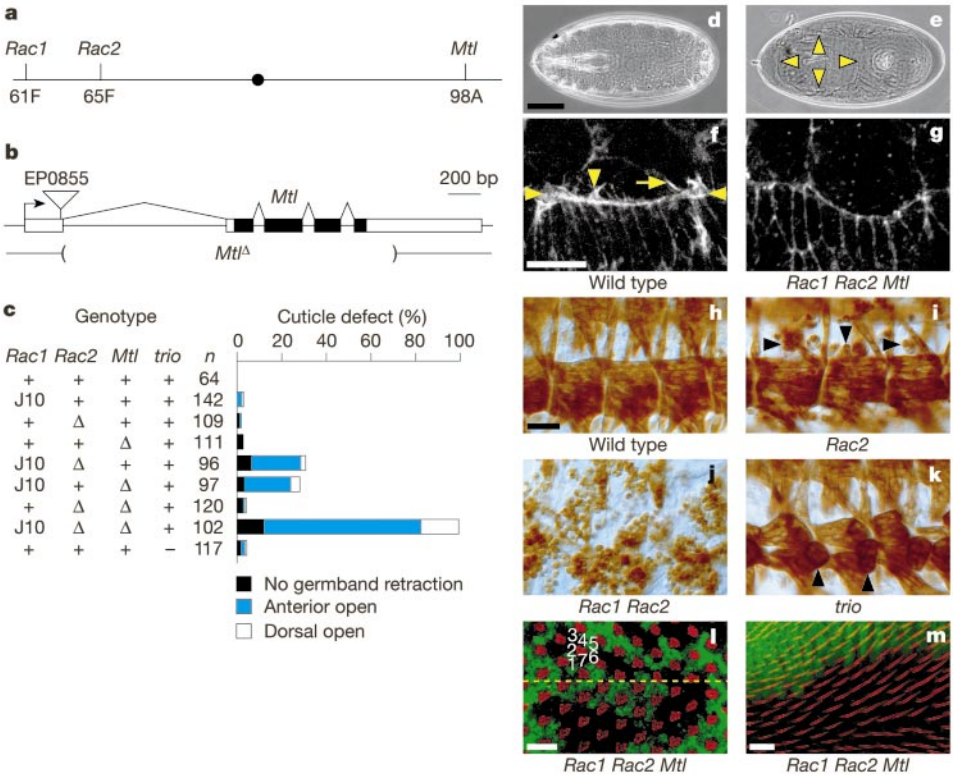

Figure 1 Rac GTPases are required for dorsal closure and myoblast fusion, but not for planar cell polarity. **a**, Location of the three *Drosophila Rac* genes on chromosome 3. **b**, Genomic organization of the *Mtl* locus. Coding regions are shaded. **c**, Quantification of cuticle defects in embryos of the indicated genotype (maternal and zygotic). *trio* embryos were $trio^{1}/trio^{1}$ maternal and $trio^{1}/trio^{8}$ zygotic. **d**–**g**, Dorsal closure in wild-type (**d**, **f**), and $Rac1^{J10}$ $Rac2^{\Delta}$ Mtl^{Δ} (**e**, **g**) embryos. **d**, **e**, Cuticle phenotypes of 22-h embryos. The cuticle of *Rac* triple mutant embryos remains open (arrowheads in **e**). **f**, **g**, F-actin accumulation at the leading edge of the epidermis of stage 14 embryos, visualized by rhodamine-phalloidin staining. The actomyosin cable (horizontal arrowheads), lamellipodia (vertical arrowhead) and filopodia (arrow) seen in wild-type embryos (**f**) are mostly lacking in *Rac* triple mutant embryos (**g**). **h**–**k**, Ventral and lateral musculature of stage 15–16 embryos, stained with anti-muscle myosin monoclonal antibody FMM5. Arrowheads indicate unfused myoblasts in a $Rac2^{\Delta}$ embryo (**i**), and detached myotubes in a *trio* mutant embryo (**k**). **l**, **m**, $Rac1^{J11}$ $Rac2^{\Delta}$ Mtl^{Δ} triple null clones in the pupal eye (**l**) and wing (**m**), at 75 and 35 h, respectively, after puparium formation, stained with rhodamine-phalloidin (red). Clones were generated using *hsFLP*, and are revealed by the lack of staining for a cytoplasmic β-galactosidase marker (green). **l**, Rhabdomeres of R1–R7 (1–7, red) display the normal trapezoidal arrangement and orientation, with mirror symmetry across the equator (yellow line). Ommatidial polarity is also normal in whole-eye clones generated with *eyFLP* (not shown). Rhabdomere morphology appears normal at this stage, but is often perturbed in adults, as has been observed on expression of dominant negative Rac1 in the eye[30]. **m**, A single distally oriented pre-hair (red) forms in each cell in the wing disc, both inside and outside the *Rac* triple mutant clones. Scale bars: **d**, 100 μm; **f**, **m**, 10 μm; **h**, **l**, 20 μm.

g). Specification of photoreceptor cell fate appears to be normal, even in triple mutant clones (Fig. 1l and data not shown). The projection defects observed in the triple mutant could be rescued by reintroducing either *Rac1* or *Mtl* specifically in the eye using a *GMR* transgene (Fig. 3g).

Together, these data establish a critical role for endogenous Rac proteins in axon guidance. The three Rac proteins have overlapping functions in axon guidance in both the CNS and visual system, just as they do in the mushroom bodies[11]. Nevertheless, some degree of specialization can be discerned. For example, axon guidance at the CNS midline depends more on Mtl than Rac1, whereas Rac1 is more important than Mtl in the mushroom bodies.

Whereas single and double mutant embryos reveal a role for Rac proteins in axon guidance, triple mutant embryos demonstrate the essential function of Rac proteins in axon growth. Severe growth defects occur in *Rac1 Rac2 Mtl* homozygous mutant embryos (Fig. 2d, i, k). In the CNS, FasII-positive axons rarely extend from one segment into the next (Fig. 2d), and very few sensory axons from the PNS reach the CNS (Fig. 2i). Specification of neuronal and glial cell fate appears relatively normal (not shown), as does dendritic growth and morphology (Fig. 2i). We quantified these axon growth defects by determining the frequency with which axons from the dorsal cluster of sensory neurons reach the lateral cluster on their path towards the CNS (Fig. 2g, k). This analysis of the PNS confirmed the general impression gained from the CNS: axon stalling is severe in *Rac1 Rac2 Mtl* triple mutants, occurs occasionally in *Rac1 Mtl* double mutants, and is rare in all other combinations (Fig. 2h, i, k).

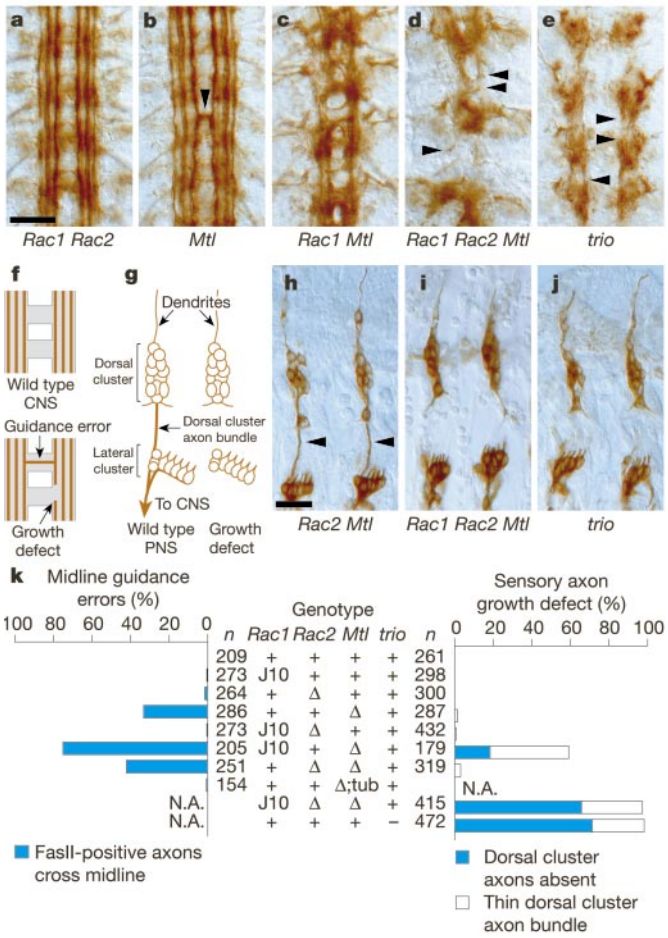

Figure 2 Axon growth and guidance defects in *Rac* mutant embryos. **a**–**e**, **h**–**j**, Late stage 16 embryos stained with anti-FasII monoclonal antibody 1D4 (**a**–**e**) or 22C10 (**h**–**j**). Stained with 1D4, the $Rac1^{J10}$ $Rac2^{\Delta}$ double mutant (**a**) is identical to wild type. In Mtl^{Δ} (**b**) and $Rac1^{J10}$ Mtl^{Δ} (**c**) mutants, some longitudinal axons are misrouted across the midline (arrowhead in **b**). Severe axon stalling is observed in $Rac1^{J10}$ $Rac2^{\Delta}$ Mtl^{Δ} (**d**) and *trio* mutant (**e**) embryos (arrowheads). Some of the stalled longitudinal axons are directed across the midline in *Rac* triple mutants (**d**), but not in *trio* mutants (**e**). **f**, Schematic of CNS axon pathways, with the three FasII-positive longitudinal axon fascicles highlighted. **g**, Schematic of dorsal and lateral peripheral nervous system (PNS). **h**, A $Rac2^{\Delta}$ Mtl^{Δ} mutant, showing the normal projection of dorsal cluster sensory axons (arrowheads). These axons fail to extend in $Rac1^{J10}$ $Rac2^{\Delta}$ Mtl^{Δ} mutants (**i**) and *trio* mutant (**j**) embryos. Panels **h**–**j** are photomontages of images acquired in multiple focal planes. **k**, Embryos were scored for midline guidance errors or sensory axon growth defects. *n* indicates the number of segments or hemisegments scored, respectively. tub, presence of a *tub-Mtl* transgene; NA, not applicable. Scale bars: 20 μm.

These data demonstrate that axon growth also requires Rac activity, but only at a very low level. This activity can be provided by any one of the three Rac proteins alone, although Rac2 is less effective than either Rac1 or Mtl. Axon growth is thus maintained at levels of Rac activity that are insufficient for accurate guidance, consistent with the idea that a low level of Rac activity is essential to drive the growth cone forward, while spatially restricted bursts of high activity may be needed to turn it. In the accompanying paper[11], we take this model one step further by showing that axon branching requires even higher levels of Rac activity.

Endogenous Rac GTPases thus function in morphogenesis of the epidermis, mesoderm, and nervous system. Are they regulated by the same or different upstream activators in each of these tissues? The guanine nucleotide exchange factor Trio activates Rac1, Rac2 and Mtl *in vitro*[10], and loss of *trio* function in the visual system results in projection errors of photoreceptor axons similar to those observed in *Rac* triple mutants[10]. Axon guidance errors and occasional stalling defects also occur in embryos lacking zygotic *trio* function[21–23]. Axon stalling becomes severe in both the CNS (Fig. 2e) and PNS (Fig. 2j, k) if the maternal *trio* function is also eliminated. As with the Rac proteins, low levels of Trio activity are sufficient but essential for axon growth. This critical requirement for Trio in axon growth is particularly striking, given that the *Drosophila* genome encodes at least 22 other Rho family GTPase exchange factors, several of which are also expressed in the developing nervous system (ref. 24 and S.H.-S. and B.J.D., unpublished data).

In the embryonic nervous system and adult visual system, loss of *trio* function thus results in defects remarkably similar to those observed upon loss of *Rac* function, consistent with the idea that Trio and Rac proteins act in a common pathway *in vivo*. We performed an epistasis experiment to test this. Overexpression of the Trio GEF1 domain using the eye-specific *GMR* promoter results in a severely disrupted eye morphology (Fig. 4a) and highly aberrant photoreceptor axon projections (Fig. 4f; see also ref. 10). If Trio signals through Rac proteins *in vivo*, then these defects should be dependent on *Rac* function. This is indeed the case. Both the eye morphology and axon projection defects are almost completely suppressed in animals homozygous for loss-of-function mutations in either *Rac1* or *Rac2* (Fig. 4b, c, g, h). *Mtl* alone does not suppress this *trio* gain-of-function phenotype (Fig. 4d, i). The *Rac1 Rac2 Mtl* triple mutant phenotype is completely epistatic to the *trio* gain-of-function phenotype (Figs 4e, j and 3e). These data demonstrate that Trio GEF1 does indeed act through Rac proteins *in vivo*, and further suggest that Rac1 and Rac2 are its preferred substrates. The *trio* loss-of-function phenotype is however much more severe than the *Rac1 Rac2* double mutant phenotype (Figs 3b, g and 2a, e, k), suggesting that endogenous Trio may also activate Mtl, at least when Rac1 and Rac2 are lacking.

Having identified Trio as the primary activator of Rac proteins during axon growth, we next investigated whether Trio is required for any of the other Rac functions. Dorsal closure occurs normally in embryos lacking both maternal and zygotic *trio* function (Fig. 1c). Myoblast fusion also appears complete in these embryos, but myotubes often fail to attach themselves correctly to the epidermis

(Fig. 1k). Thus, although it is expressed in both the epidermis and mesoderm[21–23], Trio is not required for either dorsal closure or myoblast fusion.

We have shown here that endogenous Rac proteins control cell-sheet spreading, cell fusion, and axon growth and guidance, and in the accompanying paper[11] we show that they also regulate axon branching. Each of these processes involves its own characteristic restructuring of the cytoskeleton, and hence is likely to be mediated by a different set of Rac effectors[11]. What determines which of these effector pathways will be stimulated when Rac proteins are activated? One possibility would be that distinct Rac proteins have distinct effectors. This may well be the case for myoblast fusion, which can be mediated by Rac1 or Rac2, but not Mtl. However, we find that in most cases Rac1, Rac2 and Mtl have largely overlapping functions, indicating that they also share a common set of effectors. A similar pattern of overlapping functions in diverse processes has also recently been reported for the three *C. elegans Rac* genes[25,26]. In general, the cellular response is therefore unlikely to be dictated by the specific Rac protein involved. Our results suggest an alternative possibility. We find that Trio, despite its widespread expression, is required for only a limited subset of Rac functions. This suggests that the set of effectors a Rac protein engages, and hence the cellular response it induces, might also depend on how or where it has been activated. Trio, for example, might activate Rac proteins to a level, for a duration, or in a subcellular location, that allows it to stimulate only those effector pathways that control motility and guidance.

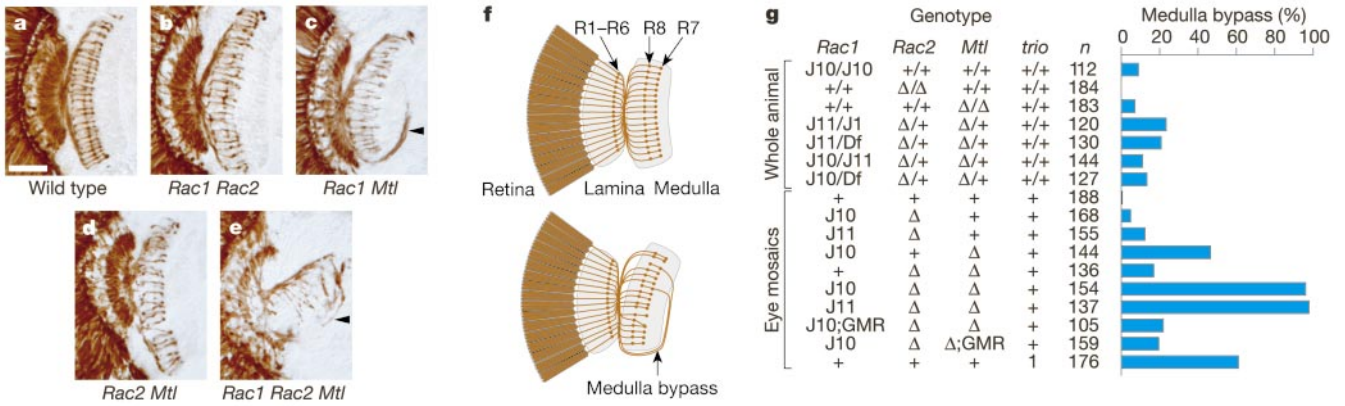

Figure 3 Photoreceptor axon projections in *Rac* mutants. **a**–**e**, Horizontal sections of adult heads of the indicated genotypes. All animals carried the *glass-lacZ* reporter and were stained with anti-β-galactosidase antibodies to visualize photoreceptors and their axons. Genotypes are as follows: **a**, *eyFLP2*; *FRT80B*/*M(3)RpS17*^{A} *FRT80B*; **b**, *eyFLP2*; *Rac1*^{J11} *Rac2*$^{\Delta}$ *FRT80B*/*M(3)RpS17*^{A} *FRT80B*; **c**, *eyFLP2*; *Rac1*^{J10} *FRT80B Mtl*$^{\Delta}$/*M(3)RpS17*^{A} *FRT80B Mtl*$^{\Delta}$; **d**, *eyFLP2*; *Rac2*$^{\Delta}$ *FRT80B Mtl*$^{\Delta}$/*M(3)RpS17*^{A} *FRT80B Mtl*$^{\Delta}$; **e**, *eyFLP2*; *Rac1*^{J11} *Rac2*$^{\Delta}$ *FRT80B Mtl*$^{\Delta}$/*M(3)RpS17*^{A} *FRT80B Mtl*$^{\Delta}$. No stalling of photoreceptor axons can be detected, but such defects cannot be excluded, in particular for the triple mutant (**e**). Arrowheads in **c** and **e** indicate axons showing the medulla bypass phenotype. These axons are not entirely contained within the sections shown. **f**, Schematic diagram of axonal projections of wild-type (top) and *Rac* or *trio* mutant (bottom) photoreceptors in the adult visual system. The termini of R1–R8 axons are indicated. **g**, Frequency of photoreceptor axons misrouting around the medulla (medulla bypass). Animals were either entirely mutant for the indicated allelic combination (upper lines), or carried whole-eye clones generated using *eyFLP* (ref. 20) (lower lines). Data for *trio* are from ref. 10. Df, *Df*(3)*Rac1* (ref. 11). GMR indicates the presence of either a *GMR-Rac1* or a *GMR-Mtl* transgene. The *Rac1*^{J11} allele has the same phenotypic strength as the deficiency (lines 4 versus 5, and 6 versus 7). *Rac1*^{J10} appears to be only slightly weaker than both *Rac1*^{J11} (lines 4 versus 6, 9 versus 10, and 13 versus 14) and the deficiency (lines 5 versus 7). These genetic data confirm that *Rac1*^{J11} is a null allele, whereas *Rac1*^{J10} behaves as a strong hypomorph[11]. Scale bar: 40 μm.

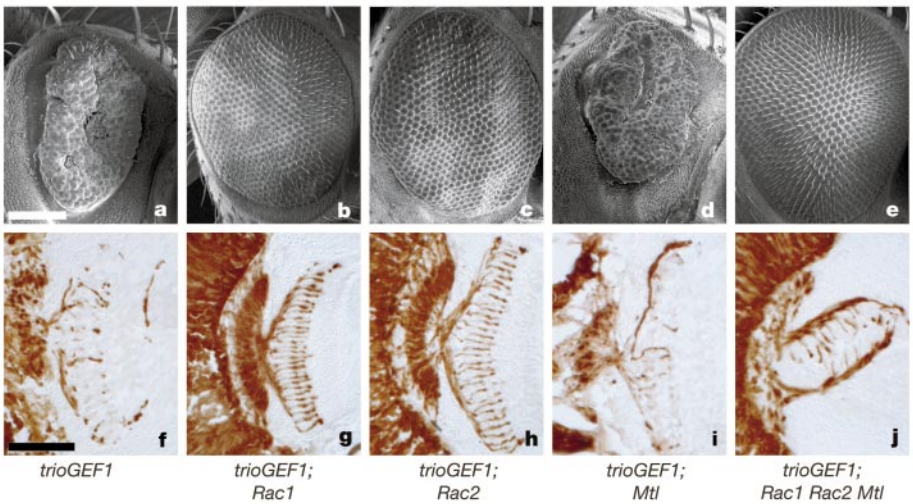

Figure 4 *Rac1* and *Rac2* suppress a *trio* gain-of-function phenotype. **a**–**j**, Scanning electron micrographs of eyes (**a**–**e**) and horizontal head sections (**f**–**j**) of animals of the following genotypes: **a**, **f**, *GMR-trioGEF1*/+; **b**, **g**, *GMR-trioGEF1*/+; *Rac1*^{J10}/*Rac1*^{J10}; **c**, **h**, *GMR-trioGEF1*/+; *Rac2*$^{\Delta}$/*Rac2*$^{\Delta}$; **d**, **i**, *GMR-trioGEF1*/+; *Mtl*$^{\Delta}$/*Mtl*$^{\Delta}$; **e**, **j**, *eyFLP2*; *GMR-trioGEF1*/+; *Rac1*^{J10} *Rac2*$^{\Delta}$ *FRT80B Mtl*$^{\Delta}$/*M(3)RpS17*^{A} *FRT80B Mtl*$^{\Delta}$. Scale bars: **a**, 100 μm; **f**, 40 μm.

Exploring the basis for specificity in Rac function is an important task for the future. □

Methods

Mosaic analysis

Eye-specific mosaics for chromosome arm 3L were generated using *eyFLP* and *FRT80B* as described[20], using *M(3)RpS17*[4] to enhance clone size. For wing and eye clones generated using *hsFLP*, heat shocks of 1 h at 38 °C were administered at 24–48 h and again at 48–72 h of development. For germline clones, *hsFLP* was used together with an ovo^{D1} insertion[27] on the *FRT80B* chromosome (gift of K. Basler). Heterozygous third instar larvae were heat shocked for 35 min at 39 °C, and adult females crossed to males carrying the appropriate third chromosome over a TM3, *Ubx-lacZ* balancer. In order to generate germline clones in a Mtl^{Δ} homozygous background, Mtl^{Δ} was first recombined onto the ovo^{D1} *FRT80B* chromosome by FLP-induced germline recombination in males.

Histology

Embryos were fixed and stained with monoclonal antibodies 1D4, 22C10, or FMM5, as described[28]. Embryonic cuticle preparations and F-actin staining were performed as described[5], using rhodamine-conjugated phalloidin (Molecular Probes, 4 U ml^{-1}). Pupal wings and eyes were prepared and stained with rhodamine-phalloidin and mouse anti-β-galactosidase (Promega, 1:200), as described[29,30]. Adult head sections were prepared and stained as described[20].

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Rac GTPases control axon growth, guidance and branching

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Growth, guidance and branching of axons are all essential processes for the precise wiring of the nervous system. Rho family GTPases transduce extracellular signals to regulate the actin cytoskeleton[1]. In particular, Rac has been implicated in axon growth and guidance[2–8]. Here we analyse the loss-of-function phenotypes of three Rac GTPases in *Drosophila* mushroom body neurons. We show that progressive loss of combined Rac1, Rac2 and Mtl activity leads first to defects in axon branching, then guidance, and finally growth. Expression of a Rac1 effector domain mutant that does not bind Pak rescues growth, partially rescues guidance, but does not rescue branching defects of *Rac* mutant neurons. Mosaic analysis reveals both cell autonomous and non-autonomous functions for Rac GTPases, the latter manifesting itself as a strong community effect in axon guidance and branching. These results demonstrate the central role of Rac GTPases in multiple aspects of axon development *in vivo*, and suggest that axon growth, guidance and branching could be controlled by differential activation of Rac signalling pathways.

The *Drosophila* genome has two *Rac* genes that share 92% amino acid sequence identity and have overlapping expression patterns[2,9,10]. A highly related *Mig-2-like* (*Mtl*) gene, the orthologue of *Caenorhabditis elegans mig-2* (ref. 4), is present on the same chromosome[11,12]. To isolate loss-of-function mutants of *Rac1* and *Rac2*, we generated small deficiencies by means of imprecise excision of nearby P-elements (Fig. 1a, b). The $Rac2^{\Delta}$ excision disrupted only the *Rac2* open reading frame (ORF) (Fig. 1a), and hence is a *Rac2*-specific null mutation, but is homozygous viable. The *Df(3)Rac1* excision disrupted the *Rac1* ORF and two adjacent genes (Fig. 1b). *Rac1* point mutations were then recovered from