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Opposing Feedbacks on Ras Tune RTK Signaling

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Abstract

Signaling in development is not always on or off; often, distinct intensity and duration of signaling leads to distinct outcomes. This is true for receptor-tyrosine-kinase (RTK) signaling in many contexts, where negative feedback often plays a role. Although such negative feedback might reduce or even turn off signaling output over time, continued signaling is often maintained for proper cell fate specification. In this issue, Sieglitz *et al.* identify a positive regulator of Ras-mediated RTK signaling that they name Rau. Rau is necessary to achieve specific signaling intensity for the differentiation of photoreceptors and of glia that wrap axons in the developing *Drosophila* eye disc. Both the negative regulator Sprouty and Rau influence signaling through the GTPase Ras: Rau forms a positive feedback loop important for counteracting the Sprouty negative feedback loop.

The signaling pathways mediated by the epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR) (simplified in Fig. 1A) play important roles in cell-to-cell communication in both vertebrates and invertebrates, regulating cell proliferation, survival, patterning, and differentiation (1, 2). Tightly controlled spatial and temporal EGFR signaling specifies numerous cell fates in the *Drosophila* eye and the *C. elegans* vulva (3–6), which have served as two main models to identify members of the EGFR pathway. These factors are highly conserved across multicellular organisms (7) and mutations in this pathway are commonly involved in human cancers [reviewed in (8)]. *Drosophila* continues to provide a genetically tractable model that has been used to study receptor tyrosine kinase (RTK) signal transduction in development using genetic, biochemical, molecular, and genomic approaches (6, 9).

One of the best-characterized examples of EGFR signaling is the specification of diverse cell fates in the *Drosophila* retina. Repeated rounds of signaling recruit cells from an undifferentiated epithelium in a stereotyped sequence and culminate in specification of the R7 photoreceptor (10). Sieglitz *et al.* (11) bring new perspective to this system by examining the role of a previously uncharacterized gene which they name *rau* (“rough” in German) for the rough eye phenotype that its disruption produces. They go on to show that RTK signaling through the FGFR receptor is also modified by Rau, which is required to induce

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the correct amount of wrapping by glial cells when they come in contact with photoreceptor axons.

The *rau* null phenotype causes loss of about 17% of R7 cells in the retina (these cells are instead recruited as cone cells, Fig. 1B). This partial phenotype indicates that Rau has a modulatory role and is most likely involved in establishing specific signaling intensity or duration. Removing one copy of the Ras signaling repressor *sprouty* rescues the corresponding *rau* phenotype (making the eye less rough), whereas removing a copy of *pointed* (which encodes Pointed-P2, a positive effector of RTK signaling) enhances it. Reciprocally, overexpression of Rau leads to the same phenotype as does *sprouty* loss of function. Similar genetic interactions are observed for the formation of wrapping glial cells, which depend on FGFR but not EGFR signaling (Fig. 1C). In these cells, initially high signaling activity is required to specify wrapping fate as migrating glial cells come into contact with photoreceptor axons. Signaling must then be carefully controlled: too much signaling (through either increased expression of *rau* or loss of *sprouty*) results in over-wrapping, and too little signaling results in under-wrapping (Fig. 1C). The authors conclude that Rau and Sprouty play antagonistic roles during eye development: Rau is part of a positive auto-regulatory loop in RTK signaling that seems to counteract the negative auto-regulatory loop mediated by Sprouty.

The authors also examine the genetic interactions between *sprouty*, *rau*, and *seven-up* (a Ras pathway target) using specification of wrapping glial cell fate. They show that *rau* and *sprouty* are both activated by FGF but respond differently to *seven-up*: *sprouty* is negatively regulated by *seven-up*, and the resulting increase in RTK signaling may allow for more *rau* expression. They go on to show biochemically that Rau interacts directly with Ras through two Ras-association domains that together prefer activated, GTP-bound Ras (RasGTP). They propose a model by which Rau provides a platform that results in local increases of RasGTP abundance, which subsequently promotes Ras signaling.

One interesting feature of both developmental contexts is the incomplete and variable phenotype – each provides a direct phenotypic outcome of what happens when the amount of signaling is increased or decreased. In the case of FGF signaling, this means over- or under-wrapping of glia around axons in a variable percentage of cases. The number of times this happens and the amount of wrapping can both be quantified. Similarly, in the induction of R7 cell fate, changes to the pathway such as over-expression or loss of Rau results in too many or too few R7 cells – but again, in just a percentage of cases. This can be quantified as an unambiguous readout of the amount of signaling, and Sieglitz and colleagues use this approach to demonstrate the role of Rau as a positive effector.

Rau appears to have a modulatory role that boosts Ras activity without being essential for signaling itself. Although the primary phenotype of the *rau* null allele is its effect on the eye, *rau* is also expressed in other developmental contexts that are known to involve RTK signaling such as in the *Drosophila* embryonic nervous system and the wing. Though loss of *rau* in the embryonic CNS lacks a clear phenotype in laboratory conditions, it might still play a role in maintaining appropriate signaling under more natural (and variable) conditions. Lower EGFR signaling recruits cone cells almost concurrently with R7 cells, and

simply increasing the amount of signaling activity causes potential cone cells to take an incorrect R7 fate. Signaling activity is again important in the specification of wrapping glia where over- or under-activation of the FGFR pathway produces defects. Rau may help provide a buffering effect in these contexts, and its loss sensitizes the system in a way that reveals underlying variability by changing the ratio of opposing outcomes.

Antagonism between two regulatory loops represents a theme often found in complex regulatory pathways that need to maintain a careful balance of activation. Though Rau does not have a direct homolog in vertebrates, it is probable that an equivalent positive feedback loop exists. Such opposing inputs might also be used when a signaling system functions in contexts where it might either require homeostatic regulation through negative feedback or, as is the case in cell fate determination, a bistable output that requires positive regulation (12). Characterization of Rau as a positive feedback mechanism moves us closer to fully understanding the mechanisms that establish a correct response to RTK-associated ligands.

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Figure 1. Positive and negative feedback in RTK signaling

(A) RTK signaling through EGFR or FGFR can both act through Ras and the subsequent phosphorylation cascade. This results in phosphorylation of mitogen-activated protein kinase (MAPK) and its translocation into the nucleus where it promotes activation of Pointed-P2, a transcriptional activator, and export of the repressor Yan from the nucleus. This can both de-repress and directly activate transcriptional targets such as *sprouty* and potentially *rau*. Rau acts in two contexts in the *Drosophila* eye as a positive effector of Ras to help counterbalance the negative feedback of Sprouty. In photoreceptor differentiation (B), a high activity of EGFR signaling and positive feedback from Rau is required for specification of the R7 cell. Without the action of Rau, or upon reduced signaling, cone cell fate is sometimes specified (right), which requires lower activity of EGFR signaling. In the specification of wrapping glia (C), high activity of FGFR signaling and Rau are required for induction of wrapping glia fate. After initial specification, signaling activity remains important: too much results in over-wrapping, whereas too little, such as through loss of positive feedback from Rau, results in under-wrapping of axons.