Senseless represses nuclear transduction of Egfr pathway activation

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Summary

The Epidermal growth factor receptor (Egfr) pathway controls cell fate decisions throughout phylogeny. Typically, binding of secreted ligands to Egfr on the cell surface initiates a well-described cascade of events that ultimately invokes transcriptional changes in the nucleus. In contrast, the mechanisms by which autocrine effects are regulated in the signal-producing cell are less clear. Ligand/receptor interactions are of critical importance during Development, as recruitment and differentiation of almost all photoreceptors are dependent on high levels of activation of the Egfr pathway (Freeman, 1994; Kumar et al., 1998; Lesokhin et al., 1999; Yang and Baker, 2001). Binding of activating ligand to Egfr results in a cascade of membranous and cytoplasmic phosphorylation events, ultimately resulting in the translocation of phosphorylated ERK to the nucleus where it phosphorylates the pathway at the level of the receptor and within the cytoplasm (Chen and Chien, 1999; Freeman, 1994; Kumar et al., 2003; Kumar et al., 1998; Lesokhin et al., 1999; Rawlins et al., 2003; Spencer and Cagan, 2003; Tio et al., 1994; Zak and Shilo, 1992). However, despite this apparent activation of the pathway at the level of the receptor and within the cytoplasm, a number of nuclear indicators of Egfr activation are either not detected or decreased in R8. First, Rough (Ro), a homeodomain-containing protein that is an early target of Egfr activation, is not expressed in R8 (Dokucu et al., 1996; Kimmel et al., 1990). Second, transcription of argos (aos), which encodes a negative regulator of Egfr signaling, is decreased in R8 relative to other photoreceptors (Lesokhin et al., 1999). As aos expression is required Egfr activation to differentiate (Fig. 1A) (Kumar et al., 1998; Yamada et al., 2003; Yang and Baker, 2001).

Although Egfr pathway signaling is not required for R8 differentiation, several lines of evidence indicate that the uppermost tiers of the Egfr pathway are activated at a high level in the differentiating R8 photoreceptor: (1) R8 is very probably exposed to high levels of secreted Spi, the Egfr ligand in the eye, as R8 itself is the major source of Spi during photoreceptor recruitment; (2) Egfr protein is ubiquitously expressed at high levels at the time when early photoreceptor fates are assumed; and (3) high levels of ERK activation, as assayed by an antibody to dpERK (dual phosphorylated ERK), are detected in R8 intermediate groups and R8 itself, but only in the cytoplasm (Chen and Chien, 1999; Freeman, 1994; Kumar et al., 2003; Kumar et al., 1998; Lesokhin et al., 1999; Rawlins et al., 2003; Spencer and Cagan, 2003; Tio et al., 1994; Zak and Shilo, 1992). However, despite this apparent activation of the pathway at the level of the receptor and within the cytoplasm, a number of nuclear indicators of Egfr activation are either not detected or decreased in R8. First, Rough (Ro), a homeodomain-containing protein that is an early target of Egfr activation, is not expressed in R8 (Dokucu et al., 1996; Frankfort et al., 2001; Kimmel et al., 1990). Second, transcription of argos (aos), which encodes a negative regulator of Egfr signaling, is decreased in R8 relative to other photoreceptors (Lesokhin et al., 1999). As aos expression is required Egfr activation to differentiate (Fig. 1A) (Kumar et al., 1998; Yamada et al., 2003; Yang and Baker, 2001).

Introduction

Interactions between receptors and their ligands are frequently used during development to distinguish one cell type from another. While much is known about the consequences of ligand/receptor binding in the signal-receiving cell, the mechanisms by which autocrine effects are prevented in the signal-producing cell are less clear. Ligand/receptor interactions are of critical importance during Drosophila eye development, as recruitment and differentiation of almost all photoreceptors are dependent on high levels of activation of the Egfr pathway (Freeman, 1996; Kumar et al., 1998; Lesokhin et al., 1999; Yang and Baker, 2001). Binding of activating ligand to Egfr results in a cascade of membranous and cytoplasmic phosphorylation events, ultimately resulting in the translocation of phosphorylated ERK to the nucleus where it phosphorylates the pathway at the level of the receptor and within the cytoplasm (Chen and Chien, 1999; Freeman, 1994; Kumar et al., 2003; Kumar et al., 1998; Lesokhin et al., 1999; Rawlins et al., 2003; Spencer and Cagan, 2003; Tio et al., 1994; Zak and Shilo, 1992). However, despite this apparent activation of the pathway at the level of the receptor and within the cytoplasm, a number of nuclear indicators of Egfr activation are either not detected or decreased in R8. First, Rough (Ro), a homeodomain-containing protein that is an early target of Egfr activation, is not expressed in R8 (Dokucu et al., 1996; Frankfort et al., 2001; Kimmel et al., 1990). Second, transcription of argos (aos), which encodes a negative regulator of Egfr signaling, is decreased in R8 relative to other photoreceptors (Lesokhin et al., 1999). As aos expression is
thought to occur proportionately to the degree of Egfr signaling in that cell, this implies that the Egfr pathway is activated at lower levels in R8 than in non-R8 photoreceptors (Golembo et al., 1996). It is probable that this reduction in Egfr signaling from cytoplasm to nucleus in R8 is developmentally important because high levels of activation of Egfr signaling induced by either ectopic expression or the Egfr\textsuperscript{E2} mutation result in the development of very few R8 photoreceptors, suggesting that high levels of Egfr signaling are not compatible with R8 differentiation (Dominguez et al., 1998; Kumar et al., 1998).

Thus, R8 serves as the signal-producing epicenter for Egfr-dependent recruitment in developing ommatidia, yet must remain refractory to the very signaling events that it initiates.

R8 development requires the actions of the proneural gene atonal (ato) and its downstream effector, senseless (sens), which encode a conserved C2H2 zinc finger transcription factor (Frankfort et al., 2001; Jarman et al., 1994; Nolo et al., 2000). Several specific substages of R8 development have been identified (reviewed by Frankfort and Mardon, 2002). In particular, there is a distinction between R8 selection, the choosing of an R8 precursor from a group of developmentally equivalent cells, and R8 differentiation, a later process by which R8 fate is ‘locked’ and the expression of neural markers is initiated. sens is required only in R8, and mutations in sens result in a total failure of R8 differentiation despite normal selection of R8 precursor cells (called presumptive R8s, or pre-R8s) (Frankfort et al., 2001; Nolo et al., 2000). Furthermore, in sens mutants, the pre-R8 cell instead consistently differentiates as a founder photoreceptor of the R2/R5 subtype (Fig. 1B) (Frankfort et al., 2001), or R2/R5 is normally the first founder photoreceptor of the R2/R5 subtype (Fig. 1B) (Frankfort et al., 2001; Nolo et al., 2000). Furthermore, in sens mutants, the pre-R8 cell instead consistently differentiates as a founder photoreceptor of the R2/R5 subtype (Fig. 1B) (Frankfort et al., 2001), Nolo et al., 2000).

Fig. 1. R8 and non-R8 photoreceptors differentiate according to distinct developmental paradigms. (A) Non-R8 photoreceptors are recruited by Egfr signaling. Spitz (Spi, purple circles) is initially secreted by R8 and binds to the Egfr receptor to induce photoreceptor differentiation (yellow). As the field of Spi expands outward from R8, photoreceptors differentiate in a stepwise fashion (R2/R5, R3/R4, R1/R6, R7). R8 differentiation does not require Egfr pathway activation (blue). (B) In wild-type ommatidia, the pre-R8 cell expresses Ro and Rough (Ro) expression is repressed. The pre-R8 cell then differentiates as an R8 photoreceptor. In sens mutant ommatidia (bottom), the pre-R8 cell expresses Ro and differentiates as a cell of the R2/R5 subtype. (C) R8 differentiation requires Ato and Sens function, while R2/R5 differentiation requires Spi/Egfr activation, which in turn induces Ro expression. Since Sens is a repressor of Ro in R8, it is possible that this prevention of Ro expression occurs via Sens-mediated repression of the Spi/Egfr pathway.

Materials and methods

Drosophila genetics and clonal analysis

All Drosophila crosses were carried out at 25°C on standard media. For clonal analyses in Figs 2 and 3, GMRP35; rho-3\textsuperscript{1} rho-1\textsuperscript{TM43} FRT80B/TM6B (Wasserman et al., 2000), w; sens\textsuperscript{E2} FRT80B/TM6B (Frankfort et al., 2001), w; egfr\textsuperscript{E2} pxx,CyO; sens\textsuperscript{E2} FRT80B/TM6B (this work), or GMRP35; rho-3\textsuperscript{1} rho-1\textsuperscript{TM43} sens\textsuperscript{E2} FRT80B/TM6B (this work) were crossed to y w hsFLP122; P[w\textsuperscript{+}=ubigFP]61EF M(3)i(55) P[w\textsuperscript{+}=ubigFP]61EF M(3)i(55) y w; ro-3\textsuperscript{1} rho-1\textsuperscript{7M43} sens E2 FRT80B/TM6B (Frankfort et al., 2001), w; egfr\textsuperscript{E2} pxx,CyO; P[w\textsuperscript{+}=shbid]; sens\textsuperscript{E1} FRT80B/TM6B (this work) (Tio et al., 1994) was crossed to y w hsFLP; P[w\textsuperscript{+}=arm-lacZ] FRT40A; P[w\textsuperscript{+}=arm-lacZ] FRT80B (this work); or GMRP35; rho-3\textsuperscript{1} rho-1\textsuperscript{TM43} sens\textsuperscript{E2} FRT80B/TM6B (this work) was crossed to y w hsFLP122; P[w\textsuperscript{+}=ubigFP]61EF M(3)i(55) P[w\textsuperscript{+}=ubigFP]61EF M(3)i(55) y w; ro-3\textsuperscript{1} rho-1\textsuperscript{7M43} FRT80B/TM6B (Frankfort et al., 2001). Clones were induced as previously described (Frankfort et al., 2001). Misexpression of sens in the egfr\textsuperscript{E2} background was accomplished through a cross between w; egfr\textsuperscript{E2} pxx,CyO; ey-GAL4 and w; egfr\textsuperscript{E2} pxx,CyO; UAS-sens. The fplout-GAL4 clones shown in Fig. 4 were generated as previously described (Dominguez et al., 1998) with crosses between y w P[w\textsuperscript{+}=actx+ca2<GALA]; hsFLP MKRS/Tb and either UAS-Egfr\textsuperscript{E2} or UAS-lacZ (Dominguez et al., 1998), UAS-Egfr\textsuperscript{E2} pnt\textsuperscript{1277} (this work), or UAS-sens; UAS-Egfr\textsuperscript{E2} pnt\textsuperscript{1277} (this work). Clonal misexpression experiments in Fig. 5 were performed essentially as described, except...
with crosses between y w hsFLP; FRT40A sca-GAL4/CyO P[w=hsFlp] females and w; M{2}24F P[w=arm-lacZ] P[w=tub-GAL80] FRT40A/v+; UAS-gene/TM6B males, where 'gene' represents Egfr
tp

Egfr act males, where 'gene' represents Egfr activation. We blocked Egfr activation by removing function of both rhomboid-1 (rho-1) and rhomboid-3 (rho-3; FlyBase: roughoid, ru). Loss of both rho-1 and rho-3 function prevents processing of secreted Egfr ligands, including Spi, and results in the loss of all ERK (MAP kinase) activation (Urban et al., 2002; Wasserman et al., 2000). Furthermore, loss of rho-1 and rho-3 phenocopies Egfr loss-of-function in that only R8 cells differentiate (Fig. 2A) (Wasserman et al., 2000). Loss of sens function results in pre-R8 differentiation as a founder R2/R5 cell which is sufficient to recruit a reduced number of photoreceptors (Fig. 2B). However, the absence of rho-1, rho-3 and sens together causes total photoreceptor loss, except for a few photoreceptors near the clonal boundary that are rescued non-autonomously by neighboring wild-type cells that produce and process Spi appropriately (Fig. 2C) (Frankfort et al., 2001). A similar phenotype is detected in tissue mutant for both spi and sens (Fig. 2D). This loss of photoreceptors seen in rho-1 rho-3 sens and spi sens mutants is not due to cell death because apoptosis was prevented in these experiments by expression of GMR-p35 (see Materials and methods) (Hay et al., 1994). Furthermore, pre-R8 selection still occurs in both rho-1 rho-3 and rho-1 rho-3 sens mutant tissue, suggesting that a potential founding photoreceptor is present (Fig. 2E,F). Therefore, our interpretation of these results is that, in the absence of sens function, pre-R8 differentiation as a founder R2/R5 photoreceptor requires activation of the Egfr signaling pathway via the Spi ligand. In other words, in sens mutants, the pre-R8 switches from a Spi/Egfr-independent R8 differentiation pathway to a Spi/Egfr-dependent R2/R5 differentiation pathway.

Sens is a negative regulator of the Egfr pathway

When rho-1 and rho-3 function are removed in sens ro double mutants, R8 differentiation does not occur (Fig. 2G). This suggests that the requirement in the pre-R8 cell for Egfr activation remains even when ro function is removed, and that the ro-independent function of sens may involve a relationship with the Egfr pathway. Specifically, as the pre-R8 normally does not require Egfr activation but becomes completely dependent on Egfr activation when sens function is removed, we hypothesized that sens normally acts as a repressor not only of ro, but also of Egfr pathway activation in R8. This potential function of sens as a repressor of Egfr signaling is supported by genetic interactions between sens and the gain-of-function EgfrElp mutation. EgfrElp homozygotes have a greatly reduced number of ommatidia with large gaps of pigmented tissue between them (Fig. 3A) (Baker and Rubin, 1989). In contrast, sens mutant tissue is disrupted in appearance but does not contain undifferentiated gaps between ommatidia (Fig. 3B) (Frankfort et al., 2001). However, when clones of sens mutant tissue are induced in a background that is heterozygous for the EgfrElp mutation, gaps of undifferentiated tissue appear between ommatidia, a phenotype very similar to that of EgfrElp homozygotes (Fig. 3C). Thus, loss of sens function strongly enhances the EgfrElp heterozygous phenotype such that it closely approximates that of EgfrElp homozygotes. If this enhancement occurs by derepression of Egfr signaling by the loss of sens function, then misexpression of sens in an EgfrElp
Fig. 2. The presumptive R8 cell requires Spitz-mediated activation of the Egfr pathway to differentiate in sens mutants. Third instar eye imaginal discs are presented with posterior to the left in this and subsequent figures. (A-C,E-G) Mutant tissue is negatively marked by the absence of GFP (green). (A-C) Neuronal differentiation is indicated by an antibody to Elav (red). (A) Single R8 neurons (overlap with Sens, blue) are detected in rho-1 rho-3 mutant tissue, which lack all activation of the Egfr pathway. This suggests that R8 differentiation does not require Egfr activation. (B) Clusters of variable numbers of neurons are detected in rho-1 rho-3 sens mutant tissue except at the clonal border, where non autonomous effects cause photoreceptor differentiation. This suggests that neuronal differentiation of the pre-R8 as an R2/R5 cell in sens mutants is dependent on Egfr activation. (C) Neurons are not detected in rho-1 rho-3 sens mutant tissue except at the clonal border, where non autonomous effects cause photoreceptor differentiation. This suggests that neuronal differentiation of the pre-R8 as an R2/R5 cell in sens mutants is dependent on Egfr activation. (D) spi sens double mutant tissue is identified by the absence of β-gal (blue) and outlined with the dotted line. (D’ sens mutant tissue is also marked by the absence of Sens (green). Elav (red) marks neurons. (D’’) Overlay of D and D’. Tissue that lacks both spi and sens function does not contain Elav-positive cells except near the borders of the clone, where non autonomous function of spi is sufficient to induce some neuronal differentiation. Differentiation of the pre-R8 as an R2/R5 cell in sens mutant tissue is therefore also dependent on spi function. (E-F) R8 selection (pre-R8) is marked by sca-lacZ (red). (E) Pre-R8s are selected in rho-1 rho-3 mutant tissue. (F) Pre-R8s are still selected in rho-1 rho-3 sens mutant tissue, indicating that the loss of neuronal differentiation in these mutants is not secondary to a failure of R8 selection. (G) Boss (red), a marker for R8 differentiation, is absent in rho-1 rho-3 sens ro mutant tissue, suggesting that the R8 rescue seen in sens ro double mutants cannot occur when Egfr signaling is absent.

Fig. 3. sens is a repressor of the Egfr pathway. (A-D) Light micrographs of adult Drosophila eyes. (A) EgfrE6 homozygotes have few ommatidia as well as prominent gaps of tissue between ommatidia (arrow). (B) sens mutant clone (unpigmented). The sens homozygous mutant tissue is roughened in appearance compared to sens heterozygous tissue, which is wild type in appearance (pigmented). (C) sens mutant clone (unpigmented) induced in an Egfr Elp heterozygous background. Overall the eye is smaller, suggesting a dominant interaction between sens and EgfrE6. In the sens homozygous mutant area, there are reduced numbers of ommatidia, as well as gaps of tissue between ommatidia, similar to EgfrE6 homozygotes (arrow, compare with A). (D) Expression of UAS-sens with ey-GAL4 in an EgfrE6 heterozygous background. Overall the eye is smaller, suggesting a dominant interaction between sens and EgfrE6. In the sens homozygous mutant area, there are reduced numbers of ommatidia, as well as gaps of tissue between ommatidia, similar to EgfrE6 homozygotes (arrow, compare with A). (E,F) Third instar expression of an enhancer trap in the nuclear effector of the Egfr pathway, pntlacZ (pnt-lacZ, red). pnt-lacZ is not expressed in Sens-expressing R8 cells (green, arrow), suggesting that the Egfr pathway is not activated to a high degree in the nucleus of R8.
homozgyote might have the opposite effect and suppress the phenotype of ommatidial loss and interommatidial gaps. Indeed, misexpression of UAS-sens with ey-GAL4 has precisely these effects on Egfr<sup>E3p</sup> homozygotes (Fig. 3D). Together, these gain- and loss-of-function experiments suggest that sens functions as a powerful negative regulator of the Egfr pathway during Drosophila eye development. Since the expression of Sens is tightly restricted to R8 and the primary sens mutant phenotype occurs in the pre-R8 cell, it is most likely that this repression occurs specifically in the differentiating R8 photoreceptor. We therefore looked at expression of an enhancer trap in pointed (pnt-lacZ), which encodes the nuclear effector of the Egfr pathway. Consistent with our hypothesis, pnt-lacZ, while expressed in many non-R8 photoreceptors as they differentiate, is not expressed in Sens-expressing R8 cells (Fig. 3E,F; Materials and methods) (Scholz et al., 1993).

**Sens blocks activation of Egfr signaling at the nuclear level**

While the Egfr pathway is probably activated at a high level at the cell membrane and in the cytoplasm of R8, expression of nuclear outputs of the pathway is low (Fig. 3E,F, see Introduction). Moreover, whereas loss-of-function mutations in all major Egfr pathway members have no effect on R8 differentiation, high levels of activation of Egfr signaling as a result of either ectopic expression or Egfr<sup>E3p</sup> mutations result in the development of very few R8 photoreceptors (Domínguez et al., 1998; Kumar et al., 1998; Lesokhin et al., 1999; Yamada et al., 2003; Yang and Baker, 2001). Thus, the reduction in Egfr activation from high cytoplasmic levels to low nuclear levels in R8 may be of developmental importance. Since Sens acts as a negative regulator of the Egfr pathway, we hypothesized

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**Fig. 4.** Sens prevents Egfr pathway activation in the nucleus. UAS constructs were ubiquitously expressed in clones using flpout-GAL4. (A-A″) Co-misexpression of UAS-Egfr<sup>act</sup> and UAS-lacZ posterior to the morphogenetic furrow (MF). Elav (red) is expressed in almost all cells within the clone (blue). Sens (green) is not detected within the clone. (B,C) Co-misexpression of UAS-Egfr<sup>act</sup> and UAS-lacZ anterior to and within the MF. (B-B″) Elav (red) is expressed within and surrounding the clone (blue). Sens (green) is not expressed within the clone but is ectopically induced non autonomously. (C-C″) dpERK (red) and Sens (green) are expressed non-autonomously. Together, B and C are consistent with the presence of ectopic MFs surrounding areas of Egfr activation. (D-D″) Misexpression of UAS-Egfr<sup>act</sup> anterior to the MF, pointed (pnt) transcription (pnt-lacZ, blue) occurs in most ectopic Elav-positive (red) cells. (E,F) Co-misexpression of UAS-Egfr<sup>act</sup> and UAS-sens anterior to the MF. (E-E″) pnt transcription (pnt-lacZ, blue) does not occur and numbers of Elav-positive cells (red) are greatly reduced in the clone, which is marked by the Sens expressing cells (green). (F-F″) dpERK (red) is expressed autonomously at a high level within the clone, which is marked by Sens-expressing cells (green). Thus, sens is sufficient to block Egfr-induced pnt transcription, photoreceptor differentiation, and ectopic MF generation, but does not prevent dpERK induction.
that Sens mediates this critical decrease in Egfr signaling from membrane/cytoplasm to nucleus in R8.

To test this hypothesis, we ubiquitously expressed an activated form of Egfr (Egfract) in small clones using flpout-GAL4. We first looked at Egfract clones positioned posterior to the morphogenetic furrow (MF). Neural differentiation occurs throughout such clones (Fig. 4A). Since ectopic Egfr activation is sufficient to induce photoreceptor differentiation prior to passage of the MF, these clones probably represent a field of cells that had already differentiated as neurons by the time the MF reached it (Domínguez et al., 1998). Consistent with the hypothesis that high levels of Egfr activation are not compatible with R8 differentiation, these clones show a cell-autonomous lack of Sens expression (Fig. 4A). Anterior to the MF, activation of Egfr signaling causes precocious neural development autonomously, and induces ectopic MFs non-autonomously. These ectopic MFs express Ato, Sens and dpERK appropriately (Fig. 4B,C) (Domínguez et al., 1998). Furthermore, the ectopic MFs express Ato, Sens and dpERK appropriately (Fig. 4B,C) (Domínguez et al., 1998). Furthermore, the ectopic MFs express Ato, Sens and dpERK appropriately (Fig. 4B,C) (Domínguez et al., 1998).

When the Egfr pathway is activated at the cell membrane or within the cytoplasm (Fig. 4F). This suggests that Sens cell-autonomously blocks transduction of the activated Egfr pathway to the nucleus and is sufficient to prevent the effects of cell membrane activation of the Egfr pathway. These results are also consistent with our proposed role for Sens as a repressor of high levels of Egfr signaling in R8.

**Sens represses pointed-P1 in R8**

If Sens acts to reduce Egfr signaling from the cytoplasm to the nucleus of R8, we hypothesized that activation of Egfr signaling downstream of the point at which Sens blocks the pathway could disrupt normal R8 differentiation, whereas activation of the pathway upstream of this point would have little or no effect. To test this hypothesis, we misexpressed members of the Egfr pathway in R8 using sca-GAL4 (Materials and methods). When Egfract or rasval12 (an activated form of Ras that functions in the cytoplasm upstream of ERK) is expressed in R8 with this system there is no appreciable effect on Sens, Boss, or Ro expression in third instar eye imaginal discs (Fig. 5A-C, not shown). This suggests that R8 differentiation proceeds normally when the Egfr pathway is activated at the cell membrane or within the cytoplasm of R8 and is consistent with our proposed role for Sens in R8. Since Sens acts as a repressor of pnt transcription, we also misexpressed both isoforms of pnt in R8. Interestingly, misexpression of the P2 isoform of pnt (pnt-P2), or an activated form of pnt-P2, also has no effect on R8 differentiation (not shown) (Halfon et al., 2000). However, misexpression of pnt-P1 causes a disruption in Sens expression such that Sens-expressing nuclei are displaced apically in the imaginal disc (Fig. 5D,E). Since photoreceptor nuclei move basally during neuronal differentiation, this implies that misexpression of pnt-P1 in R8 may disrupt R8 differentiation. Consistent with this, many sca-GAL4 × UAS-pnt-P1 adult ommatidia do not contain small rhabdomeres, suggesting an absence of R8 (Fig. 5F). Ommatidia also contain a variable number of photoreceptors and these adult phenotypes are very similar to the sens loss-of-function phenotype. These results imply that pnt-P1, but not pnt-P2, may be a target of sens repression. Misexpression of ro, an early target of Egfr signaling, has a more profound effect on R8 development as both Sens and Boss expression are absent.

![Fig. 5. Expression of nuclear effectors of the Egfr pathway](image_url)
represses the pathway at the final step – positive regulation of activation occurs all the way into the nucleus of R8, but Sens (O’Neill et al., 1994). In our model, transduction of Egfr on its own cell membrane, yet remains protected from the deleterious effects of activation of Pnt and other Egfr targets, such as Ro, in R8.

The mechanism by which Sens regulates the discrepancy between levels of Egfr activation at the receptor/cytoplasmic and nuclear levels in R8 is probably through repression of pnt transcription. This is supported by the observation that pnt transcription is not induced by misexpression of an activated form of Egfr when sens is co-misexpressed (Fig. 4). Furthermore, expression of the pnt-P1 isoform in R8 disrupts R8 differentiation (Fig. 5D-F). As misexpression of pnt-P2 has no effect on R8 differentiation, this suggests that Sens negatively regulates transcription of pnt-P1, but not pnt-P2. This mode of regulation is consistent with established models for transduction of the Egfr signal to the nucleus. Specifically, ERK phosphorylates Pnt-P2, which is thought to be a transient positive regulator of pnt-1 transcription (Brunner et al., 1994; O’Neill et al., 1994). In our model, transduction of Egfr activation occurs all the way into the nucleus of R8, but Sens represses the pathway at the final step – positive regulation of pnt-P1 by Pnt-P2 (Fig. 6). When sens function is removed, the block on pnt-P1 transcription is relieved, and Pnt-P1 can exert its transcriptional effects on the nucleus, including Ro induction.

There is evidence that pnt-P1 transcription can be regulated by Egfr signaling independently of pnt-P2 during Drosophila embryogenesis (Gabay et al., 1996). If this is the case during eye development, our model would remain essentially the same – Sens would still act as a negative regulator of pnt-P1 in R8. However, this regulation would occur independently of pnt-P2 rather than downstream of pnt-P2.

Sens is also a potent negative regulator of ro and this relationship appears to specifically affect the cell fate decision between R8 and R2/R5 differentiation (Fig. 5G,H) (Frankfort et al., 2001). Several lines of evidence suggest that Sens-mediated repression of ro is distinct from other effects of Sens in R8. First, loss of ro function does not rescue R8 differentiation in all ommatidia in sens mutants (Frankfort et al., 2001). Second, even those R8 cells that do differentiate in sens ro double mutants require Spi/Egfr pathway activation (Fig. 2G). Third, misexpression of ro in R8 causes a different phenotype than misexpression of pnt-P1 in R8. Specifically, even though Egfr pathway activation is necessary and sufficient for Ro expression, misexpression of pnt-P1 in R8 does not cause an obvious cell fate transformation from R8 to R2/R5, while misexpression of ro in R8 does (Fig. 5D-H) (Dominguez et al., 1998; Hayashi and Saigo, 2001). Indeed, R8 markers are still expressed when pnt-P1 is misexpressed in R8. However, aberrant nuclear movements and the absence of small rhabdomeres at the level of R8 in adults suggest that misexpression of pnt-P1 does perturb R8 differentiation (Fig. 5D). Together, these results suggest that Sens repression of pnt-P1 occurs independently of Sens function as a repressor of ro, and that Sens-mediated repression of pnt-P1 is probably required for normal R8 differentiation upstream or independently of cell fate determination (Fig. 6).

Since Sens acts as a transcription factor and its mammalian homolog, Gfi-1, binds directly to enhancer regions of Ets1 and Ets3, two mammalian orthologs of pnt, it is possible that Sens repression of pnt-P1 expression occurs directly (Duan and Horwitz, 2003; Nolo et al., 2000; Zweidler-Mckay et al., 1996). Gfi-1 also interacts with nuclear matrix proteins to repress transcription (McGhee et al., 2003). Thus, it is possible that Sens represses transcription of Egfr nuclear effectors via a similar mechanism. Future experiments are required to determine which of these or other mechanisms are important during R8 differentiation. However, it is likely that Sens does not act as a positive regulator of Edl/Mae, a proposed cell-autonomous repressor of Egfr signaling, because edl/mae function is not required for normal R8 differentiation (Yamada et al., 2003). Finally, it is also unlikely that sens functions as an activator of yan, which encodes a nuclear repressor of the Egfr pathway, because yan loss-of-function mutations also do not impact R8 differentiation (Lai and Rubin, 1992).

Conservation of Sens/Egfr antagonism?
The positioning of Sens repression downstream of ERK activation may help explain interactions observed between sens and Egfr pathway homologs in T-lymphocytes. In Jurkat T-cells, activation induced cell death (AICD), a process that is required to prevent non-specific activation of T-cells, is dependent, in

![Fig. 6. Model for Sens action in R8. Spi induces Egfr activation and the signal transduction cascade is induced normally. However, Sens prevents transcription of pnt-P1, thereby blocking the pathway at the final step. This relationship is likely to specifically mediate cell differentiation in R8 (see text). Sens also represses ro, an early target of the Egfr pathway. This second relationship regulates the cell fate decision in the founder photoreceptor between R8 and R2/R5 (see text).](image-url)
part, on ERK1/2 activation (van den Brink et al., 1999). Intriguingly, high levels of Gfi-1 have been shown to inhibit AICD despite high levels of ERK1/2 activation (Karsunky et al., 2002). The antagonistic relationship between Sens and the Egfr pathway in R8, in conjunction with the observation that Gfi-1 can bind to the enhancer regions of Ets1 and Ets3, suggest that this inhibition of AICD may occur via Gfi-1-mediated repression of ERK1/2 targets (such as Ets/pnt) in T-cells (Duan and Horwitz, 2003). Thus, our results may establish R8 development as a powerful and novel system with which to study mechanisms of lymphomagenesis, apoptosis and cancer.

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References


