Negative Regulation of EGFR/MAPK Pathway by Pumilio in Drosophila melanogaster

Sung Yun Kim1,2*, Ji Young Kim1,2*, Sumira Malik1, Wonseok Son3, Ki-Sun Kwon2, Changsoo Kim1*
1 Hormone Research Center, School of Biological Sciences and Technology, Chonnam National University, Yongbong-Dong, Gwangju, South Korea, 2 Aging Research Center, Korea Research Institute of Bioscience and Biotechnology, Yuseong-gu, Daejeon, South Korea, 3 Department of Biological Science, KAIST (Korea Advanced Institute of Science and Technology), Daejeon, South Korea

Abstract

In Drosophila melanogaster, specification of wing vein cells and sensory organ precursor (SOP) cells, which later give rise to a bristle, requires EGFR signaling. Here, we show that Pumilio (Pum), an RNA-binding translational repressor, negatively regulates EGFR signaling in wing vein and bristle development. We observed that loss of Pum function yielded extra wing veins and additional bristles. Conversely, overexpression of Pum eliminated wing veins and bristles. Heterozygotes for Pum produced no phenotype on their own, but greatly enhanced phenotypes caused by the enhancement of EGFR signaling. Conversely, over-expression of Pum suppressed the effects of ectopic EGFR signaling. Components of the EGFR signaling pathway are encoded by mRNAs that have Nanos Response Element (NRE)–like sequences in their 3'UTRs; NREs are known to bind Pum to confer regulation in other mRNAs. We show that these NRE-like sequences bind Pum and confer repression on a luciferase reporter in heterologous cells. Taken together, our evidence suggests that Pum functions as a negative regulator of EGFR signaling by directly targeting components of the pathway in Drosophila.

Introduction

A variety of cellular processes such as cell fate specification, proliferation, and apoptosis utilize epidermal growth factor receptor (EGFR) signaling. Upon activation, the signaling proceeds through Drk, Sos, and Ras activation, to a phosphorylation cascade involving Raf (MAPKKK) and Dsor1 (MEK). The pathway culminates in activation of rolled (rl) MAP kinase (MAPK), which phosphorylates a suite of substrates to determine a specific cellular response [1]. Since aberrant signaling results in abnormal organ formation or tumorigenesis [2], intricate spatio-temporal regulation of the signaling is essential. Thus diverse negative regulators are employed to precisely regulate EGFR signaling.

In Drosophila melanogaster, adult wing blade has five wing veins, which are differentiated in the wing imaginal disc during larval and pupal stages. EGFR signaling during the larval period promotes wing vein cell differentiation. Enhanced EGFR signaling results in the development of extra-wing veins, whereas reduced signaling results in wing vein loss [3–10]. Apparently, the levels of EGFR signaling are carefully regulated to ensure normal vein development.

Large bristles (macrochaetes) on the notum of adult flies arise from a single sensory organ precursor (SOP) cell in the wing imaginal disc during larval development. Each SOP cell is selected from a group of equipotent cells in a proneural cluster that is specified by high level expression of proneural genes such as achaete (ac) and scute (sc) [11–15]. Persistent expression of proneural genes in SOP cells requires EGFR signaling [16,17]. Reduced EGFR signaling results in loss of SOP cells in the disc and macrochaetae in the adult [4,16]. Conversely, excess EGFR signaling evokes supernumerary SOP cells by stimulating proneural gene expression [16], in turn causing the formation of extra bristles on the thorax and notum of the adult. Thus, as in the case of wing vein specification, the selection of SOP cells from proneural clusters requires precise regulation of EGFR signaling.

In our study, we observed extra wing veins and thoracic macrochaetae in pum mutants, which is reminiscent of phenotypes associated with up-regulation of EGFR signaling. Our genetic interaction analysis suggests that Pum functions as a negative regulator of EGFR signaling. Pum is a translational repressor that binds to the Nanos Responsive Element (NRE) sequence at the 3'UTR of its target mRNAs [18–20]. We demonstrated that Pum binds to potential NRE sequence found in the EGFR, Drk, Sos, and MAPK (rl) 3'UTRs and represses reporters containing these NRE sequences. This study revealed a role for Pum in formation of wing veins and bristles by negatively regulating EGFR signaling.
Results

Genetic Interaction between Pum and EGFR Signaling in Wing Vein Formation

We observed that, pum7/pum7 and pum1688/pum1688 adult “escapers” have rare extra wing veins (arrowhead in Figure 1D and data not shown). Transheterozygotes of pum alleles, pum+/pum1688, pum11/pum4, pum/pum1688, pum+/pum4, and pum+/pum1688 all displayed similar extra wing vein phenotypes (Figure 1B, 1C and data not shown). The penetrance for extra vein phenotypes is incomplete: pum+/pum1688 (68%, n = 54), pum11/pum4 (77%, n = 175), pum11/pum1688 (55%, n = 81), pum+/pum1688 (37%, n = 45). Extra wing veins also arose where pum function is reduced in wing imaginal disc via RNAi of pum (en-gal4/+; UAS-pum-IR/+) (penetrance > 90%, n = 33); these flies displayed extra-wing veins only in the posterior compartment, where en-GAL4 is active (arrowhead in Figure 1G). Extra wing veins also arise when EGFR/Ras/MAPK signaling is enhanced [3, 21]. For example, a gain of function mutant, rl[Sem] (Figure 1E) (penetrance >90%, n = 93); these flies expressed elevated MAPK activity [3, 22] produced extra vein material (arrowhead in Figure 1E). In addition, wing veins arose in the posterior compartment of the wing when Ras was expressed by en-Gal4 (en-gal4/UAS-Ras) (Figure 1H). Conversely, wing veins are lost when EGFR signaling is reduced (Figure S1) or Pum is overexpressed (Figure 2D).

We explored genetic interactions between Pum and EGFR signaling. Toward this end, we examined the effect of eliminating one copy of pum* on the wing vein phenotypes associated with rI[Sem]. Eliminating one copy of pum (pum+/pum) by itself does not produce ectopic wing vein (data not shown). However, eliminating one copy of pum greatly enhanced the extra wing vein phenotype in rI[Sem] flies (rI[Sem]/+; pum+/+) (arrowhead in Figure 1F) (penetrance > 90%, n >10). Likewise, greatly increased ectopic wing veins were generated when ras activation was combined with pum knock-down (en-gal4/UAS-Ras; UAS-Pum-IR/+). Taken together these results indicate that reduction of pum synergistically enhanced EGFR signaling, consistent with the idea that pum negatively regulates EGFR signaling in wing vein formation.

If pum negatively regulates EGFR signaling, over-expression of pum should override EGFR signaling. We tested this hypothesis and found that ectopic expression of EGFR under ser-Gal4 control (active in the dorsal compartment during the 2nd instar and the dorso-ventral boundary in the third instar) resulted in extra wing veins around the wing boundary (arrowhead in Figure 2B). Ectopic expression of Pum via ser-Gal4 resulted in development of a distal wing notch (Figure 2A). Co-overexpression of pum with EGFR (UAS-pum/+; ser-GAL4/UAS-EGFR) (Figure 2C) suppressed the development of ectopic veins, generating wing indistinguishable from those in which Pum alone is mis-expressed. Likewise, co-ectopic expression of pum with rI[Sem] (UAS-Pum/UAS-rI[Sem]; dppInd-Gal4/+ (Figure 2D) resulted in suppression of ectopic veins caused by rI[Sem] overexpression (UAS-rI[Sem]/+; dppInd-Gal4/+). Further, both loss- and gain-of function of Pum function modify EGFR pathway activity in a manner that suggests negative regulation by Pum.

Pum Negatively Regulates EGFR/Ras/MAPK Signaling in Bristle Formation

The wild-type notum bears macrochaetes at specific position (circles, Figure 3A), whereas escapers of pum homozygote mutants and transheterozygotes of pum alleles (pum13/pum13, pum1688/pum1688, pum+/pum; and pum+/pum) have extra macrochaetes (arrowhead in Figure 3B-E, M; Table 1). The penetrance for this phenotype ranges from 58% to 99%: pum13/pum13 (99%, n = 175), pum1688/pum1688.
Pum Activity in the Wing Disc

Although EGFR pathway component expression and activity have been well characterized in imaginal discs, Pum activity in the discs has not been well documented. By histochemical methods, we found that Pum is uniformly expressed in wing imaginal discs (not shown). To distinguish uniform expression from uniform background, we performed two additional experiments. Using the dpp-GAL4 driver that is active near the anterior-posterior (A/P) compartment boundary (Figure 4A), we over-expressed either wild type Pum or Pum RNAi. As shown in Figure 4, ectopic Pum antigen is detected where dpp-GAL4 is active in the former experiment; conversely the antigen is specifically depleted in the latter experiment. We conclude that Pum is expressed throughout the wing disc and thus available to regulate EGFR pathway components.

We also assayed Pum activity in wing discs using a GFP reporter mRNA bearing NRE sequences in its 3'-UTR [23]. Modulating the level of Pum near the A/P compartment boundary via dpp-GAL4 regulates accumulation of GFP encoded by this reporter (Figure 4B, C) demonstrating that Pum is active in the wing disc. Furthermore, over-expression of Nos, which is a cofactor of Pum in other tissues, negatively regulates GFP (Figure 4D). Thus, Pum is both expressed and active in the wing disc.

Pum can Repress Translation of EGFR and Rl through Binding to NRE Sequences in their 3'-UTRs

The genetic analysis described above indicates that Pum down-regulates EGFR signaling. Thus we searched the 3'-UTRs encoding EGFR components for potential NRE sequences [27]. Pum binds to the NREs of EGFR and Rl (Figure 4E, F) as a first step in determining whether regulation by Pum might be direct [24,25]. A genome-wide screen had previously shown that some mRNAs of EGFR pathway can be co-precipitated with Pum [24,25]. We identified two putative NRE-like sequences in each of the EGFR, Raf, and Drk 3'-UTRs, termed NRE 1 and NRE 2; Ras, Rolled (Rl), and Sos each possess a single putative NRE-like sequence. We next examined whether Pum binds directly to these putative NREs. Using a well-characterized yeast three-hybrid assay for Pum binding [26] we found that Pum bound to the EGFR NRE1, Rl NRE, Sos NRE, and Drk NRE1 sequences (Figure 5A; Table 2). Binding was abolished by mutation of the NREs. A genome-wide screen had previously shown that some mRNAs of EGFR pathway can be co-precipitated with Pum.
Pumilio Regulation of EGFR/MAPK Signaling

A. WT
B. pum^{1688}/pum^{3}
C. pum^{3}/pum^{Mac}
D. pum^{1}/pum^{Mac}

E. pum^{1688}/pum^{Mac}
F. Sca>Egfr
G. Sca>Egfr; pum^{1688}/+
H. Sca>Egfr; pum^{Mac}/+

I. Sca>Pum
J. Sca>Pum+Egfr
K. C253>Pum^{HD}
L. C253>pum^{HD} +Egfr

M. Number of thoracic bristles

N. Number of thoracic bristles

- WT
- 1688/1688
- 1688/Msc
- 1/Msc
- 1688/3/Msc

- WT
- sca>egfr 1688
- sca>egfr 1/Msc
- C253>Pum
- C253>pum^{HD}
- C253>pum^{HD} +Egfr
Discussion

We have shown that, in the absence of Pum, extra bristles and wing veins develop, while over-expression of Pum eliminates bristles and wing veins. Several lines of evidence show that the role of Pum is to negatively regulate development of wing veins and bristles. First, loss- and gain-of Pum function produced aberrant wing vein and bristle phenotypes that are inverse to those produced by altered EGFR signaling. Second, reduction of Pum activity greatly enhanced phenotypes associated with reduced EGFR signaling. Third, concomitant expression of Pum suppressed phenotypes associated with ectopic EGFR signaling. In support of the genetic conclusion, we show that Pum binds the NRE-like sequence of EGFR, Rl, Sos, and Drk mRNAs and represses translation of a reporter containing these sequences in heterologous cells, suggesting that Pum is a negative regulator of EGFR signaling.

Materials and Methods

Drosophila Strains

The pum alleles were used as follows; pumG12, pumA9, pum1, pum2 and pum7 [30–34]. The UAS-PumH flies (a gift from Y. Jan) can drive expression of the RNA binding region of Pum (1092–1427) in vivo RNA interference) lines were obtained from VDRC RNAi [35]. UAS-Pum-IR, UAS-EGFR-IR, UAS-Raf-IR and UAS-Raf-IR (in vivo RNA interference) lines were obtained from VDRC RNAi library [36]. UAS-NosH1 line was obtained from H. Lin. EGFR

Table 1: The number of macrochaetes reveals genetic interaction between Pum and EGFR.

| Genotype                        | Average number of macrochaetes * | s.d.   | n    | P     |
|---------------------------------|----------------------------------|--------|------|-------|
| w1118                           | 8.00                             | (± 0.00) | 46  |       |
| pum1688/+; pum1688              | 9.86                             | (± 1.95) | 28  | <0.0001** |
| pum1/+; pum1688                 | 10.21                            | (± 1.63) | 29  | <0.0001 |
| pum1/pum1                       | 10.31                            | (± 1.36) | 51  | <0.0001 |
| pum1688/+; pum1688              | 13.86                            | (± 1.30) | 29  | <0.0001 |
| pum1688/+                         | 10.81                            | (± 1.55) | 52  | <0.0001 |
| pum1688/+                       | 10.58                            | (± 0.94) | 52  | <0.0001 |
| sca-GAL4/UAS-EGFR               | 9.37                             | (± 1.58) | 40  |       |
| pum1088/TM3                     | 8.27                             | (± 1.15) | 22  |       |
| sca-GAL4/UAS-EGFR; pum1088/+    | 13.43                            | (± 1.40) | 10  |       |
| pum1688/TM3                     | 8.15                             | (± 0.37) | 20  |       |
| sca-GAL4/UAS-EGFR; pum1688/+    | 12.50                            | (± 0.58) | 8   |       |
| sca-GAL4/UAS-Pum                 | 0.35                             | (± 0.61) | 17  |       |
| sca-GAL4/UAS-Pum; UAS-EGFR/+    | 0.88                             | (± 0.70) | 17  |       |
| C253-GAL4/UAS-EGFR              | 8.08                             | (± 0.28) | 38  |       |
| C253-GAL4/UAS-Pum               | 5.97                             | (± 1.11) | 60  |       |
| C253-GAL4/UAS-Pum; UAS-EGFR/+   | 6.33                             | (± 0.99) | 24  | 0.167 |
| C253-GAL4/UAS-Pum10             | 2.73                             | (± 0.98) | 26  |       |
| C253-GAL4/UAS-Pum10; UAS-EGFR/+ | 3.42                             | (± 0.79) | 21  | 0.013 |

*Bristles circled in the Figure 3A were counted, s.d., standard deviation, N, number of flies counted. P, P-value by student’s t-test.

**pum mutants compared to w1118

doi:10.1371/journal.pone.0034016.t001

To define Pum’s role in the development of wing veins and bristles precisely, we attempted to locate Pum protein and measure Pum activity through a GFP-NRE construct in the 3rd-instar larval and pupal wing imaginal discs where wing vein and SOP cells are specified. We obtained a low-level ubiquitous expression of Pum and broad Pum activity, suggesting that Pum might function as a general attenuator of EGFR signaling.

Our discovery of negative regulation of EGFR signaling by Pum is not confined to Drosophila somatic cells, since it has also been reported in germline cells of C. elegans, cultured human stem cells, and yeast cells [28,29]. Thus, it is likely that Pum regulation of EGFR signaling is universal and involves diverse developmental contexts, ranging from C. elegans to Drosophila and humans.
pathway alleles and transgenes were used as follows: \( \alpha^{\text{Sem}} \); UAS-EGFR, UAS-\( \alpha^{\text{Sem}} \); UAS-Ras [9]; Ga4 lines, sea-Ga4 and C253-Ga4 drive expression in proneural clusters [15,16,38]; dppdisk-Ga4 [39], ser-Ga4, and en-Ga4 drive expression in a stripe at the anterior-posterior boundary, dorso-ventral boundary, and in the posterior compartment of wing pouch, respectively. They are described in detail at FlyBase (http://flybase.bio.indiana.edu).

UAS-Pum lines were constructed as follows; the full-length Pum coding sequence was removed from pOT2-pum (LD44635) (Drosophila Genomics Resource Center, Bloomington, IN) via digestion with EcoRI, XbaI digestion. The ends were filled using Klenow fragment. The pum sequence was then cloned into the XhoI site (blunted with Klenow) of pINDY5 [40] to create UAS-Pum. The construct was verified by DNA sequencing. Five independent transgenic flies were produced and used in this study.

Yeast Three-hybrid Assays

The C-terminal Puf region (1093~ 1427) of the Drosophila Pum was generated from the LD44635 by polymerase chain reaction (PCR) and cloned into EcoRI/XhoI sites of pACT2 AD vector (Clontech) to produce Ga4 transcriptional activation domain (GAD-Puf (GAD-Puf)). The NRE and its mutant fragments were generated by oligomer dimerization, and inserted into the Smal/ SphI sites of pHIA/MS2-2 to express NRE-MS2 RNA for three-hybrid test [41]. The sequences of the primers were shown in the Table 2. All constructs were verified by DNA sequencing. pHI/MS2/\( \beta \)NRE and pHI/MS2/\( \beta \)NRE mt produce \( \beta \)NRE-MS2 RNA and \( \beta \)NREmt-MS2 RNA, respectively. Yeast strain, YPH-500 (MATa, ura3-52, lys 2-80, ade2-101, trp1-D63, his3-D200, leu2-D1), was used to analyze RNA-protein interaction and three-hybrid assays were performed as described [41]. In brief, plasmid expressing the LexA-Puf fusion protein, GAD-MS2 coat protein, and an NRE-MS2 RNA were co-transformed into yeast strain YPH-500 harboring the lexAop-LacZ reporter. Liquid assays for \( \beta \)-galactosidase activity of three or more transformants were carried out as described [27,42].

Luciferase Reporter Assays

A full-length Pum from LD44635 was cloned into the EcoRI/XhoI sites of pcDNA3 (Invitrogen) by PCR. The same NRE.
The 3rd instar larval wing discs were dissected in PBS. The tissues were then exposed to 4oC for at least 15 minutes with gentle rocking in 4% formaldehyde in PBS. After fixation, the tissues were washed three times in PBT (PBS, 0.1% Tween-20) at RT for 15 minutes. The tissues were then blocked for 1 hour by 5% normal Goat serum in PBS. After fixation, the tissues were washed three times in PBT (PBS, 0.1% Tween-20) at RT for 15 minutes. The subsequent washes and mounting were performed according to the VectaShield Mounting medium (Vector Laboratories). The following antibodies were used: Anti-rabbit Alexa 488 conjugated (1:200); anti-rat Rhodamine conjugated (1:200); and anti-mouse Alexa 568 conjugated (1:200) (molecular Probes). The fragments used for the yeast three-hybrid assay were inserted into the BamHI/Xhol sites of pcDNA3-LUC [27]. HEK293 cells were maintained with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum for 24 h and transiently transfected with the appropriate set of reporter and expression plasmids using SuperFect reagent (Qiagen). For reporter assays, 24 h after transfection, cells were harvested and assayed for luciferase activity as described previously [27,42]. The results from triplicate samples were averaged and normalized to the mean + SD values obtained from at least three independent experiments performed in triplicate (+, P < 0.05). The P-values were obtained by student's t-test in SigmaPlot.

**Table 2. Pum binding and repression of NRE-like sequences of EGFR and its transducers.**

| 3'UTR | UGUANAUAA* | Binding** | Repression*** |
|-------|-------------|-----------|---------------|
| hb NRE | GUCAAAAUUGUA[CAUA][AGCGG | +++ | Y |
| hb NRE mt | GUCAAAAU[aca][AUCGA][AGCGG | - | N |
| egr NRE 1 | CCAUAGA[UUGUA][AUACUCU | +++ | Y |
| egr NRE 1 mt | CCAUAGA[aca][AUACUCU | - | N |
| egr NRE 2 | UCCUGGCUUGUA[UAGCCAG | - | ND |
| ras NRE | CACGCCAUUGUA[AGAAUGU | - | ND |
| raf NRE 1 | UUGUCCUCUGUA[AGAAGGA | - | N |
| raf NRE 1 mt | UUGUCCUC[aca][ACUA][AGAAGGA | - | N |
| raf NRE 2 | AGCCCAUG[UGUA][ACUGC | - | ND |
| raf NRE 2 mt | AGCCCAUG[ACA][ACUGC | - | ND |
| rl NRE | UAAGAAACG[GUCA][UAAUGA | ++ | Y |
| rl NRE mt | UAAGAAACG[aca][UAAUGA | - | N |
| sos NRE | AUAAUAAUUGUA[AGCAGA | +++ | Y |
| sos NRE mt | AUAAUAAU[aca][UAAUGA][AGCAGA | - | N |
| drk NRE 1 | AUCUAGA[UAAU][AUUUUG | +++ | Y |
| drk NRE 1 mt | AUCUAGA[aca][UAAU][AUUUUG | - | N |
| drk NRE 2 | GCCGCCACUGUA[AGAAUGAU | - | ND |

*NRE consensus sequences (UGUANAUAA).

**Puf binding to NRE-like sequence determined by yeast three hybrid assay as shown in Figure 5A, +++; strong binding; ++, moderate binding.

***Pum repression of a reporter containing NRE-like sequence as determined in Figure 5B, Y; repression; N, no repression; ND, not determined.

**Figure 5. Pum binds to the potential NRE-like sequence of EGFR signaling components.** (A) (Upper) Schematic drawing of the yeast three-hybrid assay. An RNA containing the NRE and MS2 sequence recruits both Gal4 transcriptional activation domain (GAD)-MS2 coat protein (CP) fusion protein (GAD-MS2 CP) and lexA DNA binding domain (DBD)-Puf fusion protein (LexA-Puf). The resultant ternary complex leads to the expression of lacZ reporter. (Lower) Yeast YPH500 cells harboring the lexAop-lacZ reporter, LexA DBD-Puf and GAD-MS2 CP were transformed with vectors that allow for expression of diverse NRE-MS2 transcripts as indicated. Liquid β-galactosidase assays were carried out for transformants. The mean ± SD values were obtained from at least three independent experiments performed in triplicate (*, P < 0.05). The P-values were obtained by student's t-test in SigmaPlot.
stained images were processed via the LSM 510 confocal microscope (Zeiss).

Supporting Information

Figure S1 Reduction of EGFR signaling causes loss of wing veins. Wing veins are lost by the reduction of EGFR signaling (en-GAL4/UAS-EGFR-IR (A), en-GAL4/+; UAS-Ras-IR/+ (C), en-GAL4/UAS-Raf-IR (E)). Concomitant reduction of Pum does not overrule vein loss by reduced EGFR signaling (en-GAL4/UAS-EGFR-IR, UAS-Pum-IR/+ (B), en-GAL4/+; UAS-Pum-IR/UAS-Ras-IR (D), en-GAL4/UAS-Raf-IR; pum-IR/+ (F)). (TIF)

References

1. Perrimon N (1994) Signalling pathways initiated by receptor protein tyrosine kinases in Drosophila. Curr Opin Cell Biol 6: 269–266.
2. Downward J (2003) Targeting Ras signalling pathways in cancer therapy. Nat Rev Cancer 3: 11–22.
3. Brunner D, Oellers N, Szabad J, Biggs WH, 3rd, Zipursky SL, et al. (1994) A gain-of-function mutation in Drosophila MAP kinase activates multiple receptor tyrosine kinase signaling pathways. Cell 76: 879–888.
4. Clifford RJ, Schubbach T (1989) Coordinate and differentially mutable activities of torso, the Drosophila melanogaster homolog of the vertebrate EGFR receptor gene. Genetics 123: 771–787.
5. Diaz-Benjumea JJ, Hafen E (1994) The sevenless signalling cassette mediates Drosophila EGF receptor function during epithelial development. Development 120: 569–578.
6. Martin-Blanco E, Roch F, Noll E, Baume A, Dufy JB, et al. (1999) A temporal switch in DER signaling controls the specification and differentiation of veins and interveins in the Drosophila wing. Development 126: 5739–5747.
7. Sturivant MA, Roux M, Bier E (1993) The Drosophila rhomboid gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway. Genes Dev 7: 961–973.
8. Sawamoto K, Okano H, Kohayakawa Y, Hayashi S, Mikoshiba K, et al. (1994) The function of argos in regulating cell fate decisions during Drosophila eye and wing vein development. Dev Biol 164: 267–276.
9. Karim FD, Ruhim GM (1998) Ectopic expression of activated Ras induces hyperplastic growth and increased cell death in Drosophila imaginal tissues. Development 125: 1–9.
10. Marenda DR, Vraillas AD, Rodrigues BA, Cook S, Powers MA, et al. (2006) MAP kinase subcellular localization controls both pattern and proliferation in the developing Drosophila wing. Development 133: 43–51.
11. Cubas P, de Celis JF, Campuzano S, Modolell J (1991) Proneural clusters of achaete-scute expression and the generation of sensory organs in the Drosophila imaginal wing disc. Genes Dev 5: 996–1000.
12. Skarnes WB, Carroll SB (1991) Regulation of achaete-scute gene expression and sensory organ pattern formation in the Drosophila wing. Genes Dev 5: 984–995.
13. Bodner R, Carretto R, Jan YN (1989) Neurogenesis of the peripheral nervous system in Drosophila embryos: DNA replication patterns and cell lineages. Neuron 3: 21–33.
14. Hartenstein V, Posakony JW (1989) Development of adult sensilla on the wing and notum of Drosophila melanogaster. Development 107: 389–405.
15. Cui J, Modolell J (1998) Proneural gene self-stimulation in neural precursors: an essential mechanism for sense organ development that is regulated by Notch signaling. Genes Dev 12: 2036–2047.
16. Cui J, Martin-Blanco E, Modolell J (2001) The EGF receptor and N signalling pathways act antagonistically in Drosophila mesothorax bristle patterning. Development 128: 299–308.
17. zur Lage PI, Powell LM, Prentice DR, McLaughlin P, Jarman AP (2004) EGF receptor signaling triggers recruitment of Drosophila sense organ precursors by stimulating proneural gene autoregulation. Dev Cell 7: 687–696.
18. Degen KA, Aggarwal AK, Whitarton RP (2002) Transpositional repressors in Drosophila. Trends Genet 18: 572–577.
19. Whitarton RP, Sonoda J, Lee T, Patterson M, Murata Y, et al. (1998) The Pumilio RNA-binding domain is also a translational regulator. Mol Cell 1: 863–872.
20. Zanore PD, Bartel DP, Lehmann R, Williamson JR (1999) The PUMILORNA interaction: a single RNA-binding domain monomer recognizes a bipartite target sequence. Biochemistry 38: 596–604.
21. Ciapponi I, Jackson DB, Moshk M, Bohmann D (2001) Drosophila Fos mediates ERK1 and JNK signals via distinct phosphorylation sites. Genes Dev 15: 1540–1553.
22. Oellers N, Hafen E (1996) Biochemical characterization of rolledsem, an activated form of Drosophila mitogen-activated protein kinase. J Biol Chem 271: 24938–24944.

Acknowledgments

We thank R. P. Wharton for providing vectors of yeast three-hybrid system as well as helpful comments and discussion. We thank Y. Jan for pum mutants and UAS-PumIR flies, P. MacDonald for rat anti-Pum antibody, J. Kimble for yeast three-hybrid, and the Bloomington stock centers for fly stocks, and the Berkeley Drosophila Genome Project for the LD4H65 cDNA.

Author Contributions

Conceived and designed the experiments: SYK JK CK. Performed the experiments: SYK JK SM WS KK. Analyzed the data: SYK JK CK. Wrote the paper: SYK JK CK.

Author Contributions

Conceived and designed the experiments: SYK JK CK. Performed the experiments: SYK JK SM WS KK. Analyzed the data: SYK JK CK. Wrote the paper: SYK JK CK.