Supplemental Material

Or85e promoter deletion series

The wild type *Or85e* 3.1 kb promoter–GAL4 construct has been described in Goldman et al. (2005) and has been shown to recapitulate the endogenous expression pattern of *Or85e* in the maxillary palp. This plasmid was used to generate all the smaller promoter deletion products by 5' blunt- 3'NotI cloning of fragments into the blunt-NotI sites of pG4PN (Dobritsa et al., 2003). pG4PN is a P-element transformation vector which is designed to contain promoter sequences upstream of the yeast *GAL4* coding sequence. The fragments generated were ~ 2.4 kb (EcoRI – NotI); ~1.9 kb (HindIII–NotI); ~1.5 kb (XhoI–NotI); ~0.95 kb (NheI-NotI); ~0.45 kb (ScaI-NotI).

The ~0.35 kb and ~0.09 kb promoters were made by Hi-fidelity PCR amplification from the *Or85e* 3.1 kb-promoter-*GAL4* plasmid where the 5' primer was engineered to contain KpnI sites and the 3' primer to contain NotI sites. The PCR products were AT –cloned into pGEM-T Easy and then subcloned directionally into the KpnI–NotI site of pG4PN.

Or46a-promoter derivatives

The wild type *Or46a* 1.9 kb promoter–GAL4 construct has been described in Goldman et al. (2005). The *Or46a* 0.4kb-GAL4 construct was made by cloning the 400 bp EcoRI/NotI fragment from the wild-type *Or46a* 1.9kb-GAL4 into pG4PN.

In the 1.9(Dyad)-GAL4 construct, the pair of Dyad 1 elements present in an inverted repeat separated by 5 bp (TTAACCAACGTTAGGTCGTCTAATGACATTTTAA), were mutated by replacing 6 bp in the middle with a Spe1 site which was incorporated at the ends of the primers and, replacing 5 bp to the right by primer-mediated PCR mutagenesis. The 1.9 kb promoter was PCR-amplified in two parts and the two fragments AT–cloned into pGEM-T Easy. Upon ligation of the two fragments a product was obtained that differed from the one originally planned. Sequencing indicated that the SpeI site present in the multiple cloning site of pGEM-T Easy was cut instead leading not only to the desired mutation, but also a 45 bp insertion between the two extremities of the site.

Or71a-promoter derivatives

The *Or71a* promoter-*GAL4* construct containing 2.3 kb of upstream and 1.4 kb of downstream sequence (*Or71a* 5'+3') has been described in Goldman et al. (2005) and has been shown to recapitulate the endogenous expression pattern in the maxillary palp. In the *Or71a* 5'+3'(Oligo)-*GAL4* construct, the two clustered Oligo-1 sites in the 3' region (TTATAAGCACTTTGCTTTGGAATAATG*GAATTC*TTATAA) were mutated to (TTATCAGCACTTTGCTTTGGAATAATGGAA*AA*TTCGCCCCTT)by deleting the last 6 bases of the element on the right (underlined) and engineering a 1 bp change in the element on the left (underlined). This was accomplished by PCR amplification of a 1.4 kb 3' promoter fragment in 2 parts. A long primer was designed, starting from the EcoRI site (italicized sequence) heading left, containing mismatched nucleotides for the bases in the left element. The other primer started immediately downstream of the right element. The two fragments were PCR-amplified from the *Or71a-GAL4* plasmid using the above

mentioned primers in combination with the original upstream (SpeI) and downstream (BamHI) primers. These fragments were AT-cloned into pGEM-T Easy. Next, the left side fragment was SpeI/EcoR1 cloned into an appropriately oriented SpeI/EcoRI site lying on the 5' side of the right hand fragment containing pGEM-T Easy vector. The EcoRI site for the plasmid containing the right hand fragment was from the pGEM multiple cloning site. This ligation resulted in a vector called p71aM3, which had a 6 bp deletion from the right hand element. The 1.4 kb SpeI / BamHI fragment from p71aM3 was cloned using SpeI /BamHI sites downstream of the GAL4 in pG4PN to give p71M3-G4PN. Finally the 2.3 kb upstream KpnI /Not I fragment from the *Or71a-GAL4* plasmid was KpnI / Not I cloned upstream of the p71M3-G4PN plasmid to produce the *Or71a* 5'+3'(oligo) construct.

***Or85e*-promoter derivatives**

In the 0.45(Dyad)-*GAL4* construct, a pair of Dyad-1 elements in an overlapping inverted repeat (TTATTTGTGTGCTAGAGCAAACATAA), were mutated to (ATTATTTGTGGCTAGCAGCAGCAAACATA) by replacing 6 bp in the center with a Nhe1 site. This was achieved using a PCR cloning strategy similar to the ones described above.

In the 0.45(Oligo)-*GAL4* construct the Oligo-1 element (CTTATAA) was mutated to (CGCTAGC) by replacing 6 bases with a NheI restriction site using a PCR cloning strategy similar to the one described earlier..

In *Or85e*(pb2A-1)-*GAL4* the pb2A-1 box (TTTATTTGCATA) was mutated to (TTTGGATCC_ _A) by replacing the 6 bases in the middle with a BamHI restriction site using a PCR cloning strategy similar to the one described earlier.

In *Or85e*(pb2A-2)-*GAL4* the pb2A-2 element (AGTTTTTA) was mutated to (GGATCCTA) by replacing the 6 bases in the middle with a BamHI restriction site using a PCR cloning strategy similar to the one described above.

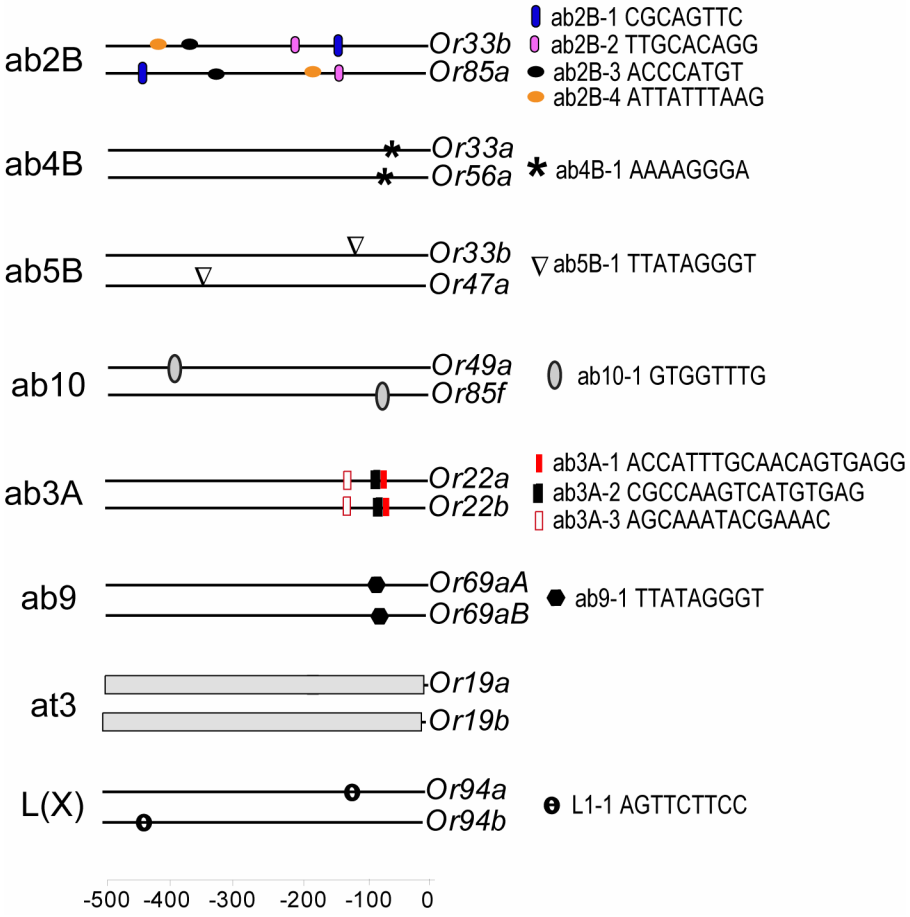
For 0.9(+Dyad)-*GAL4*, a pair of oligonucleotides were designed for the region of the *Or85e* upstream sequences with the pair of overlapping Dyad-1elements. When the oligonucleotides were annealed, they were designed to contain EcoRI sites at the ends. The annealed oligonucleotides were cloned into the upstream EcoRI site of the *Or85e* 0.09kb-*GAL4* construct.

Sequence for pb2A-2 minimal promoter construct

DNA inserted in the EcoR1 site of *pPTGAL* (Sharma et al., 2002) or the *Or85e* 0.09kb-*GAL4* construct:
GAATTC*TAAAAACTC*ATTAACTTT*TAAAAACTC*ATTAACTTT*TAAAAACTC*ATTAA CTTT*TAAAAACTC*AATTC*TAAAAACTC*ATTAACTTT*TAAAAACTC*ATTAACTTT*TAA AAACTC*ATTAACTTTTAAAACTGAATTC. The seven pb2A-2 box sequences are italicized, and each half-site of the two Dyad-1 sequences is underlined.

Reference

Sharma, Y., Cheung, U., Larsen, E. W., and Eberl, D. F. (2002). PPTGAL, a convenient Gal4 P-element vector for testing expression of enhancer fragments in drosophila. Genesis *34*, 115-118.



Supplementary Figure 1. Shared sequence motifs present in the upstream regions of coexpressed *Or* genes of the antenna and larva. ORNs are indicated in all cases in which A and B ORNs have been distinguished by physiological analysis; otherwise only the sensillum designation is indicated. L(X) indicates a larval ORN.