Supporting Information

Papadopoulos et al. 10.1073/pnas.0914595107

SI Text

Materials and Methods. ***Cloning Procedure and Fly Transgenesis.*** The fly stocks used were: *CyO/Sp*; *dpp*^{blink}*-Gal4/TM6B* (1); *ubi-histoneH2B-mRFP1/CyO*; *Dr/TM6B* (a kind gift of Yohanns Bellaïche); *Dll-Gal4(MD23)/CyO*; *Dr/TM6B* and *Dll-Gal4(MD713)/Dll-Gal4(MD713)* (2); *CyO/Sp*; *heat-shock-Gal4* (Bloomington stock center, stock number 1799); *CyO/Sp*; *grn-lacZ/TM6B* (3, 4); *UAS-Scr*; *fr10/TM3* (a kind gift of Deborah Andrew); and *Dll-Gal4(MD23)/CyO*; *grn-lacZ/TM6B*.

Immunohistochemistry. Third instar wandering larvae were dissected at room temperature (RT) in Grace's medium (Gibco) and fixed for 10 min in Grace's medium containing 5.3% EM-grade paraformaldehyde (Electron Microscopy Science, PA), freshly used every 10 days. After twice washed for 15 min in PBT (PBS containing 0.1% TritonX-100) and blocked for at least 30 min in PBT, containing 5% normal goat serum (NGS) and 20 μg/mL NaN_3, samples were incubated with primary antibodies in blocking solution overnight. Subsequently, samples were washed 3 times for 20 min in PBT and secondary antibodies were applied in PBS for 4 h at RT. After 3 washes for 20 min with PBT and equilibration in PBS, discs were dissected from the fixed carcasses and mounted in Vectashield (Vector Laboratories). Images were obtained within 24 h using a Leica SP5 Confocal setup. Antibody dilutions used were: rat anti-dCREB-A 1/2000 (Deborah Andrew), rat anti-Dan 1/300 (Steven Cohen), rabbit anti-Salm 1/25 (Reinhard Schuh), guinea pig anti-Hth 1/2000 (Richard Mann), mouse anti-Dll 1/1000 (Steven Cohen), mouse anti-Elav 1/100 (Developmental Studies Hybridoma Bank, University of Iowa), rabbit anti-GFP 1/500 (Molecular Probes), mouse anti-GFP 1/250 (Molecular Probes), rabbit anti-Scr 1/300 (5), and mouse anti-Ey 1/100.

Scanning Electron Microscopy (SEM). Adult flies were fixed for 2 h in 2.5% glutaraldehyde containing 0.1% TritonX-100, washed once for 30 min in PBS, twice for 30 min in water, and passed through increasing dilutions of ethanol in water (30%, 50%, 70%, 90% ethanol in water) for 15 min each time. Finally, cuticles were completely dehydrated in 100% ethanol overnight. After removal of the ethanol and complete air-dying, the heads were dissected and mounted on a SEM block, subjected to gold-coating and observed at the Center for Microscopy of the University of Basel (ZMB) using a Philips XL30 FEG ESEM electron microscope.

High-Resolution APD Imaging. High-resolution APD imaging was performed on a uniquely modified ConfoCor3 instrument (Carl Zeiss, Jena, Germany), consisting of an inverted microscope for transmitted light and epifluorescence (Axiovert 200 M); a VIS-laser module comprising the Ar/ArKr (458, 477, 488 and 514 nm), HeNe 543 nm and HeNe 633 nm lasers; and the scanning module LSM 510 META. The instrument was modified to enable detection using silicon Avalanche Photo Detectors (SPCM-AQR-1X; PerkinElmer, USA) for imaging and FCS. Images were recorded at a 512×512 pixel resolution. The C-Apochromat $40 \times /1.2$ W UV-VIS-IR objective was used throughout.

1. Staehling-Hampton K, Jackson PD, Clark MJ, Brand AH, & Hoffmann FM (1994) Specificity of bone morphogenetic protein-related factors: cell fate and gene expression changes in Drosophila embryos induced by decapentaplegic but not 60A. *Cell Growth Differ* 5(6):585–593.
2. Calleja M, Moreno E, Pelaz S, & Morata G (1996) Visualization of gene expression in living adult Drosophila. *Science* 274(5285):252–255.
3. Grieder NC, Morata G, Affolter M, & Gehring WJ (2009) Spalt major controls the development of the notum and of wing hinge primordia of the Drosophila melanogaster wing imaginal disc. *Dev Biol* 329(2):315–326.
4. Wagner-Bernholz JT, Wilson C, Gibson G, Schuh R, & Gehring WJ (1991) Identification of target genes of the homeotic gene Antennapedia by enhancer detection. *Genes Dev* 5(12B):2467–2480.
5. LeMotte PK, Kuroiwa A, Fessler LI, & Gehring WJ (1989) The homeotic gene Sex combs reduced of Drosophila: Gene structure and embryonic expression. *EMBO J* 8(1):219–227.
6. Zhao JJ, Lazzarini RA, & Pick L (1996) Functional dissection of the mouse Hox-a5 gene. *EMBO J* 15(6):1313–1322.
7. Tour E, Hittinger CT, & McGinnis W (2005) Evolutionarily conserved domains required for activation and repression functions of the Drosophila Hox protein Ultrabithorax. *Development* 132(23):5271–5281.

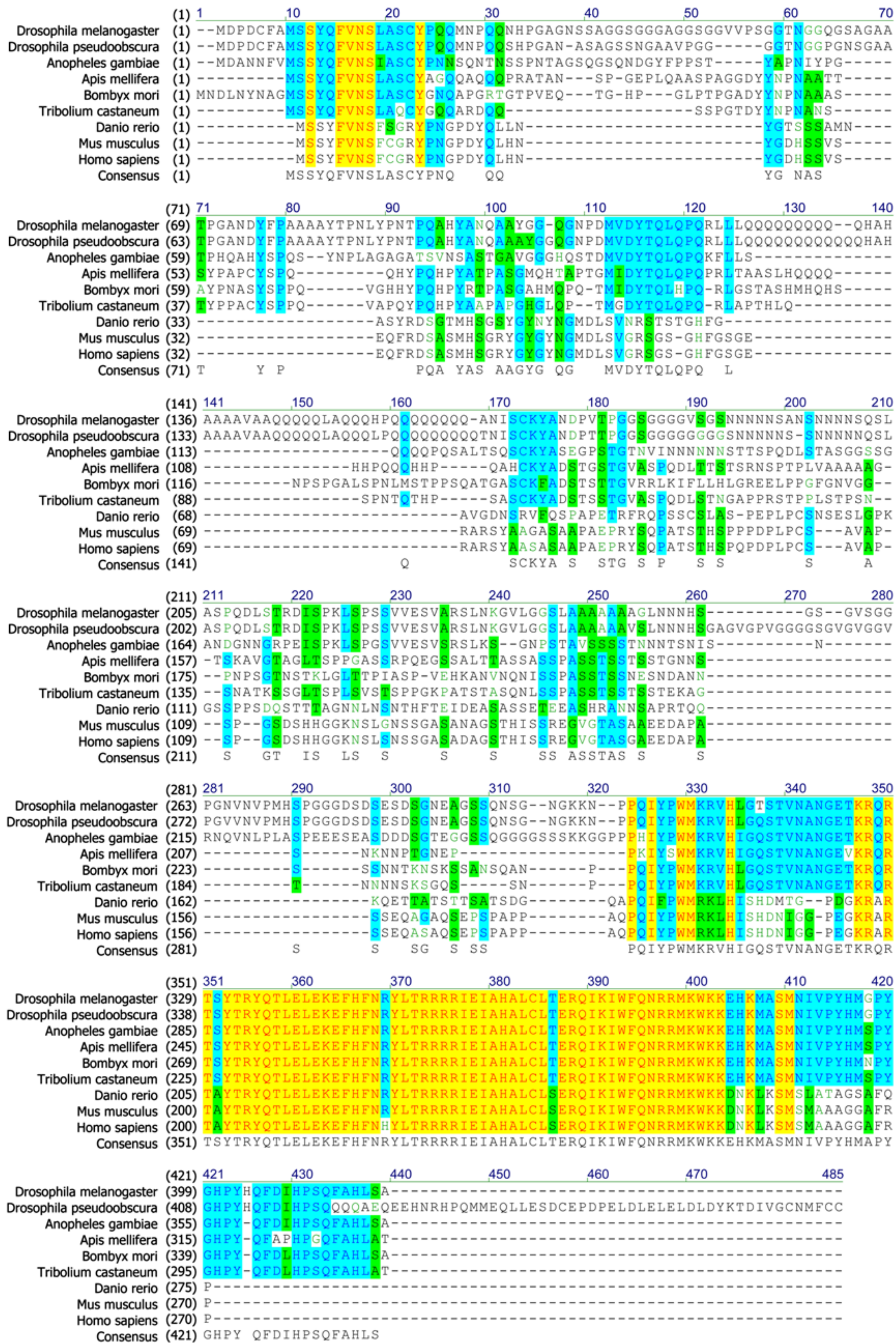

Fig. S1. Alignment of Scr amino acid sequence in insects and vertebrates. The YPWM motif, the homeodomain and the N-terminal MSSYFVNS motif are conserved among all animals examined, yet only the first two play a role in transcriptional specificity of Scr in vivo (see text for more details). The MSSYFVNS motif participates in enhancing transcriptional potency in the mouse Scr homolog Hoxa5 (6). The DYTQL motif, also described to enhance transcriptional activation (7), is only conserved among insect species. Little is known about the function of the SCKYA motif. Note the high conservation of the linker between HD and YPWM motif in insects. The same applies to the C-terminal sequences of Scr.