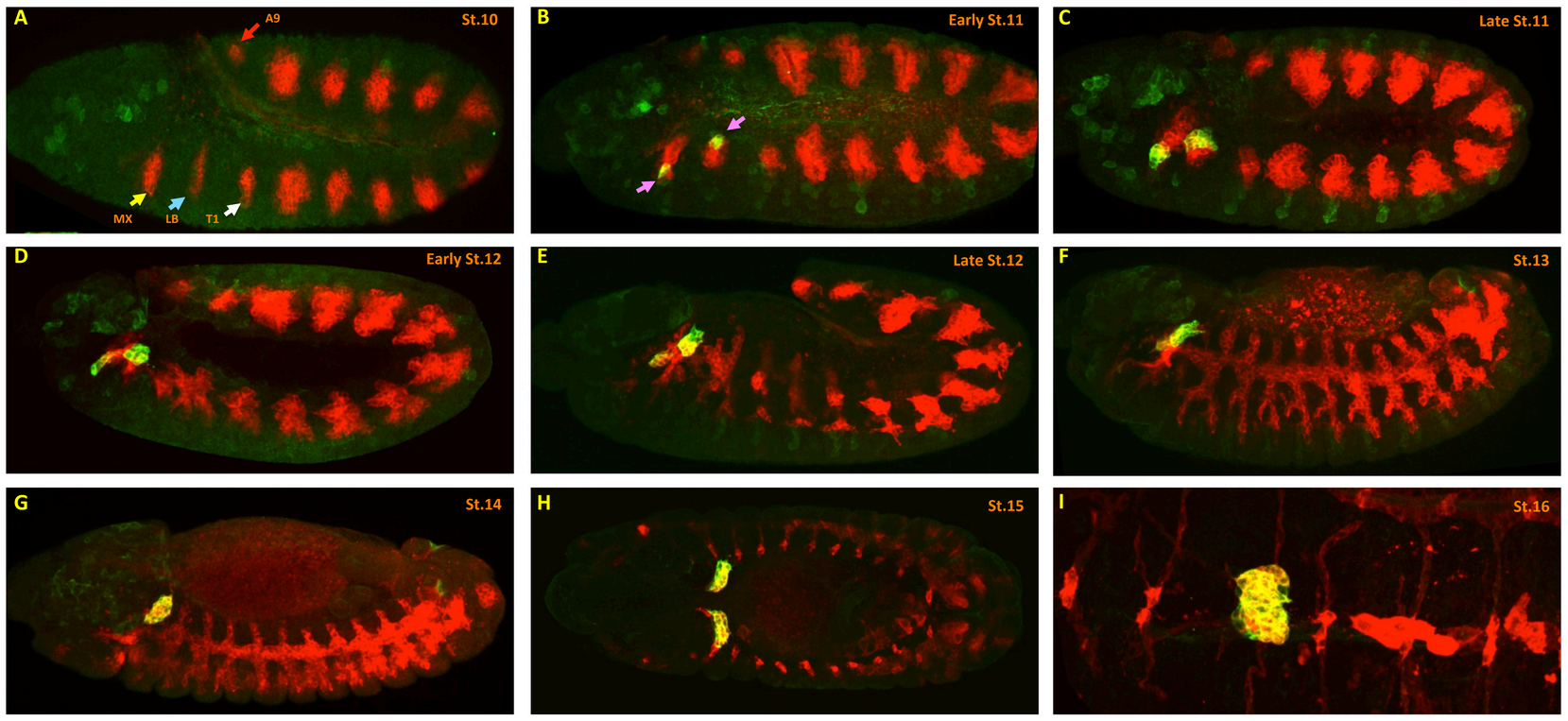
DIVERGENT GENE NETWORKS SELECT ENDOCRINE GLANDS VS. TRACHEA FROM A COMMON SEGMENTALLY REPEATED PRECURSOR IN *DROSOPHILA*

Carlos Sanchez-Higueras[a], Sol Sotillos[a] and James Castelli-Gair Hombria[a]

[a]*CABD, CSIC/JA/Universidad Pablo de Olavide, Seville, Spain*

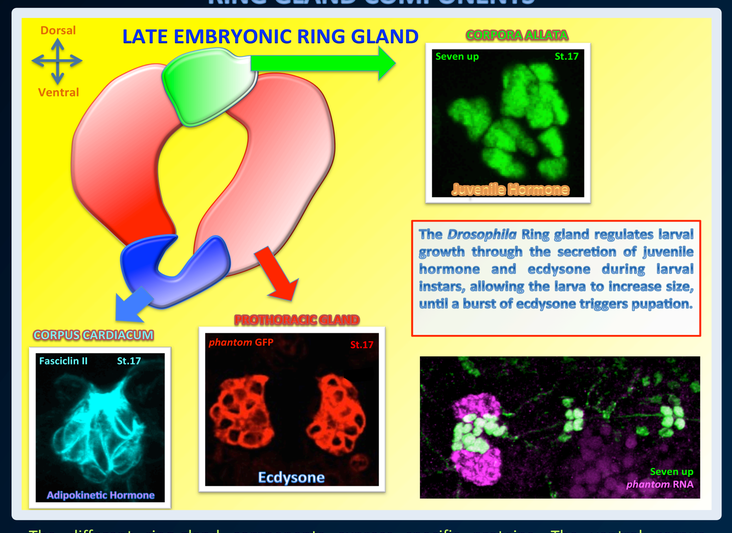
ABSTRACT. The main endocrine organ of *Drosophila*, the ring gland, is formed by the fusion of the corpora allata (producing Juvenile Hormone), the prothoracic gland (Ecdysone) and the corpus cardiacum (Adipokinetic hormone and others). The embryonic origin of the corpus cardiacum from cephalic mesodermal cells has been established, but the origin of the corpora allata (ca) and prothoracic gland (pg) is unknown. We demonstrate that the corpora allata and prothoracic gland develop from cephalic ectodermal cells that in other segments of the body give rise to the trachea. We identify Hox and Vvl as common primary genes required for trachea, corpora allata and prothoracic gland specification; as well as Snail as a specific corpora allata and prothoracic gland gene. Snail controls the ephitelial to mesenchymal transition (EMT) that is one of the major differences between the ring gland and trachea development. We also show that the trachea can be converted into corpora allata or prothoracic gland and vice versa. Our data indicate that endocrine glands and trachea evolved by the divergence of a homologous segmentally repeated structure.

CORPORA ALLATA AND PROTHORACIC GLAND MORPHOGENESIS



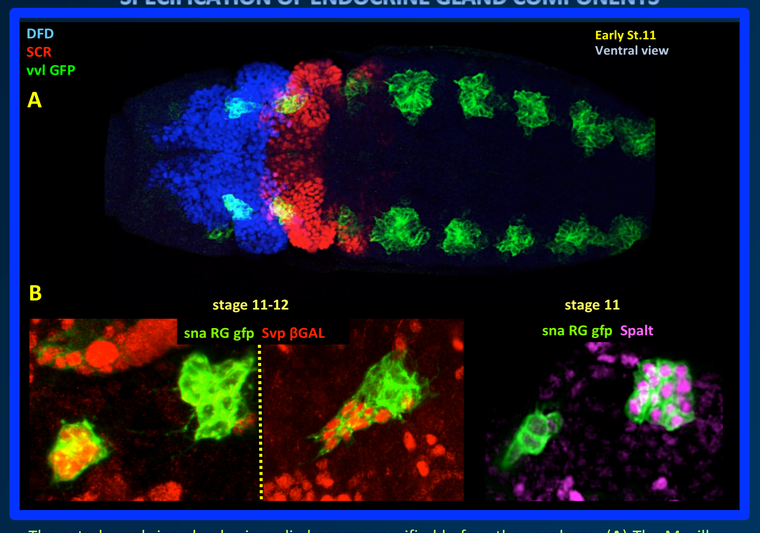
(**A**) The *ventral veinless* (*vvl*) enhancer vvl1+2.mCherry labels both the trachea primordia in T2 to A8 segments and cells at homologous positions in the Maxillary (Mx, yellow arrow), Labial (Lb, blue arrow), T1 and A9 segments. With the exception of the T1 and A9 patches (white and red arrows) that remain on the surface all other cells invaginate. (**B**) The Maxillary and Labial patches invaginate and activate the *snail* gene in a subpopulation of cells (magenta arrows) that go through EMT and fuse. The sna-rg-EGFP specific marker allows following the fate of these cells that will become the corpora allata and prothoracic gland primordia (**C-F**)**.** The coalesced corpora allata and prothoracic gland primordia migrate towards the dorsal midline **(G)** where they fuse to the contralateral cluster and to the corpus cardiacum generating the ring gland (**H-I**).

RING GLAND COMPONENTS



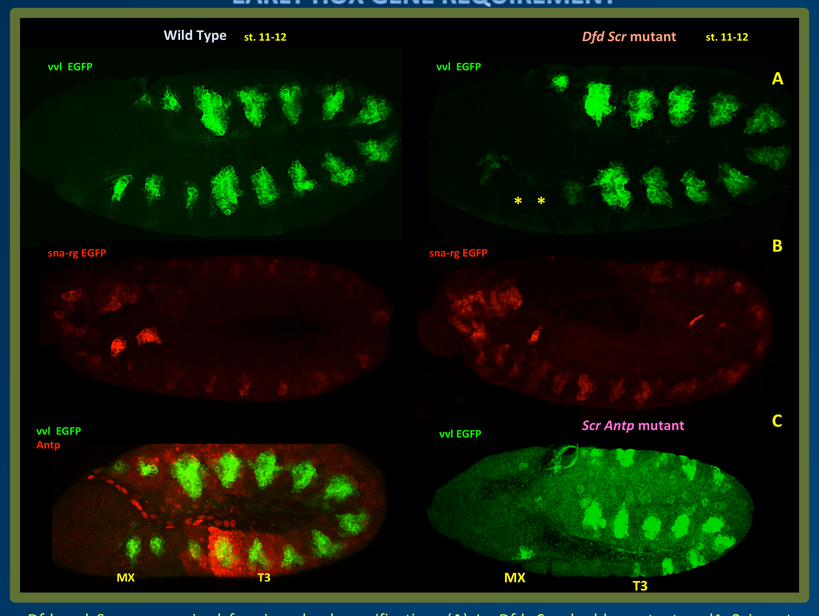
The different ring-gland components express specific proteins. The ventral corpus cardiacum is labelled by **Fasciclin II**; the prothoracic gland by **both Spalt** and the Ecdysone producing enzyme **phantom**; and the corpora allata by **Seven-up** (Svp). **Phantom** and **Svp** are expressed in non overlapping cells.

SPECIFICATION OF ENDOCRINE GLAND COMPONENTS



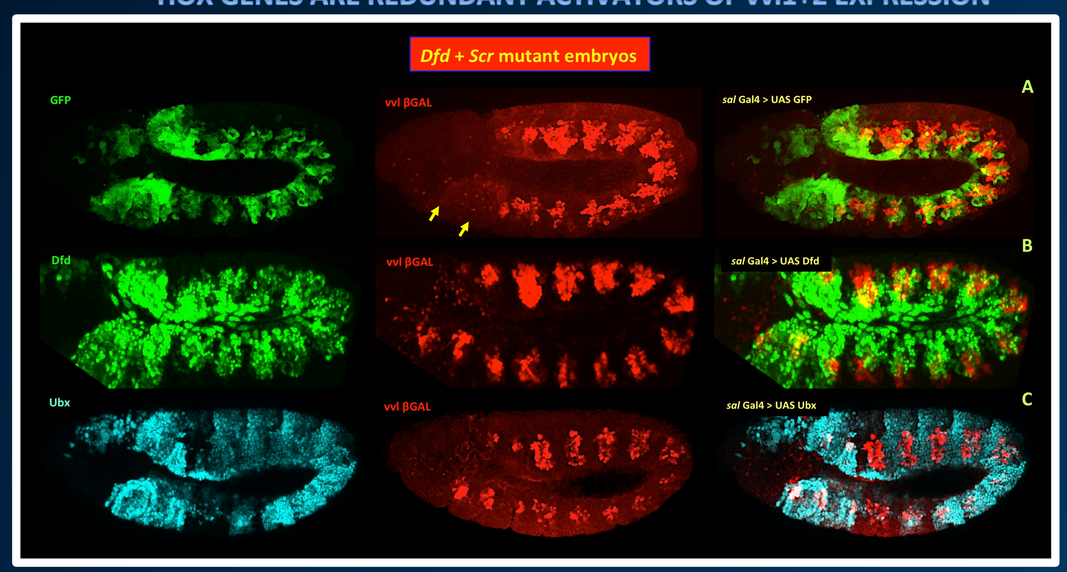
The ectodermal ring gland primordia become specified before they coalesce. (**A**) The Maxillary primordium expresses **Dfd** and the Labial primordium **Scr**. (**B**) Before coalescence both primordia are specified with the Maxillary primordium expressing Svp and the Labial primordium **Sal** at stage 11.

EARLY HOX GENE REQUIREMENT



Dfd and Scr are required for ring gland specification. (**A**) In *Dfd, Scr* double mutants vvl1+2 is not expressed in the Maxillary and Labial segments (*) and sna-rg is also downregulated (**B**). Dfd mediates these functions in the Maxillary primordium and Scr in the Labial primordium (data not shown). (**C**) vvl1+2 expression also requires Hox input in tracheal segments. This image shows a Scr, Antp double mutant that is missing besides the Labial and the T1 patches the tracheal T2 patch.

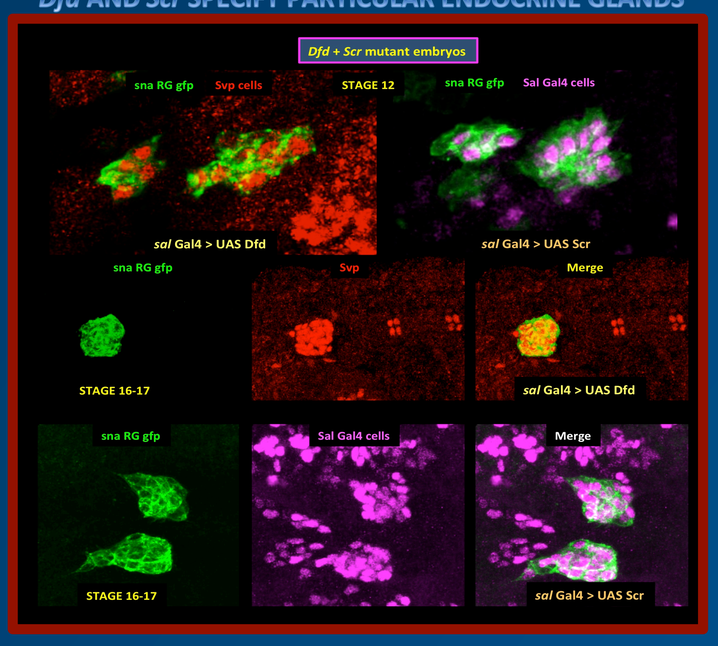
HOX GENES ARE REDUNDANT ACTIVATORS OF vvl1+2 EXPRESSION



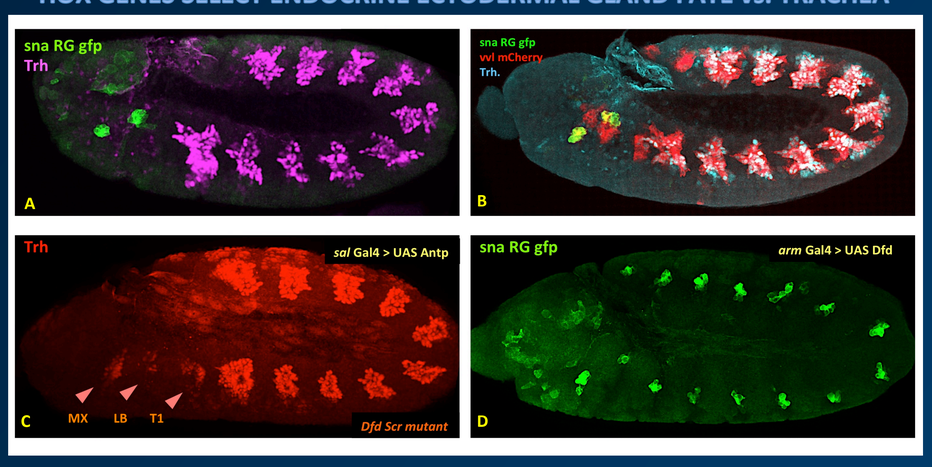
The lack of Maxillary and Labial vvl1+2 expression seen in Dfd, Scr embryos is not rescued in control embryos expressing GFP (**A**) but can be rescued by expression of cephalic Hox genes (Dfd shown in **B**) and by any trunk Hox gene (Ubx shown in **C**).

In *Dfd*, *Scr* double mutants, ectopic Dfd rescues vvl1+2 and *sna* expression, but both primordia become corpora allata. Ectopic Scr rescues vvl1+2 and *sna* expression but induces both primordia to become prothoracic glands. Central Hox genes do not rescue *sna* expression (data not shown). Thus, each cephalic Hox gene specifies the development of a different endocrine primordium.

Dfd AND *Scr* SPECIFY PARTICULAR ENDOCRINE GLANDS



HOX GENES SELECT ENDOCRINE ECTODERMAL GLAND FATE vs. TRACHEA



Endocrine ectodermal glands express Sna while trachea express Trachealess (Trh) (**A**). Both primordia express Vvl (**B**) and T1 and A9 only Vvl. Ectopic central Hox can specify trachea along the vvl1+2 cells (**C**), while ectopic Dfd can specify gland along the vvl1+2 trunk cells (**D**).

CONCLUSION: RING GLAND & TRACHEA DIVERGENT EVOLUTION

We propose that the corpora allata, the prothoracic gland and the trachea have evolved from an ancestral homologous segmentally repeated structure. This is reflected by (1) their primordia still forming in segmental homologous positions, (2) the use of common early developmental programs including JAK/STAT signalling and Hox protein, (3) their similar early developmental behaviour (4) the capacity of ring gland and tracheal primordia to acquire either tracheal or endocrine fate depending on the Hox gene expressed. The trachea have maintained their epithelial character and evolved respiratory functions under the control of Trachealess/Tango/Vvl proteins. In the endocrine primordia the Hox genes Deformed (Dfd) and Sex Combs Reduced (Scr) respectively induces a Snail dependent epithelial to mesechymal transition as well as specify both glands by activating Svp in the corpora allata and Sal in the prothoracic gland.