ELSEVIER

Mechanisms of Development 123 (2006) 893–906

www.elsevier.com/locate/modo

Fate map of the distal portion of *Drosophila proboscis* as inferred from the expression and mutations of basic patterning genes

Keiichiro Yasunaga, Kaoru Saigo, Tetsuya Kojima *

Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Received 25 November 2005; received in revised form 21 August 2006; accepted 24 August 2006
Available online 26 August 2006

Abstract

The late-third-instar labial disc is comprised of two disc-proper cell layers, one representing mainly the ventral half of the anterior compartment (L-layer) and the other, the dorsal half of the anterior compartment and most, if not all, of the posterior compartment (M-layer). In the L-layer, *Distal-less* represses *homothorax* whereas no *Distal-less*-dependent *homothorax* repression occurs in the M-layer where *Distal-less* is coexpressed with *homothorax*. In wild-type labial discs, *clawless*, one of the two homeobox genes expressed in distal cells receiving maximum (Decapentaplegic + Wingless) signaling activity in leg and antennal discs, is specifically repressed by *proboscipedia*. A fate map, inferred from data on basic patterning gene expression in larval and pupal stages and mutant phenotypes, indicates the inner surface of the labial palpus, which includes the pseudotracheal region, to be a derivative of the distal portion of the M-layer expressing *wingless*, *patched, Distal-less* and *homothorax*. The outer surface of the labial palpus with more than 30 taste bristles derives from an L-layer area consisting of dorsal portions of the anterior and posterior compartments, each expressing *Distal-less*. Our analysis also indicates that, in adults and pupae, the anterior–posterior boundary, dividing roughly equally the outer surface of the distiproboscis, runs along the outer circumference of the inner surface of distiproboscis.

Keywords: Proboscis; Labial disc; *en*; *dpp*; *wg*; *Dll*; *hth*; *cll*; *pb*; Anterior–posterior boundary

1. Introduction

Insect appendages diversify according to their position along the body axis; for instance, a pair of antennae on the antennal segment, mouth parts in the gnathal segments, three pairs of legs in thoracic segments and analia in the terminal segment. Despite these differences, insect appendages are considered to be basically homologous in structure and to derive from a common ancestor through modification of a basic patterning mechanism. This may be supported by the discovery of homeotic genes whose mutation causes a transformation of one appendage into another (reviewed by Emerald and Cohen, 2001; Angelini and Kaufman, 2005).

The mechanism for appendage development has been extensively studied using *Drosophila* leg as a model system (for review, see Morata, 2001; Kojima, 2004). Adult legs differentiate from leg discs, sac-like structures comprised of a monolayered cell population invaginated inside from the ectoderm during embryogenesis. The leg disc consists of anterior and posterior compartments, each derived from different cell lineages (Steiner, 1976). *engrailed* (*en*) and *hedgehog* (*hh*) are expressed specifically in the posterior compartment and secreted Hh activates *decapentaplegic* (*dpp*) expression dorsally, *wingless* (*wg*) expression ventrally and *patched* (*ptc*) expression dorsally and ventrally in the anterior compartment cells along the anterior–posterior (A–P) boundary (Lee et al., 1992; Tabata et al., 1992; Tashiro et al., 1993; Basler and Struhl, 1994; Tabata and Kornberg, 1994). *ptc* encodes the Hh receptor, while *dpp* and *wg*, respectively, TGF-β and Wnt family members.

---

* Corresponding author. Present address: Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan. Tel./fax: +81 4 7136 3657.

E-mail address: tkojima@k.u-tokyo.ac.jp (T. Kojima).

0925-4773/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mod.2006.08.008

The antagonistic action of Dpp and Wg provides the dorsoventral information (Struhl and Basler, 1993; Brook and Cohen, 1996; Jiang and Struhl, 1996; Penton and Hoffmann, 1996), while their cooperative action determines the proximal–distal (P–D) information (Diaz-Benjumea et al., 1994; Lecuit and Cohen, 1997). According to high, middle and low levels of (Dpp + Wg) signaling activity, *Distal-less* (*Dll*), *dachshund* (*dac*) and *homothorax* (*hth*) are expressed in the distal, middle and proximal regions, respectively (Abu-Shaar and Mann, 1998; Wu and Cohen, 1999).

The proboscis, a highly specialized appendage for feeding and drinking, derives from a pair of labial discs (Bodenstein, 1950; Fristrom and Fristrom, 1993). On the outer surface of the labial palpus, the distal portion of the proboscis (Ferris, 1950), stereotypically arranged taste sensilla are formed and function to perceive various taste stimuli (Amrein and Thorne, 2005). Situated on the inner surface of the palpus are six rows of pseudotracheae and taste pegs (Ferris, 1950; Falk et al., 1976), all used to direct food into the pharynx. Clonal analysis has shown the A–P boundary to run lengthwise along the proboscis and divide in half the outer surface of the palpus (Struhl, 1977, 1981), although the A–P boundary on the inner surface has yet to be assigned owing to lack of appropriate genetic markers for this analysis.

The proboscis is transformed into other appendages through homeotic mutation. Complete loss of *proboscipedia* (*pb*) activity causes the proboscis to transform to a distal prothoracic leg (Kaufman, 1978), while *pb* hypomorphs or *pb* and *Sex combs reduced* (*Scr*) double mutants possess the proboscis with antennal features (Kaufman, 1978; Percival-Smith et al., 1997). *pb* and *Scr* have been shown to repress the expression of leg or antennal genes such as *dac* and *spalt*, and modify *Dll* expression in the labial disc. The proboscis may thus be a highly modified appendage lacking regulatory capacity in proper segment specification (Abzhanov et al., 2001). Furthermore, selector function of *hth* and *spineless* in combination with these homeotic genes specify identity of the labial palpi and, might be partially transmitted through diffusible signaling molecules (Joulia et al., 2005, 2006). Specifically, the attenuation of *dpp* and *wg* expression by *pb* through Hh signaling may also be essential to labial disc development (Joulia et al., 2005).

Unlike other discs, labial disc morphogenesis occurs mainly during pupal stages (Madhavan and Schneiderman, 1977; Kumar et al., 1979), thus making difficult the recognition of any morphological traits of the proboscis in late-third-instar labial discs. This has hindered detailed clarification of numerous fundamental properties of labial disc development. Expression of basic patterning genes such as *en*, *hh, dpp*, *wg*, *Dll* and *hth* in the larval labial disc has been described (Abzhanov et al., 2001; Joulia et al., 2005). However, we do not know about exact spatiotemporal expression patterns of these genes and correspondence between gene expression in developing tissues and adult proboscis structures.

The present study examines in detail spatiotemporal expression patterns of basic patterning genes as well as certain distal-region-specific genes during larval and pupal stages of labial disc development. Unlike leg and antennal discs, the labial disc possesses two disc-proper cell layers, one representing mainly the ventral half of the anterior compartment and the other, the dorsal half of the anterior compartment and most, if not all, of the posterior compartment. The fate map as deduced from basic patterning gene expression and mutant analysis, indicated the inner surface of the labial palpus, which includes the pseudotracheal region, to derive from a distal part of the anterior-ventral region. The outer surface of the labial palpus with taste sensilla appeared to derive from the larval-labial-disc area consisting of dorsal regions of both anterior and posterior compartments, each expressing *Dll*.

The present results make possible detailed analysis of proboscis development and provide a starting point for further investigation of the mechanism for appendage specification.

2. Results

2.1. Third-instar expression of dpp and en occurring primarily in cells belonging to the lateral disc layer completely devoid of wg expression

The proboscis forms from a pair of labial discs (Fig. 1A). In late third instar, the labial disc, attached to the larval mouthpart via a stalk, has the shape of a partially flattened balloon and consists of two cell layers, lateral (L) and medial (M) layers (Figs. 1A and A′). In contrast to other imaginal discs, late-third-instar labial discs may not possess a peripodial membrane (Joulia et al., 2005). The L-layer is thinner than the M-layer and associated with an apical cleft, the lateral furrow (see Figs. 1A′ and B). During early pupal stages, peripodial-membrane-like cells (large flat cells) become discernible in a region of the L-layer near the point of stalk-attachment or the proximal end of the larval disc (Abzhanov et al., 2001).

dpp, *en* and *wg* expression in the larval labial disc was first recognized by Joulia et al. (2005). To clarify the relationship between unusual disc-layer structures and basic patterning gene expression, *en*, *dpp* and *wg* expression in the late-third-instar labial disc was re-investigated using *lacZ* enhancer trap lines and antibodies. *wg* expression could be detected only in M-layer cells (Figs. 1C and E–G), while *dpp* and *en*, mainly in L-layer cells (Figs. 1B, D and F). Despite this apparently unusual expression of *en*, *dpp* and *wg*, a distal M-layer view of the gene expression (Fig. 1F) was similar to that in the leg/antennal flat disc-proper region, indicating the relative location of *dpp*, *en* and *wg* expression in the labial disc to be basically the same as that in leg and antennal discs. As with leg and antennal discs (Lee et al., 1992; Tabata et al., 1992; Tashiro et al., 1993), *hh* was co-expressed in all *en*-expressing labial-disc cells (Joulia et al., 2005) and *ptc* expression was noted