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Short Report

The regulation of *homothorax* and the control of eye-head proportion in *Drosophila*

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ABSTRACT In the fruit fly *Drosophila melanogaster*, eye and head grow up to a constant, species-specific, size proportion. Eye and head originate from the same pool of cells, so the correct number of cells must be allocated to one of either fate during development. Two signaling pathways are instrumental in this allocation: *wingless (wg)* promotes head while *decapentaplegic (dpp)* promotes eye. Key to understand how eye and head growth is coordinated is to find the genes these two antagonizing signaling pathways act upon. One of these genes seems to be the transcription factor *homothorax (hth)*. In the absence of *hth*, the head capsule does not develop and ectopic eye tissue forms. Here we present data showing that *wg* and *dpp* signaling might be independently activating and repressing *hth*, respectively. The nuclear factor Dac is required for *hth* repression downstream of *dpp*.

Introduction

Shape is one of the most distinctive features of the individuals of a species, and results from the action of gene networks that coordinate the relative growth of body parts during embryonic development. In the adult, organs and body structures usually bear constant size relationships with one another. Since shape varies with the evolution of organisms, the mechanisms that ensure a constant, species-specific, proportion of organs are likely to be the ones that change to mold new morphologies, new species. In some cases, the size of an organ relates non-linearly to the size of the animal (allometry). But in others, organ proportions are maintained despite large changes in overall body size. For example, larvae of the fruit fly *Drosophila melanogaster* reared under starving conditions grow into minute adults, and yet they have perfectly proportioned organs. What are the mechanisms that control proportionate growth? Holometabolous insects are a good material for the study of this problem: the adult (imago) of these insects develops from modules, clusters of cells called imaginal discs. Each imaginal disc will give rise to a set of body structures. For example, in *Drosophila* most structures of the fly's head (head capsule, eyes, antennae and maxillary palps) derive from a single pair of "eye-antennal" imaginal disc. During metamorphosis, the discs -which grow inside the larva- assemble into an adult, while larval tissues are histolysed. The fact that several organs develop from a single cluster of cells may make the final size of each of these organs dependent on each other. This is the case in *Onthophagus* horned beetles: in males, the bigger the horns, the smaller the neighboring structures (like eyes). This phenomenon has been called "allocation trade-off" (Emlen, 2001 and references therein). Something similar occurs in some species of sexually dimorphic flies of the genus *Delia*: the overgrown eyes of males are compensated by a stunted head capsule, when compared to females.

The goal of our work is to understand the mechanisms that control how the relative size of organs is established during development. To this aim, we have resorted to the fruit fly *Drosophila melanogaster*. In particular, we are studying the development of two fly's organs: eye and head capsule.

Eye and head capsule derive from the eye-head imaginal primordium (E-HIP), which is part of the eye-antennal imaginal disc. During early larval life all cells of the E-HIP express the same set of transcription factors (including the Pax-6 *eyeless (ey)* and *homothorax (hth)* genes (Fig. 1A)). *ey* selects the competence of the primordium to differentiate into eye. By mid- larval life eye differentiation starts at the posterior pole of the primordium and moves forward in a wave-like fashion (Fig. 1B). The *decapentaplegic* (*dpp*; a TGF-β-like molecule) and *hedgehog (hh)* signaling pathways are the eye-forming motors. On the other hand, the *wingless (wg)* signaling pathway is required for the development of the head capsule and to prevent widespread eye differentiation. At the end of development, eye-promoting and eye-repressing (or head promoting) pathways reach an equilibrium, resulting in the head to eye size proportion that is characteristic of the species (Fig. 1C) (for a review see Treisman and Heberlein, 1998). One important question is to know which genes these pathways act upon to promote one of either fate. The transcription factor *hth* has been shown to be activated by *wg* and to be required for head capsule development (Pichaud and Casares, 2000). *hth* also interacts genetically with mutations that affect eye-head proportions (F Pichaud and FC, unpublished). In addition, *hth* is normally required to prevent ectopic eye development from the ventral head. *hth* is turned off in the cells to become eye tissue; otherwise, eye differentiation is blocked (Pichaud and Casares, 2000; Pai *et al.* 1998). Therefore, *hth* is a likely candidate to be target of both, head and eye forming pathways. Here we present data on how *hth* fits into the eye-head genetic network.

Results and Discussion

dpp *signaling clears* hth *from the prospective eye directly, not through* wg *repression*

dpp signaling represses *wg*, and is required for initiation of eye differentiation and, to a lesser extent, for its progression (Treisman and Heberlein, 1998). We tested the effects of *dpp* signaling on *hth* in two ways: (i) Removal of the *dpp* receptor *thick-veins (tkv),* which

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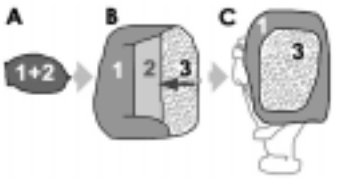

Fig. 1. Development of the eye-head imaginal primordium (E-HIP). (A) *During early larval life, all cells express* hth *(1) plus* ey *(2).* **(B)** *Eye differentiation (pattern of circles) starts during late larval life, driven by* dpp *and* hh *signaling pathways (arrow). Anterior to the differentiating eye, the primordium gets subdivided into two main regions: (2), where* ey *and other genes (like* dac*) define the area still competent to become eye and (1), expressing* hth, *that will develop into head capsule.* **(C)** *Adult derivatives of the E-HIP: (1) head capsule and (3) eye.*

abolishes *dpp* signaling, results in *hth* de-repression in the mutant cells. (ii) Ectopic expression of a constitutively active *tkv* molecule represses *hth* cell autonomously in the prospective head. These two results show that *dpp* signaling represses *hth*. It is known that *wg* activates *hth* and that *dpp* represses *wg*. So, in order to test whether *hth* repression by *dpp* is direct or indirect through *wg* repression, we generated cells doubly mutant for *wg* and *mad* (a nuclear transducer of *dpp* signal). These cells still de-repress *hth,* which indicates that *dpp* is required for turning *hth* off regardless of the activity of *wg*.

Fig. 2. *hth* in eye-head determination. *The* dpp *and* hh *signaling pathways make eye differentiation to progress in cells that are made competent by the expression of* ey. *Of these signals, only* dpp *represses* hth. *This repression is mediated in part by* dac. *The eye blocking gene* wg *maintains* hth *expression, and might therefore counteract the* dpp *pathway.*

By contrast the *hh* pathway, which is also required for eye differentiation, does not affect *hth* expression.

dac *is required, downstream of* dpp*, to repress hth*

dac is a nuclear factor-coding gene required for eye differentiation downstream of *dpp* (Treisman and Heberlein, 1998). (Dac forms a protein complex with eyesabsent (Eya) and sine oculis (So) that, in ey-expressing cells, selects the eye pathway). We have observed that *dac* is expressed abutting the *hth* domain, making it a likely candidate to be a *hth* repressor. In *dac1/dac3* mutant animals there is almost no eye differentiation, and most or all cells in the E-HIP express *hth*. When *dac* is ectopically expressed, it is able to repress *hth* in regions far from *wg*-expressing areas. These results indicate that *dac* is required for *hth* repression during normal regionalization of the E-HIP.

hth *is required, but not sufficient, to repress* dac *in the prospective head*

It is possible that not only the *dpp* pathway down-regulates *hth* expression, but that *hth* participates in repressing the eye-promoting pathway as well. We have tested this hypothesis by inducing clones of cells lacking *hth* function in the prospective head and examined the expression of the eye-promoting gene *dac*. These experiments show that *hth*- ventral clones de-repress *dac*, indicating a requirement for *hth* to keep *dac* off in the head. Nevertheless, ectopic *hth* clones do not repress *dac* in the prospective eye, which suggests that other genes collaborate with *hth* to repress eye fates in the prospective head.

hth *is needed for the normal activation of* wg *targets in the dorsal eye*

In addition to *hth* role in ventral regions of the E-HIP, we have observed that *hth*- clones within the dorsal eye develop abnormally (Pichaud and Casares, unpublished). Dorso-ventral (DV) patterning of the eye is a *wg* dependent process. Thus, *wg* activates the expression of the iro-C transcription factors (*ara, caup and mirr)* dorsally, prior to the onset of eye differentiation, at a stage when *hth* is expressed in most cells of the primordium. Notch-signaling activation takes place at the interface between iro-C-expressing and non-expressing cells, and this activation results in cell proliferation and DV polarity (Dominguez and De Celis, 1998; Maurel-Zaffran and Treisman, 2000). In dorsal *hth*- clones the expression of a *mirr*-LacZ reporter is weakened in a cell-autonomous way. The apposition of strongly and weakly *mirr*-expressing cells could give rise to the observed effects of *hth*- clones. This result suggests that *hth* contributes to the activation of *mirr* by *wg*.

The results described indicate that the head-promoting *wg* pathway and the eye-promoting *dpp* pathway antagonize, at least partly, through the activation and repression of *hth* expression, respectively. The nuclear factor Dac is required downstream of *dpp* to repress *hth*. Therefore, determining the domain of expression of *hth* is key in the allocation of the cells of the E-HIP to become head capsule or eye. Still, our knowledge of how this process takes place is fragmentary. Thus, *hth* is required only ventrally for eye repression, so it is likely that an analogous function is performed dorsally by a yet unidentified gene. Also, *hth* is necessary, but not sufficient, to fully repress the eye-promoting pathway. Again, this would suggest that *hth* works jointly with other unknown genes.

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