Shavenbaby Couples Patterning to Epidermal Cell Shape Control. Chanut-Delalande H, Fernandes I, Roch F, Payre F, Plaza S (2006) PLoS Biol 4(9): e290

Question (from Introduction): How does *svb* control the formation of denticles and dorsal hairs during epidermal differentiation?
Broader context (from Introduction): how is cell shape specified during development?

Discussion

Using denticle pattern as a readout of developmental cues, generations of fly geneticists have collectively gained a rare insight into the mechanisms acting to specify different cell populations. Quite paradoxically, how this pattern information is connected to **cell shape** remodeling for denticle formation has so far remained an unsolved question.

We show here that a single regulator, **Shavenbaby**, previously shown to integrate multiple patterning cascades, governs epidermal cell remodeling through the [A]transcriptional control of several classes of effectors, acting directly in various cellular functions. This *svb*-regulated set of effectors constitutes a [B]developmental module used in different tissues during development to produce cuticle extensions. Modification of *svb* expression has allowed [C]the concerted evolution of this developmental module to produce morphological diversification during the evolution of insect species.

[A1]*svb* Controls Several Aspects of Actin Organization

One of the first recognizable signs of the morphological differentiation of epidermal cells is the formation of an apical bundle of microfilaments in denticle cells [22,23]. These early steps of denticle formation depend on *svb,* which is necessary and sufficient to promote the formation of epidermal actin bundles [14]. Our results show that *svb* controls the transcription of several genes involved in different steps of actin assembly/organization. First, we found that *svb* directs the expression of *shavenoid/kojak,* a gene producing strong denticle defects when mutated and recently shown to encode a protein reported to associate with actin [32], but whose biochemical function is unknown. Second, Svb also directs the expression of *singed* and *forked,* coding respectively for the *Drosophila* putative homologs of Fascin and Espin, two proteins that crosslink parallel actin filaments and promote the formation of bundles of microfilaments [36,37]. The Forked and Singed proteins sequentially accumulate in growing denticles, a situation reminiscent of that of wing hair formation [38], suggesting that these proteins play similar roles in the formation of adult and embryonic epidermal extensions. Accordingly, the inactivation of *sn* and *f* alters denticles, strongly suggesting that denticle formation indeed involves parallel actin bundles, as shown for wing hairs.

Several cytoskeletal regulators such as dAPC, Enabled, Diaphanous/Formin, and the Arp2/3 complex, accumulate in denticles [14,23], suggesting that they are involved in denticle formation, although their respective functions

Francoise Chanut 9/27/06 6:52 AM
Comment: Brief reminder of the context of the study, including a restatement of the larger question (how does development specify cell shape?).

Francoise Chanut 9/27/06 7:02 AM
Comment: One of the key words used throughout the text.

Francoise Chanut 9/27/06 9:23 AM
Comment: Answer to the question stated in the Introduction. Note the signal: we show here, which announces a statement of results. Note the use of the present tense. Note how the paragraph summarizes all the results that will be discussed, in the order in which they will be discussed.

Francoise Chanut 9/27/06 7:03 AM
Comment: The main player in this study.

Francoise Chanut 9/27/06 7:56 AM
Comment: Informative title with active verb

Francoise Chanut 9/27/06 7:18 AM
Comment: First result to be discussed—part of topic A (transcriptional targets of svb)

Francoise Chanut 9/27/06 7:12 AM
Comment: Two sentences of background. Note embedded references.

Francoise Chanut 9/27/06 7:13 AM
Comment: Signal that we are now talking about the current study.

Francoise Chanut 9/27/06 7:16 AM
Comment: Two arguments to substantiate the claim made in the preceding sentence. Note the use of First and Second to orient the reader.

Francoise Chanut 9/27/06 7:22 AM
Comment: Signal for interpretation or speculation.

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Comment: Broadening the result's implications: comparison with other findings, speculation.

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remains to be evaluated. Whereas *svb* does not control the expression of those ubiquitous actin-associated factors, it is possible that *svb* regulates their activity, or subcellular localization, indirectly. Consistent with this hypothesis, we have previously shown that dAPC-2 is specifically relocalized in *svb*-induced ectopic epidermal extensions [14]. In addition, we show here that *svb* directs the epidermal expression of Wasp, a key activator of the Arp2/3 actin nucleator complex, which is well known to trigger the formation/elongation of actin filaments. Moreover, it has been shown in vitro that Fascin switches the activity of the Arp2/3 complex from the formation of a mesh-like branched network to parallel microfilaments [39], therefore suggesting that *svb* targets can regulate both the formation of actin filaments and their reorganization, at least in part, through a tight control of the activity of the Arp2/3 complex during denticle formation.

Taken together, these results show that *svb* controls the expression of several cytoskeletal factors which, probably by modifying the activity of housekeeping actin-remodeling machinery, act together to trigger the formation of apical cell extensions. Whereas few molecules are sufficient to promote actin organization in vitro, our studies indicate that, in vivo, many players are required to make a simple cellular extension. Pursuing the identification of novel genes regulated by *svb* should provide a means of identifying additional factors required for actin remodeling in vivo.

A2Denticle Formation Requires a Specific Membrane/Cuticle Interaction

A surprising outcome of our studies is that denticle formation requires a specific regulation of the membrane/cuticle interaction. We show that *svb* directs the expression of *m* in trichome cells. Miniature is a single-pass membrane protein, with a short cytoplasmic tail and a large extracellular region that contains a conserved Zona Pellucida (ZP) domain [28]. ZP domains were initially identified in the three major proteins of the zona pellucida, the extracellular envelope of mammalian oocytes (see [30] for a recent review), and they are thought to be components of apical matrices [40]. We show that Miniature is required for the correct formation of denticles, revealing a novel aspect of ZP protein function in the formation of polarized cellular extensions. The absence of *m* severely impairs the interaction between the plasma membrane and cuticle layers in denticle cells, a defect likely due to a disorganization of the extracellular matrix. In the embryonic epidermis, Miniature is required for the continuous membrane/cuticle interaction that is specific to denticles, whereas only the tips of microvilli contact cuticle in naked regions [5]. The accumulation of Miniature at the base of denticles reveals the existence of a denticle-specific membrane subdomain, suggesting that additional membrane proteins might be involved in denticle formation. We found that two other ZP genes are regulated by *svb* (Table S1) and analysis of their individual role in denticle formation is under way. Our findings shed light on the importance of membrane proteins and their interaction with extracellular matrices, an aspect of cell-shape control hardly accessible to cell-culture approaches.

Francoise Chanut 9/27/06 7:29 AM
Comment: Topic sentence states what the paragraph is going to discuss. Note the question implicit in "remains to be evaluated" and placed in a power position (end of sentence.)

Francoise Chanut 9/27/06 7:30 AM
Comment: Note the signals (consistent with, in addition, moreover) that signal the accumulation of evidence.

Francoise Chanut 9/27/06 7:34 AM
Comment: Conclusion statement.

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Francoise Chanut 9/27/06 7:31 AM
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Francoise Chanut 9/27/06 7:35 AM
Comment: Speculation/vision/where might this study go next.

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Francoise Chanut 9/27/06 7:37 AM
Comment: Second part of A. Other result pertaining to transcriptional targets of svb.

Francoise Chanut 9/27/06 7:37 AM
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Francoise Chanut 9/27/06 7:38 AM
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Comment: Wrap-up or concluding sentence, summarizes the information presented in the paragraph and points out the superiority of the approach used here.

Future analysis of Miniature targeting to the denticle should help to understand the mechanisms required for localized cell-shape modification during morphogenesis.

svb also regulates the expression of *y,* a gene encoding an apically secreted protein that associates with cuticle and is required for the production of black pigments [24]. While pigmentation per se is not related to cell morphogenesis, the role of Yellow in the catecholamine pathway remains elusive; it could be involved in denticle hardening, since *y* mutant larvae display defects, in the morphology of denticles, that have been proposed to account for their abnormal locomotor activity [41]. In addition, *svb* could directly regulate the local protein composition of cuticle, as suggested by the identification of an additional target encoding a putative chitin-binding protein (Table S1).

[B]Svb Governs a Morphological Module

Experimental evidence suggests that *svb* is situated at the bottom of regulatory cascades determining trichome patterning and is in turn directly responsible for triggering the cellular program of denticle formation (Figure 8). First, *svb* remains the most-downstream regulator determining the pattern of denticles and dorsal hairs, despite the unprecedented extent of genetic screens based upon cuticle observation (which identified most members of the Wg and DER pathways). Second, among mutations producing trichome defects, *svb* mutants display the strongest phenotype, in which most denticles and dorsal hairs are replaced by naked cuticle. Third, we show that *svb* directs the expression of genes involved in various aspects of denticle formation, including control of the cytoskeleton, membrane/matrix organization and cuticle differentiation. Finally, we provide evidence for a direct control of one of the targets *(m)*. We have defined a 400-bp *m* enhancer reproducing the endogenous expression pattern of *m* in the epidermis and show that the Svb transcription factor binds specifically to this evolutionarily conserved region. Substituting 2-bp in this *cis*-regulatory element preventing Svb binding is sufficient to abrogate its in vivo enhancer ability, thus suggesting that direct binding of Svb mediates the control of *m* epidermal expression. Several putative *svb* binding sites have been detected in evolutionarily conserved regions of other *svb* targets. Whether they are all required for *svb* transcriptional regulation remains to be tested. Further dissection of the *m* enhancer, as well as those of other *svb* targets, should lead to the definition of a functional *cis*-regulatory element responsible for *svb* control. This outcome should facilitate the identification by bioinformatic approaches of additional *svb* responsive enhancers and target genes.

During epidermal differentiation, regulatory regions governing *svb* transcription integrate outputs from many signaling pathways (Wg, Hh, and DER) and positional cues to define the precise subset of epidermal cells that express *svb*. The Shavenbaby transcription factor triggers in turn the expression of different classes of genes encoding cellular effectors. They are directly involved in distinct aspects of trichome formation, including the

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Francoise Chanut 9/27/06 7:43 AM
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Francoise Chanut 9/27/06 7:45 AM
Comment: This result is not as directly related to the question asked (not to do really with cell shape). Which is why this result is discussed last (rather than first) in this section.

Francoise Chanut 9/27/06 7:46 AM
Comment: Signal for speculation.

Francoise Chanut 9/27/06 7:57 AM
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Francoise Chanut 9/27/06 7:47 AM
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Francoise Chanut 9/27/06 7:48 AM
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Comment: Paragraph organized in a chronological manner. The first sentence introduces the topic (multiple signaling pathways and positional cues) .

reorganization of actin *(singed, forked, wasp,* and *shavenoid)*, extracellular matrix *(m)* and cuticle *(y)*, likely through modifying the activity of ubiquitous cellular machineries. Additional cytoskeletal factors or regulators (independent of *svb*) might be required for the fine sculpturing of each kind of trichome, characteristic of a given body region. Modifications of *svb cis*-regulatory regions thus provide a rich source of plasticity to evolve the trichome pattern and generate morphological diversification throughout species.

We propose that *svb* directly controls the expression of a set of "effector" genes, all required for a concerted modification of cell shape and cuticle organization to achieve the formation of denticles (Figure 8). How many genes are regulated by *svb* to promote remodeling of epidermal cells? Our analysis, which covers approximately 25% of the total number of *Drosophila* genes, has led to the identification of 11 downstream targets, suggesting that *svb* activates the expression of numerous additional genes to trigger the formation of embryonic epidermal cell extensions.

Several results suggest that this *svb*-controlled module is used in different developmental programs that produce cuticle extensions. Although signaling pathways act differently in ventral and dorsal embryonic regions, we show that *svb* is also required to express the same target genes for the formation of denticles and dorsal hairs. These results show that the *svb* targets identified so far act collectively to promote the formation of various epidermal extensions, despite the fact that they display different shapes. In addition, *svb* mutations also affect the formation of adult wing hairs and antennae laterals [14], which are known to require the activity of several *svb* targets identified in the embryonic epidermis (*m, sn*, *f, sha* for adult wing hairs, and *sn, f, sha* for antennae laterals). How can the same set of *svb*-regulated effectors participate in the formation of epidermal extensions of diversified morphology? Additional cytoskeletal factors can be differentially expressed in distinct epidermal regions independently of *svb*. It is also possible that other regulators modulate the response to *svb* (cf. the weak expression of *f* and *wsp* in naked cells expressing ectopically *svb,* when compared to that of *sn, m, sha* and *y*). Finally, upstream signaling pathways certainly contribute to the sculpting of each kind of trichome through the regulation of the expression and/or activities of cellular effectors. Recent studies have shown that members of the planar polarity pathway (PCP) are indeed involved in defining denticle polarity in response to signaling pathways [23]. Interestingly, one of the identified *svb* targets, *shavenoid,* has recently been reported to interact with PCP in the adult wing, raising the possibility that such a dialog also occurs during embryonic epidermal cell remodeling. *svb* thus appears to govern a **morphological module** responsible for the major switch from smooth surface to trichome, the precise **shape** of which is finely sculptured by independent intrinsic factors and activities.

Francoise Chanut 9/27/06 7:54 AM
Comment: Last sentence summarizes the implications of the results described in the paragraph.

Francoise Chanut 9/27/06 7:54 AM
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Comment: Repeating the key word from the title.

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Comment: Key word throughout the paper.

[c]Evolutionary Perspectives

The pattern of trichomes has been modified several times during the evolution of insects. Across the genus *Drosophila,* at least four independent evolutionary transitions have led to the loss (to various extents) of dorsal hairs. Nevertheless, evolution of the pattern of dorsal hairs and denticles results from the modification of *svb* expression in all studied cases [17–19]. Although the expression of patterning genes is unchanged in all species examined [42], the difference in the dorsal hair pattern between *D. melanogaster* and *D. sechellia* is due only to the modification of *shavenbaby*'s response to signaling pathways [16]. Our analysis further shows that the restriction of *svb* expression in *D. sechellia* embryos causes, in turn, the restriction of the dorsal expression of *m, sn, f, sha,* and *wsp* genes (Figure S2). Therefore, all these *svb* targets display a concerted modification of their expression in *D. sechellia,* bringing additional evidence that together they constitute a developmental module. Consistent with this interpretation, we show that individual inactivation, or experimental modifications of the expression, of any of the *svb* target genes identified so far are not sufficient to modify the trichome pattern. These data indicate that denticle formation requires multiple factors that act collectively to remodel epidermal cell shape. This requirement for many genes to build a cuticular extension doubtless constitutes a developmental constraint, explaining why modifications of the expression of *svb,* the factor that governs this entire set of genes, are required for the trichome pattern to evolve.

Evolutionary modifications of *cis*-regulatory elements of *lin-48,* the putative *svb* homolog in worms, were responsible for the difference in the position of the excretory duct between *Caenorhabditis elegans* and *C. briggsae* [43]. Although accumulated data thus demonstrate the particular role of *svb* genes in morphological diversification between relatively close species, how they are related to the evolution of animal forms across more distant phyla remains an open question. Several features of *svb* function have been conserved in mammals, including the role of one of its homologs, *m-ovo1,* in the differentiation of epidermal derivatives [44] and its regulation by the Wnt pathway [45]. Our identification of genes regulated by *svb* in flies now opens the way to evaluate the contribution of the different parameters contributing to the role of *svb* in morphological evolution, from the modification of its response to signaling pathways to that of its cellular targets.

Francoise Chanut 9/27/06 8:07 AM
Comment: Third topic of the discussion. Vague title. One can ask why? (hint: "Perspectives" signals speculation or vision, not hard facts.

Francoise Chanut 9/27/06 8:02 AM
Comment: Classic topic sentence: announcing the content of the paragraph.

Francoise Chanut 9/27/06 8:08 AM
Comment: Paragraph unfolds according roughly to a pro/con pattern.

Francoise Chanut 9/27/06 8:08 AM
Comment: Broadening of the interpretation to other animals.

Francoise Chanut 9/27/06 8:09 AM
Comment: Speculation/vision/further studies.