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Cuticle itself as a central and dynamic player in shaping cuticle

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The wide variety of external morphologies has underlain the evolutionary success of insects. The insect exoskeleton, or cuticle, which covers the entire body and constitutes the external morphology, is extracellular matrix produced by the epidermis. How is cuticle shaped during development? Past studies have mainly focused on patterning, differentiation and morphogenesis of the epidermis. Recently, however, it is becoming clear that cuticle itself plays important and active roles in regulation of cuticle shape. Studies in the past several years show that pre-existing cuticle can influence shaping of new cuticle, and cuticle can sculpt its own shape through its material property. In this review, I summarize recent advances and discuss future prospects.

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Introduction

Insect cuticle is extracellular matrix secreted by the epidermis. It consists mainly of proteins and the polysaccharide chitin. It covers the entire body surface and ectodermal invaginations. Cuticle thus forms the external morphology of insects. The cuticle protects insects from adversities, supports locomotion and acts as an interactive interface with environments. For all these functions, the precise shaping of the cuticle is critical. How are the diverse shapes of cuticle created during development?

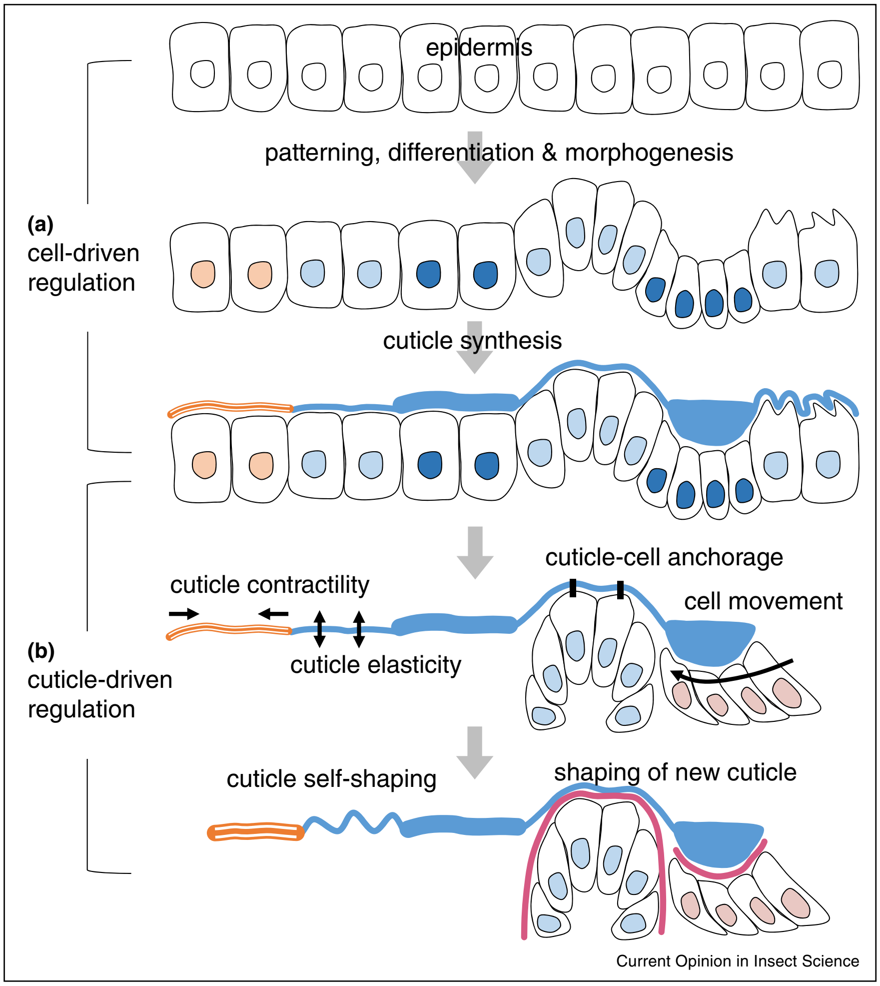
Classically, researchers have focused on the importance of patterning, differentiation and morphogenesis of epidermal cells in controlling cuticle shape [1]. In a stereotypical view, the shape of the epidermis and kinds and amounts of cuticular components to be deposited by each cell is determined prior to cuticle synthesis. Cuticle is subsequently produced as a faithful readout of the shape and the differentiation pattern of the epidermis (Figure 1a). Past studies have shown that regulation at the cellular level is indeed important for cuticle shaping [2–5]. Recent findings, however, demonstrate pivotal contributions of cuticle itself in controlling the shaping of cuticle. They show that (1) pre-existing cuticle can influence shaping of new cuticle, and (2) cuticle can sculpt its own shape through its material property (Figure 1b). Here I briefly summarize those studies, mainly from *Drosophila melanogaster* but relevant to all insects, and discuss future prospects.

Pre-existing cuticle affects the shape of the new cuticle

Contribution of old cuticle

During every molt, the old cuticle detaches from the epidermis (apolysis) and new cuticle is synthesized. This gives insects opportunities to change form. An example of the old cuticle giving a morphogenetic cue to the underlying cellular tissue, thereby ultimately controlling the shape of the new cuticle, is found in the development of the adult wing of *D. melanogaster* [6••,7•]. The wing epidermis conducts two rounds of cuticle synthesis: pupal cuticle synthesis during the prepupal and early pupal stages, and adult cuticle synthesis in the late pupal stage. After pupal cuticle apolysis, proximodistal elongation of the wing epidermis converts the round pupal wing into the long and thin shape that prefigures the adult wing, and this elongation is driven by cell contraction in the hinge region [8]. It is now shown that localized anchorage of the wing epidermis to the overlying pupal cuticle during hinge contraction is critical for elongation of the wing epidermis ([6••,7•], Figure 2). At the onset of hinge contraction, the wild-type wing epidermis is anchored to the pupal cuticle at the margin. Dumpy (Dp), a long fibrous transmembrane protein of at least 0.8 μm that mediates cuticle-epidermis anchorage in the larva [9–11], is primarily found in the apical extracellular matrix connecting the wing margin to the pupal cuticle. In *dp* mutants, the wing is not attached to the pupal cuticle, and over the course of hinge contraction, the entire wing epidermis retracts into a truncate shape. The attachment is essential for elongation of the wing epidermis, as shown by laser ablation of the Dp-containing matrix [7•] or by mathematical modeling [6••,7•]. Furthermore, experimental alteration of the spatial distribution of Dp results in wing shape changes predicted by mathematical modeling [6••]. As hypothesized, modulation of Dp distribution

Figure 1



Mechanisms for precise shaping of cuticle. **(a)** Cell-centered mechanisms. Patterning, differentiation and morphogenesis of epidermal cells determine the shape of the epidermis and kinds and amounts of cuticular components to be deposited by each cell. **(b)** Examples of cuticle-centered mechanisms. Cuticle material properties influence how cuticle remodels its own shape (left), and sequential cuticle deposition allows the pre-existing cuticle to influence shaping of new cuticle (right).

may have contributed to the evolution of appendage shapes [6••].

Early-deposited cuticle affecting the shape of late-deposited cuticle

In general, three distinct layers are recognized in the cuticle: the outermost layer called the envelop, the epicuticle beneath it, and the inner layer called the procuticle [2,12]. Temporally dynamic gene expression within the cuticle synthesis period of every molting cycle (for example, [13–17]) is assumed to correspond to the multilayer organization of the cuticle [2]. The dynamics of the adult wing cuticle formation in *D. melanogaster*, which takes approximately 2 days, is documented in [18••]. RNAseq data show that a number of genes encoding cuticular proteins, chitin-modifying proteins and others show dramatic changes in expression levels during the period. Interestingly, knocking down genes expressed specifically during deposition of the envelop, such as *dusky-like* (encoding a transmembrane Zona Pellucida domain protein [4]), *ectodermal* (an ectodermally expressed gene encoding a secretory protein [19]), and *CG8213* (encoding a serine protease [20]) often results in abnormal morphologies of the procuticle, the inner layer synthesized more than 10 hours later.

Another case of a part of cuticle synthesized earlier affecting the shape of one produced later is found in the formation of ball-and-socket joints in the *D. melanogaster* tarsus ([21••], Figure 3). In every joint, the ball-shaped cuticle and the socket-shaped cuticle are apposed in precisely reciprocal shapes, permitting free movement and tight interlocking. At the initial stage of joint cuticle formation, each joint region appears as a simple fold of