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Origin of Ventral Epidermis in a Silverfish, *Lepisma saccharina* Linnaeus (Insecta: Zygentoma, Lepismatidae)

Mika MASUMOTO and Ryuichiro MACHIDA

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan
Current address: Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano 386–2201, Japan
E-mail: masumoto@sugadaira.tsukuba.ac.jp (MM)

Abstract

The origin of the sternum or ventral epidermis in *Lepisma saccharina* was examined. The ventral epidermis is initially formed by dermatoblasts that differentiate ventral to neuroblasts. This structure is ephemeral, being named the "provisional ventral epidermis." Soon after the provisional ventral epidermis forms, the appendicular bases start to extend medially and migrate over the epidermis, eventually covering most of it. Thus the definitive ventral epidermis or the sternum is largely, if not exclusively, derived from the appendicular bases. The present study strongly supports the participation of appendages in the sternal formation, which insect morphologists have postulated.

Introduction

Insect morphologists have long postulated the participation of appendicular bases in the formation of the sternum, although their theories may differ in terms of details (cf. Matsuda, 1970). Weber (1952) examined the insect sternum in light of evolution and concluded that the appendicular bases contribute to the sternal formation, deducing that a major part of the sternum or "ventral epidermis" of insects is represented by a "secondary sternum" derived from the appendicular bases. However, Matsuda (1970) later warned "workers have tended to indulge in speculations, extending and modifying the subcoxal theory, without really looking for reliable developmental facts in support."

Our embryological knowledge of the sternal formation remains rather insufficient. As for the origin of the sternum in insects, embryology has merely revealed that the ventral epidermis is formed by the proliferation of ectodermal cells located ventral to the neuroblasts, or "dermatogene cells" or "dermatoblasts" (cf. Johannsen and Butt, 1941; Anderson, 1972a, b). Machida's (1981) embryological study on Archaeognatha revealed that the definitive ventral epidermis derived from the appendicular bases, whereas the dermatoblast-line cells only function as a provisional epidermis. Recently, based on detailed observations of mainly external features of the embryogenesis of Grylloblattodea, Uchifune and Machida (2005) precisely identified the origin of sclerites and confirmed that a major part of the ventral epidermis or the eusternum is subcoxal in origin. Our ongoing study has successfully revealed that at least a majority of the definitive ventral epidermis of Zygentoma (*Lepisma saccharina* Linnaeus), which represents the most basal clade of Dicondylia, is derived from the appendicular bases as well.

Materials and Methods

Adults of *Lepisma saccharina* collected in gravel under the Shinkoji Temple, Sanada, Nagano, were reared in the laboratory at room temperature (18–25℃). The eggs they laid were incubated at room temperature.

All eggs were punctured with a fine needle, fixed with a chilled Karnovsky's fixative for 24 h, and stored in a 0.1 M HCl-sodium cacodylate buffer (pH 7.2) at 4℃. The embryos were dissected out of the fixed eggs, embedded in a water miscible epoxy resin, Quetol 651 (Nisshin EM, Tokyo), and cut into sections 2 μm thick. Sections were stained with toluidine blue O. For transmission electron microscopy, the dissected embryos were postfixed with 1% OsO_4 for 3 h and embedded in Quetol 651. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed

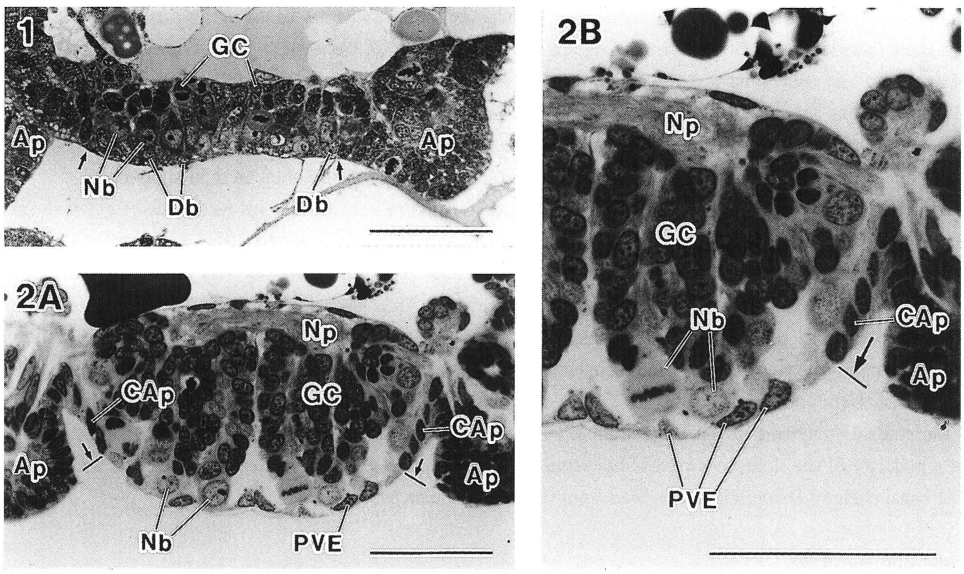
under an LEM-2000 transmission electron microscope (TOPCON, Tokyo).

Results and Discussion

In *Lepisma saccharina* embryos at diapause, the neuroblasts differentiate, and the ectodermal cells are distinguishable ventral to the neuroblasts, being referred to as "dermatoblasts" (Fig. 1). The repeated tangential divisions (divisions with the fissure plane tangential to the surface of the given structure) of neuroblasts results in the segregation of ganglionic cells arranged in a column perpendicular to the embryonic venter (Fig. 2). The dermatoblasts undergo radial divisions (divisions with the fissure plane parallel to the surface of the given structure) although not so actively, to form a unicell-layered epidermis, or the "ventral epidermis," beneath the neuroblasts at the embryonic venter (PVE in Fig. 2). The dermatoblast-line cells are clearly distinguishable from the neuroblasts in cellular size and in nuclear size and stainability: the former are smaller with a more basophilic and smaller nucleus (Figs. 2, 4). This ventral epidermis derived from the dermatoblast-line cells is, in this paper, referred to as the "provisional ventral epidermis" (Figs. 1–5), since it is an ephemeral structure, eventually to be concealed by the cells derived from the appendicular bases (cf. Figs. 2–5).

Soon after the differentiation of the provisional ventral epidermis, the appendicular bases start to extend over the structure (Fig. 2). Figure 3, showing TEMs of an embryo in the same stage of development, clearly demonstrates that the cells from the appendicular base (CAp) have started to extend medially and migrate over the provisional ventral epidermis. Figure 4 shows a transverse section of a slightly developed embryo, in which the appendicular bases are shown to have extended over the provisional ventral epidermis up to the level of the medialmost neuroblasts.

In the following stage of development, the ventral surface of the embryo was entirely covered by the definitive ventral epidermis, and the sternum had formed (Fig. 5). We have not made any conclusive observations concerning the closing of the definitive ventral epidermis, but it is highly likely that the appendicular elements from both sides fuse medially with each other to complete the epidermis. Once the definitive ventral epidermis has formed, the neuroblasts are no longer recognizable (Fig. 5). The provisional ventral epidermis cannot be distinguished either, instead, a fine



Figs. 1, 2 Cross sections of *Lepisma saccharina* embryos.

Fig. 1 A cross section through the thorax of a 9-day embryo in the katatrepsis stage. The ventral surface of the body is exclusively represented by the cells referable to as dermatoblasts or the provisional ventral epidermis. Arrows indicate the boundary between dermatoblasts and appendages.

Fig. 2 A cross section through the metathorax of a 12-day embryo yet to begin the definitive dorsal closure. A. Up to the levels shown by arrows, the cells have medially extended from the appendicular bases. B. Enlargement of A.

Ap: appendage, CAp: cells from the appendicular base, Db: dermatoblast, GC: ganglionic cell, Nb: neuroblast, Np: neuropile, PVE: provisional ventral epidermis. Bars = 50 μm.

cellular layer or the neurilemma is observed to surround the ganglia (Fig. 5). It may be that the provisional ventral epidermis has transformed into the neurilemma.

The present study clearly demonstrated that the appendicular elements largely, if not exclusively, contribute to the formation of the definitive ventral epidermis or the sternum in *Lepisma saccharina*, as suggested by Machida (1981) in Archaeognatha and Uchifune and Machida (2005) in Grylloblattodea. This may well support the argument in insect morphology that the appendages participate in the formation of the sternum (cf. Matsuda, 1970). A full understanding of the sternal formation in *Lepisma saccharina* will be crucial to clarifying the issues concerned.

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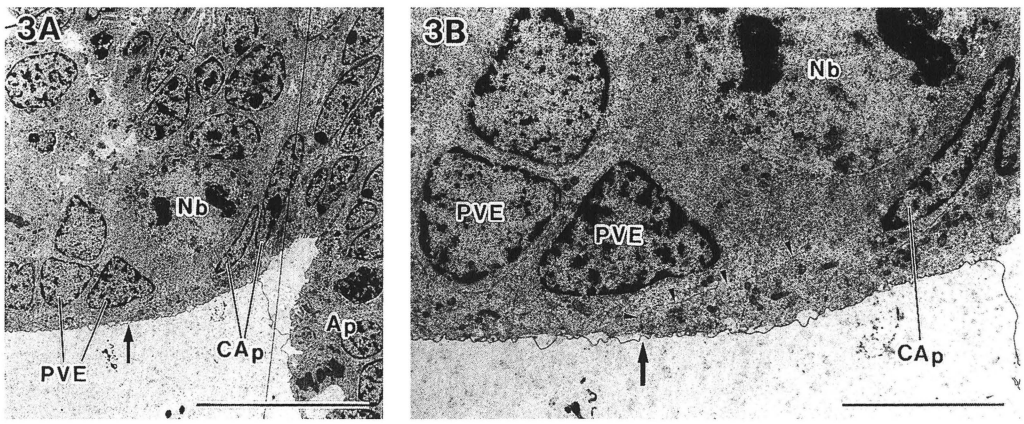
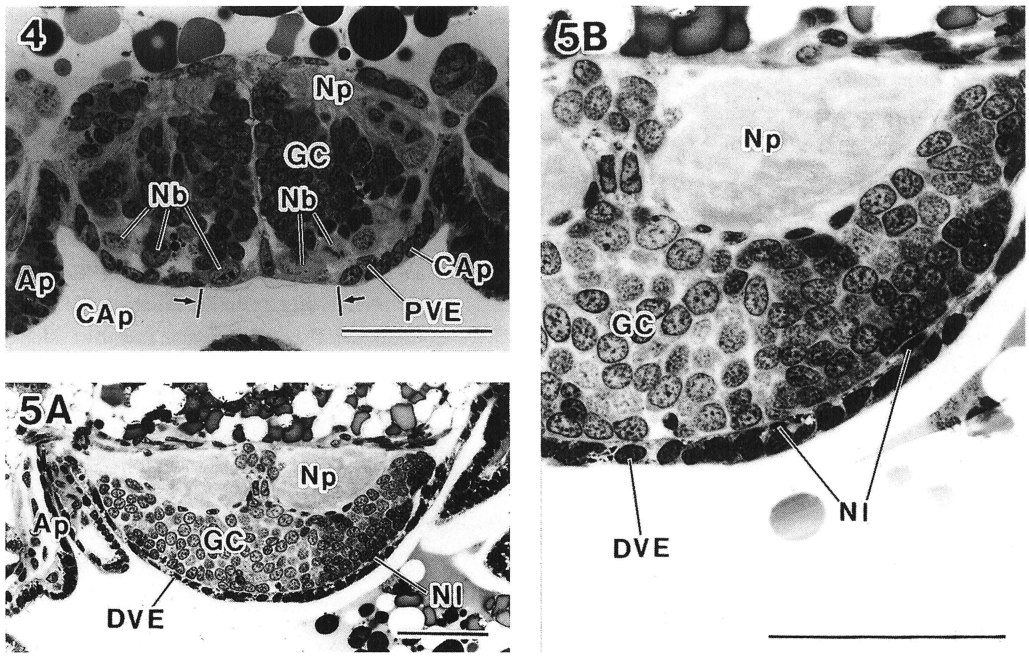


Fig. 3 TEMs of a section through the metathorax of a *Lepisma saccharina* embryo at approximately the same stage as that shown in Fig. 2. A. An arrow shows the front of the migrating cells extending from the appendicular base over the provisional ventral epidermis. B. Enlargement of A. Arrowheads show the boundary between the provisional ventral epidermis and cells from the appendicular base: concerning the arrow, the same as in A. Ap: appendage, CAp: cells from the appendicular base, Nb: neuroblast, PVE: provisional ventral epidermis. Bars = A, 20 μm; B, 10 μm.



Figs. 4, 5 Cross sections of *Lepisma saccharina* embryos.

Fig. 4 A cross section through the mesothorax of a 14-day embryo which has just started the definitive dorsal closure. The cells derived from the appendicular bases have extended up to the levels shown by arrows. The dermatoblasts or the cells of the provisional ventral epidermis are observed to lie dorsally to the cells derived from the appendicular bases.

Fig. 5 A. A cross section through the metathorax of a 24-day embryo proceeding with the definitive dorsal closure in the abdomen. The definitive ventral epidermis has formed. The provisional ventral epidermis is no longer distinguishable, instead the neurilemma is observed. B. Enlargement of A.

Ap: appendage, CAp: cells from the appendicular bases, DVE: definitive ventral epidermis, GC: ganglionic cell, Nb: neuroblast, Nl: neurilemma, Np: neuropile, PVE: provisional ventral epidermis. Bars = 50 μm.