Arthropod Structure & Development 47 (2018) 180–198

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

# Book lung development in juveniles and adults of the cobweb spider, *Parasteatoda tepidariorum* C. L. Koch, 1841 (Araneomorphae, Theridiidae)

Roger D. Farley*

*Department of Biology, University of California, Riverside, CA 92521, USA*

ARTICLE INFO

*Article history:*
Received 26 October 2017
Accepted 2 January 2018
Available online 1 February 2018

*Keywords:*
Morphogenesis
Epithelium-to-mesenchyme transition
Mesenchyme-to-epithelium transition
Lumen formation
Secretion
Planar cell polarity

ABSTRACT

Light and transmission electron microscopy were used to study the development of new book lung lamellae in juvenile and adult spiders (*Parasteatoda tepidariorum*). As hypothesized earlier in a study of embryos, mesenchyme cells dispersed throughout the opisthosoma (EMT) are a likely source of precursor epithelial cells (MET) for the new lamellae. The precursor cells in juveniles and adults continue many of the complex activities observed in embryos, e.g., migration, alignment, lumen formation, thinning, elongation, and secretion of the cuticle of air channel walls and trabeculae. The apicobasal polarity of precursor cells for new channels is apparently induced by the polarity pattern of precursor cells of channels produced earlier. Thus, new air and hemolymph channels extend and continue the alternating pattern of older channels. At sites more distant from the spiracle and atrium, new channels are usually produced by the mode II process (intracellular alignment and merging of vesicles). These air channels have bridging trabeculae and are quite stable in size throughout their length. At sites closer to the spiracle and atrium, new channels may be produced by mode I (coalescence of merocrine vesicle secretion). This raises the hypothesis that structural and functional differences in mode I and II channels and differing oxygen and fluid conditions with distance from the spiracle and atrium determine the mode of formation of new channels. Observations herein support an earlier hypothesis that there is some intercellular apical/apical and basal/basal affinity among the opposed surfaces of aligned precursor cells. This results in the alternating pattern of air channels at the apical and hemolymph channels at the basal cell surfaces.



## 1. Introduction

Light (LM) and transmission electron microscopy (TEM) were used herein to continue a comparative investigation of the development of book gills in the horseshoe crab (Farley, 2010, 2012) and book lungs in the scorpion (Farley, 2005, 2008, 2011) and spider (Farley, 2015, 2016). The spider species of these investigations is very prolific, has a relatively short life cycle and can be maintained in a laboratory colony (McGregor et al., 2008; Miyashita, 1987; Hilbrant et al., 2012). This has enabled some detailed analysis of cellular activity as lamellae are formed in embryos and early postembryonic stages (Farley, 2015, 2016) and also in the present investigation as the book lungs are enlarged in juveniles and adults. In these chelicerate respiratory organs, epithelial precursor cells with apicobasal polarity align in rows and gradually form a polarized planar tissue with hemolymph channels that alternate with air or water channels. These book-like structures are a model system for studies of tissue morphogenesis (McGregor et al., 2008; Hilbrant et al., 2012; Farley, 2015, 2016) and gene expression (e.g., Damen et al., 2002; Simonnet et al., 2006; Pechmann et al., 2010), and information is provided for hypotheses about evolutionary relationships (e.g., Dunlop, 1998, 2010; Scholtz and Kamenz, 2006; Kamenz and Prendini, 2008; Dunlop and Lamsdell, 2017)).

At least in some species studied so far, spider embryos have a small bilateral cluster of initial temporary (IT) lamellae that form in the second opisthosomal (O2) segment just posterior to the O2 limb buds (Wolff and Hilbrant, 2011; Mittmann and Wolff, 2012; Farley, 2016). There is variable expression of these lamellae among the species, and if present they are replaced by advanced embryo (AE) lamellae that develop within the O2 limb buds (Pechmann et al.,

* Fax: +1 951 827 4286.
*E-mail address:* roger.farley@ucr.edu.

https://doi.org/10.1016/j.asd.2018.01.002

2010; Farley, 2016). The distal end of the O2 limb buds joins with the ventral surface of the O2 segment, and the limb bud/book lung tissue is internalized by covering with an epidermal layer.

Farley (2016, Fig. 16) provided evidence and a proposal that cells of the opisthosomal epidermis in spider embryos undergo an epithelial-to-mesenchyme transition (EMT) and migrate anteriorly, forming entapophyses (slender stands of cells and reticular fibers). There may be cuticular invagination at some entapophysis sites leading to muscle (Lankester et al., 1885), but entapophyses are often just cells and fibers from proliferation and inward migration of epidermal cells. In addition to the mesoderm that is produced during gastrulation (Mittmann and Wolff, 2012), entapophyses are a likely source of mesenchyme cells dispersed throughout the abdomen where they provide precursors for developing organs. Such EMT is a basic feature of metazoan embryogenesis as described in recent reviews (e.g., Lee et al., 2006; Acloque et al., 2009; Thiery et al., 2009; Lim and Thiery, 2012). Entapophyses are reported to be the source of cells for the lateral tracheae in the O3 segment of spider embryos (Purcell, 1909, 1910).

In histological sections of spider embryos, an entapophysis is commonly seen attached at the base of each O2 limb bud, a site which is also the medial-posterior margin of a developing book lung (Farley, 2016). Here, the entapophysis cells are hypothesized to undergo a mesenchyme-to-epithelial transition (MET) and become book lung precursors within the limb bud; the entapophysis/mesenchyme cells may also induce some limb bud cells to become precursors. Whatever their origin, the precuror cells align and start producing lumina that become the alternating air and hemolymph channels.

In spider embryos, the apicobasal polarity of the book lung precursor cells becomes evident as the cells begin to form lumina (Farley, 2015, Fig. 16; 2016). In electron micrographs, clear spaces for the primordial air channels develop at the opposed apical ends of aligned precursor cells while hemolymph spaces develop usually later at the basal end of the precursor cells.

Electron micrographs also show that different modes of lumen formation are used, depending on location and probably other conditions within the primordial book lung (Farley, 2015, Fig. 16, 2016). Especially at the posterior end of the book lung where the spiracle, atrium and earliest lamellae are formed, the lumina may be produced by *cord hollowing* among the aligned cells (Andrew and Ewald, 2010). This results from the extracellular coalescence of merocrine secretion from vesicles. This process, labeled as mode I by Farley (2015, Fig. 16) is also used to increase the size of the air channel as the molt removes the earlier air channel cuticular wall and trabeculae (Farley, 2016).

Another very common mode of lumen formation is *cell hollowing*, the intracellular alignment and merging of vesicles (Andrew and Ewald, 2010). This was labeled as mode II by (Farley, 2015, Fig. 16, 2016). The mode II process may be used for the initial formation of some lamellae in the embryo and also, as shown herein, for lengthening and adding new lamellae as the book lung is increased in size during and after the molt. New lamellae are most commonly mode II at the anterior, dorsal and medial aspects of the book lung, at sites more distant from the spiracle and atrium.

Mode III is the development of thin spaces between aligned precursor cells, possibly by diffusion of fluid in response to an osmotic gradient (Farley, 2015, 2016). This is commonly evident in the initial development of hemolymph channels. Cell death is also likely involved in clearing space for passage of air and hemolymph since cell debris is often seen in developing spiracles, atria and air and hemolymph channels.

As hypothesized earlier (Farley, Fig. 16), book lung precursor cells align in rows and have some type of affinity between their opposed apical surfaces and also their opposed basal surfaces. The apical surfaces produce air channels while the hemolymph channels are formed at the basal surfaces. Results herein show that cells with these affinities may be able to induce similar properties in nearby cells, so there is propagation and repetition of the alternating pattern of air and hemolymph channels.

The various modes of lumen formation observed in earlier stages (Farley, 2015, 2016) are continued in juveniles and adults as the book lungs are lengthened and made wider with new-growth lamellae. The precursor cells appear to come mainly from mesenchyme cells dispersed within the opisthosoma and at the periphery of the book lung chamber. As these mesenchyme cells become precursor cells and part of the book lung, their polarity and alternating channels continue the pattern in the earlier adjacent channels. The initial round or ovate precursor cells undergo striking changes in shape and behavior as they become very thin and elongate and part of the air channel walls with supporting cuticle and trabeculae. These cuticular structures are replaced during the molt while the cellular pillar trabeculae in the hemolymph channels continue development.

## 2. Materials and methods

### 2.1. Experimental animals

A culture of *Parasteatoda tepidariorum* was maintained with procedures like those described earlier for this species (Mittmann and Wolff, 2012; Farley, 2015, 2016). Egg sacs and newly hatched spiderlings were kept in small petri dishes at 25–30 °C. Advanced juveniles and adults were separated into individual plastic containers (280 ml) with paper on the bottom and a partially opened bottom of a styrofoam cup for cover, web attachment and support for a small pad of moist paper. These spiders were maintained at 22–24 °C with a 12:12 h photoperiod. They were fed, containers cleaned and the papers changed once each week. Spiderlings were given a flightless strain of freshly killed fruitflies (*Drosophila hydei*) and small crickets while juveniles and adults were given freshly killed crickets of larger size. In experiments with long and short photoperiods, Miyashita (1987) reported four to six molts in 30–80 days from cocoon to adult in the males of this species while there are five to seven molts in 40–100 days for females.

### 2.2. Histology

A fine pin was inserted into the prosoma of juvenile and adults specimens, and each individual with inserted pin was immersed in physiological saline (Schartau and Leidescher, 1983). Fine forceps and scissors were used to separate the book lungs from nearly all the exoskeleton, and a pin was inserted in any remaining cuticle at the posterior end of the book lung. The pins were used to secure the specimen and prevent floating of excised lung tissue with air inside. The pinned tissue was immersed in Bouin's fixative. After 12–24 h, the embryos were washed in physiological saline (Schartau and Leidescher, 1983), removed from the pins, dehydrated in a graded sequence of ethanol and embedded in paraffin. Sections (8 μm) were stained with Harris' hematoxylin and eosin Y. Bouin's fixative, staining solutions and procedures were obtained from American Master Tech Scientific, Lodi, CA.

### 2.3. Electron microscopy

The book lungs were dissected in physiological saline (Schartau and Leidischer, 1983) as described above. The pinned book lung tissue was immersed in glutaraldehyde fixative (2–4 °C) for 12–24 h. The fixative consisted of 2% glutaraldehyde and 0.1 M

sodium cacodylate. Tissues were washed in a buffer solution consisting of 0.1 M sodium cacodylate, 0.05% calcium chloride and 0.23 M sucrose. The pinned book lungs were postfixed (12–24 h) in 2% osmium tetroxide, 0.1 M sodium cacodylate, 0.05% calcium chloride and 0.14 M sucrose. The tissues were washed again in buffer solution, the pins were removed and the tissue was dehydrated in a graded series of acetone. The book lungs were embedded in Spurr (1969) plastic, modified for a new replacement component (Ellis, 2006).

Semi-thin and ultrathin sections were cut on a RMC MT-X microtome (Boeckeler Instruments). The latter sections were collected on grids, stained with alcoholic uranyl acetate and lead citrate (Reynolds, 1963) and examined at 120 kv as recommended for the electron microscope that was used, FEI Technai 12 (formerly Philips). To locate suitable tissues for thin sectioning, semi-thin sections (1.0 μm) were cut and stained with warming of a mixture of 0.5 g toluidine blue, 0.5 g sodium borate and 20 ml methanol in 180 ml $H_20$.

## 3. Results

### 3.1. Changes in the atrium

The air and hemolymph channels extend longitudinally in the book lung while the atrium is a transverse channel leading to the spiracle at the posterior end of the book lung (Fig. 1A). The atrium is initially very small in the embryos and early instars (Farley, 2015, 2016), but it is increased in size as the spider molts and more air and hemolymph channels are added to the book lung. In electron micrographs of atrial development in embryos and early instars, the precursor cells at the ends of the hemolymph channels appear to release vesicles of fluid that produce the transverse atrial channel. In the earlier studies and observations herein, this process of lumen formation (mode I, coalescence of vesicle secretion) is also observed in the development of some air and hemolymph channels. As explained below, mode I is probably not used to increase the size of the atrium with each molt since air now flows through the spiracle and atrium, and drying rather than fluid accumulation is likely.

The precursor cell bodies (C) of the air and hemolymph channels remain within the hemolymph channel (Fig. 1A), and those that line the ventral wall (V) of the air channel produce peg and branching trabeculae that may also interconnect (Farley, 2015; Brunelli et al., 2015) as they form a cuticular layer that holds open the channel for passage of air. With trabeculae attached only to one wall, the air channels produced by mode I are highly variable in size.

The air channels are open to the atrium (Fig. 1A) while the hemolymph channels are enclosed by a layer of epithelial cells, and those at the posterior end (C') prevent flow of fluid into the atrium. These cells also produce long, branching trabeculae (T) that extend from the ventral air channel wall and curve a short distance anteriorly on the dorsal wall (D) of the air channel. The posterior ends of the numerous alternating hemolymph and air channels thus form a wall of trabeculae at the anterior atrial margin.

In preparation for the molt in juvenile spiders, there appears to be a retraction and/or death of the cells at the posterior end of each hemolymph channel (Fig. 1B). This leaves an anterior atrial layer of exuvial trabeculae (ET) initially formed by those cells. A similar layer of exuvial trabeculae (ET′) is formed by epithelial cells at the posterior atrial wall. New viable cells (C') farther anterior in the book lung secrete new trabeculae, and there is a layer of new trabeculae at the posterior atrial wall. Presumably, the atrial exuvia is removed at ecdysis.

Fig. 2A shows the atrial exuvia at higher magnification. From the ventral air channel wall (V) just anterior to the atrial lumen, single strands of exuvial cuticle have peg and branching trabeculae barely evident at this magnification. Attached posterior to this is a sac-like length of long, complex exuvial trabeculae (ET) that enclosed the cells that produced these cuticular trabeculae. As shown in Fig. 1B, a new layer of viable cells with their traberculae is now farther anterior (not evident in Fig. 2A) as the new posterior end of the hemolymph channels.

Farther anterior of the atrium, Fig. 2B shows the book lung lamellae while in the premolt condition with exuvia still in the book lung. The ventral air channel walls (V) have a single strand of exuvial cuticle (T1) and a new replacement cuticle (T2), both with attached exuvial peg and branching trabeculae. In this example, the exuvial layer (T1) still adheres to the new-growth layer of similar replacement trabeculae (T2), but presumably the exuvial layer will eventually be shed. The external opisthosomal exuvium (OE) in Figs. 1B and 2A appears to be connected through the spiracle to the cuticle of the outer book lung wall and the exuvial cuticle walls and trabeculae of the air channels. Apparently all this exuvia is connected and pulled through the narrow spiracle opening, leaving larger atrial and air channel lumina.

Fig. 3 is an electron micrograph showing a remaining precursor cell in a long exuvial trabeculae at the end of a hemolymph channel (Fig. 1A). It appears the shape of these cells determines the shape of the trabeculae, as the cells extend cytoplasmic processes (asterisk) that produce the trabecular branches (Br). Then, the precursor cell may withdraw and/or die as in Figs. 1B and 2A, leaving a nearly empty trabecula except for distorted cells and cell debris. The cuticle wall consists of an epicuticle (E) and underlying procuticle (Pr); fine microfibers are often evident in the latter when viewed at higher magnification. The cell in Fig. 3 has produced some microfibers (Mf), probably cuticulin as described for insect cuticle (Merzendorfer, 2006; Moussian, 2010, 2013).

### 3.2. New and old lamellae as the animal grows

Before and after each molt, new lamellae appear to be forming especially at the dorsal, anterior and/or medial margins of the book lung. Fig. 4 is a typical example, where the earliest formed lamellae (right) are at the posterior end of the book lung with air channels (A1) formed by the mode I process. Since the peg and branching trabeculae only extend from the ventral air channel wall, the size of these air channels is quite variable. As shown in Fig. 1A and B, 2A and 3, the hemolymph channels in Fig. 4 end in a layer of cells (C) with large and complex trabeculae (T) that extend into the atrium where they later become exuvia and are replaced in the molt.

In the medial aspect (left) of the juvenile book lung of Fig. 4, there are more recently produced, new-growth air channels (A2) with lumina formed by mode II. The mode II air channels in this example were produced at the anterior end of the book lung, and they extend its full length. The hemolymph channels are variable in size, but were likely formed along with the air channels or shortly thereafter, so they are clear for the passage of fluid and have numerous pillar trabeculae (T') holding them open. At the site of this section, the atrium needs to be enlarged in later molts since the atrium does not yet extend medially to the posterior end of the new mode II air channels. The mode II air channels are quite uniform in width throughout their length since they have bridging trabeculae that connect with both opposed walls of the channel.

Fig. 5 shows entapophyses extending inward from the external opisthosomal wall just posterior to the atrium and trabeculae (T) of the right book lung. The entapophysis consists of reticular fibers (F) and strands of cells (C) that apparently originated by EMT from peripheral epithelial cells (Ep). In advanced embryo and later stages, such entapophyses along with a dispersion of mesenchyme

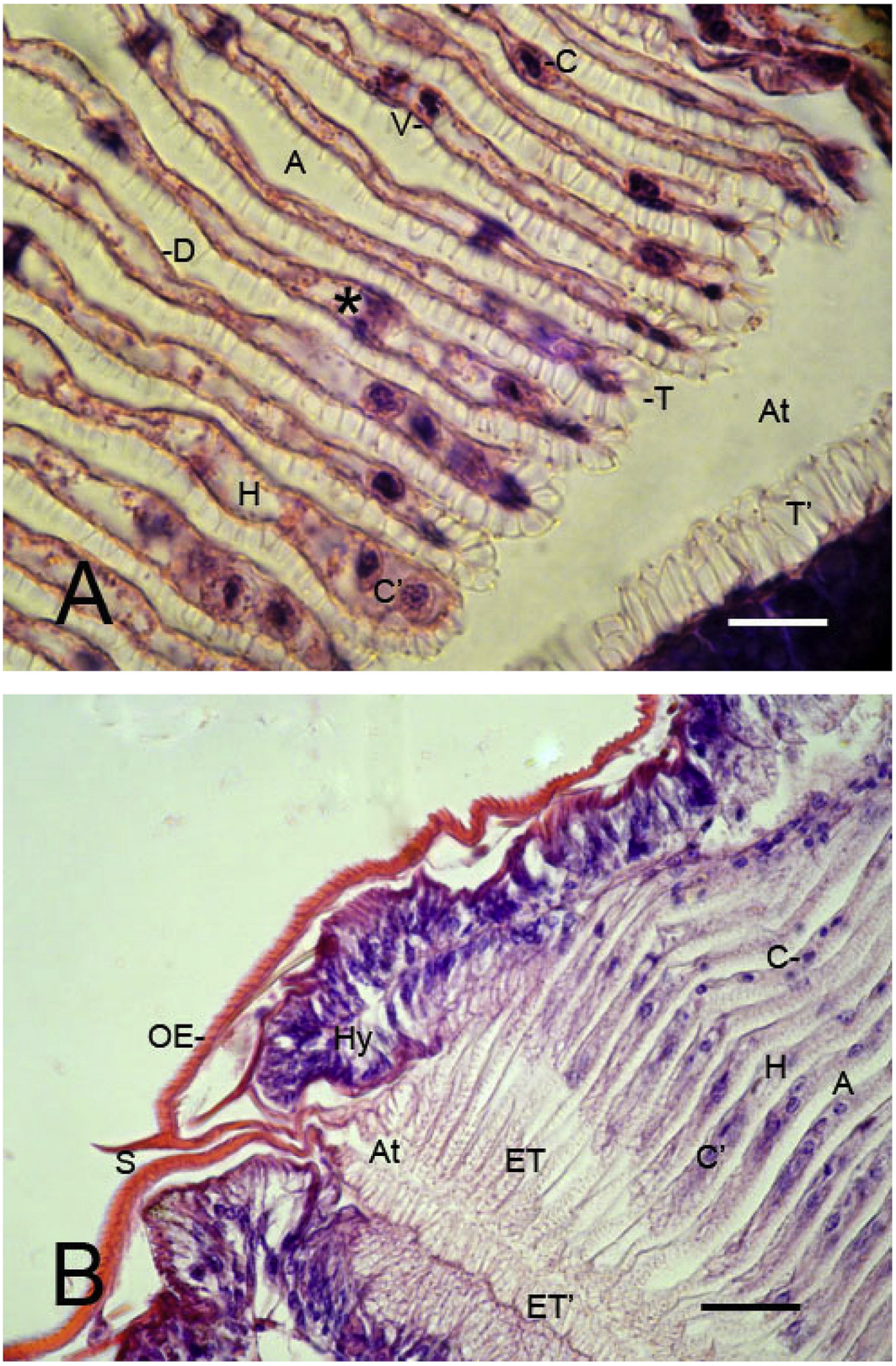
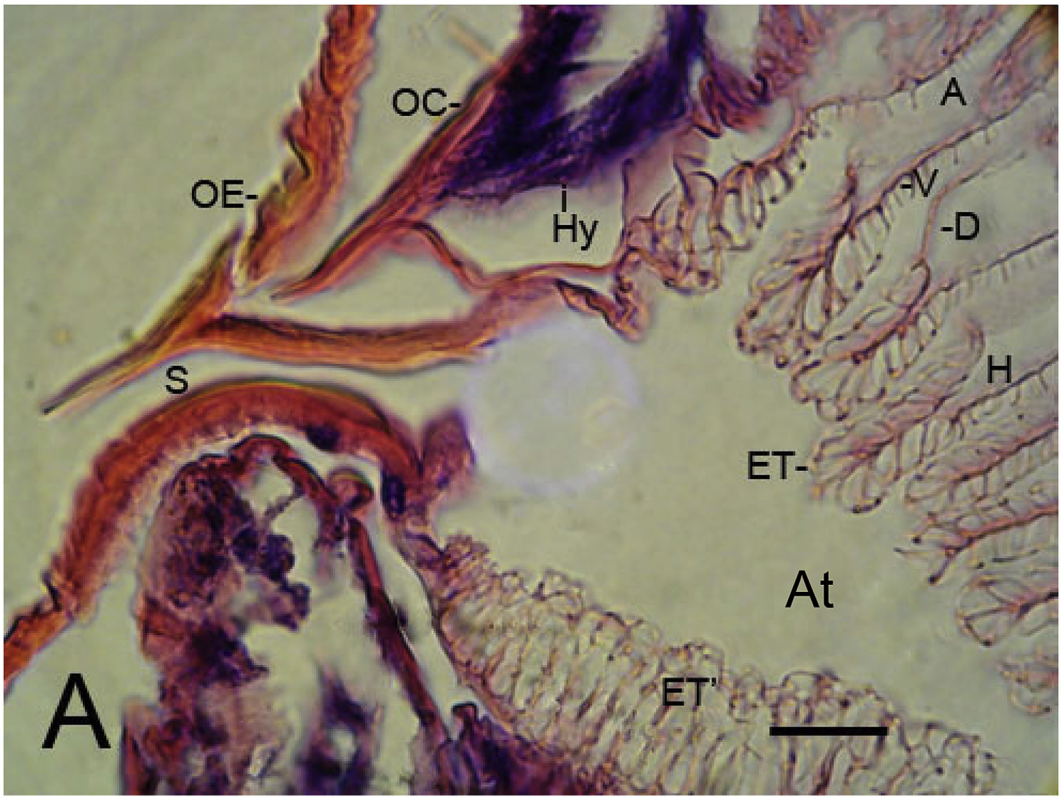



**Fig. 1.** The atrium (At) at the posterior end of book lungs where air channels (A) are commonly produced by mode I. LMs. *Parasteatoda tepidariorum*, para-saggital sections. **A:** Adult male. The ventral air channel wall (V) with attached trabeculae and the thin dorsal cuticular wall (D) of the air channels are all secreted by precursor cells (C) that remain within the hemolymph channel (H). The latter channels are nearly clear for passage of fluid except for precursor cells and some pillar trabeculae (asterisk). Cells (C′) at the posterior end of each hemolymph channel produce a sac-like layer of large, branching trabeculae (T) that curve around the channel end and line the dorsal air channel wall for a short distance. Spiracle at right, not shown. T′, long trabeculae at posterior atrial wall. Scale, 20 μm. **B:** Before ecdysis in molting juvenile. The air channels are clear for passage of air, but the hemolymph channels (H) still consist of strands of cells with lumina slow to develop, typical of mode I process. Earlier cells produced exuvial trabeculae (ET) at the anterior atrial wall and then died or withdrew, leaving a larger atrium when the exuvium is removed. Viable cells (C′) at new posterior end of hemolymph channels have produced new trabeculae. ET′, posterior exuvial trabeculae; Hy, hypodermis with new replacement cuticle; OE, opisthosomal exuvium; S, spiracle. Scale, 40 μm.



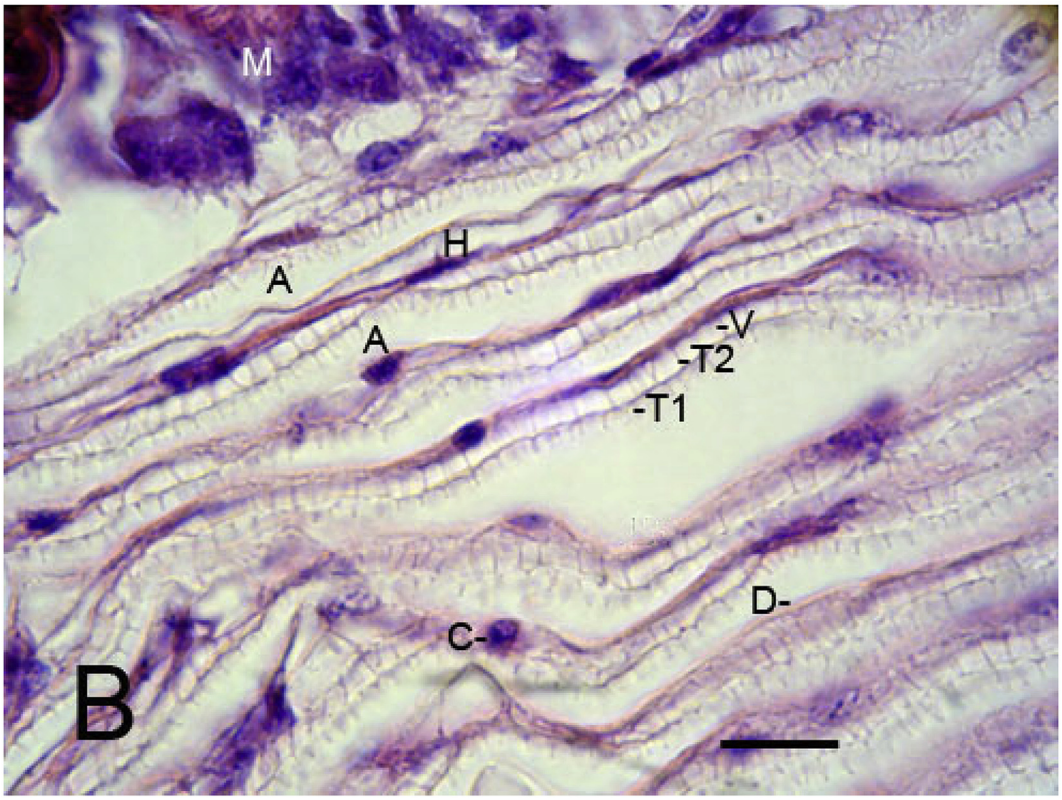




**Fig. 2.** Higher magnification, before ecdysis in molting juveniles with exuvium in the atrium (At) and farther anterior among the lamellae. LMs. *Parasteatoda tepidariorum*, para-saggital sections, air channels produced by mode I. **A:** Precursor cells at posterior end of former hemolymph channels (H) produced a layer of branching trabeculae, now exuvial (ET), that curve around channel ending. Those cells apparently died and/or withdrew, leaving the sac-like exuvium of the ventral wall (V) of the former air channels and a short length of exuvial trabeculae at adjacent dorsal air channel wall (D). A, air channel; ET′, exuvial trabeculae at posterior atrial wall; Hy, hypodermis; OE, opisthosomal exuvium; OC, new opisthosomal cuticle. **B:** The ventral wall (V) of each air channel has an exuvial strand of cuticle with attached trabeculae (T1) about to be separated from new replacement cuticle (T2) with similar structure. The dorsal wall (D) of each air channel has a thin layer of cuticle (Fig. 7). The precursor cell bodies (C) are thin and elongate within hemolymph channels (H) with some lumina starting to form. M, mesenchyme and/or precursor cells at book lung margin. Scales, 20 μm.

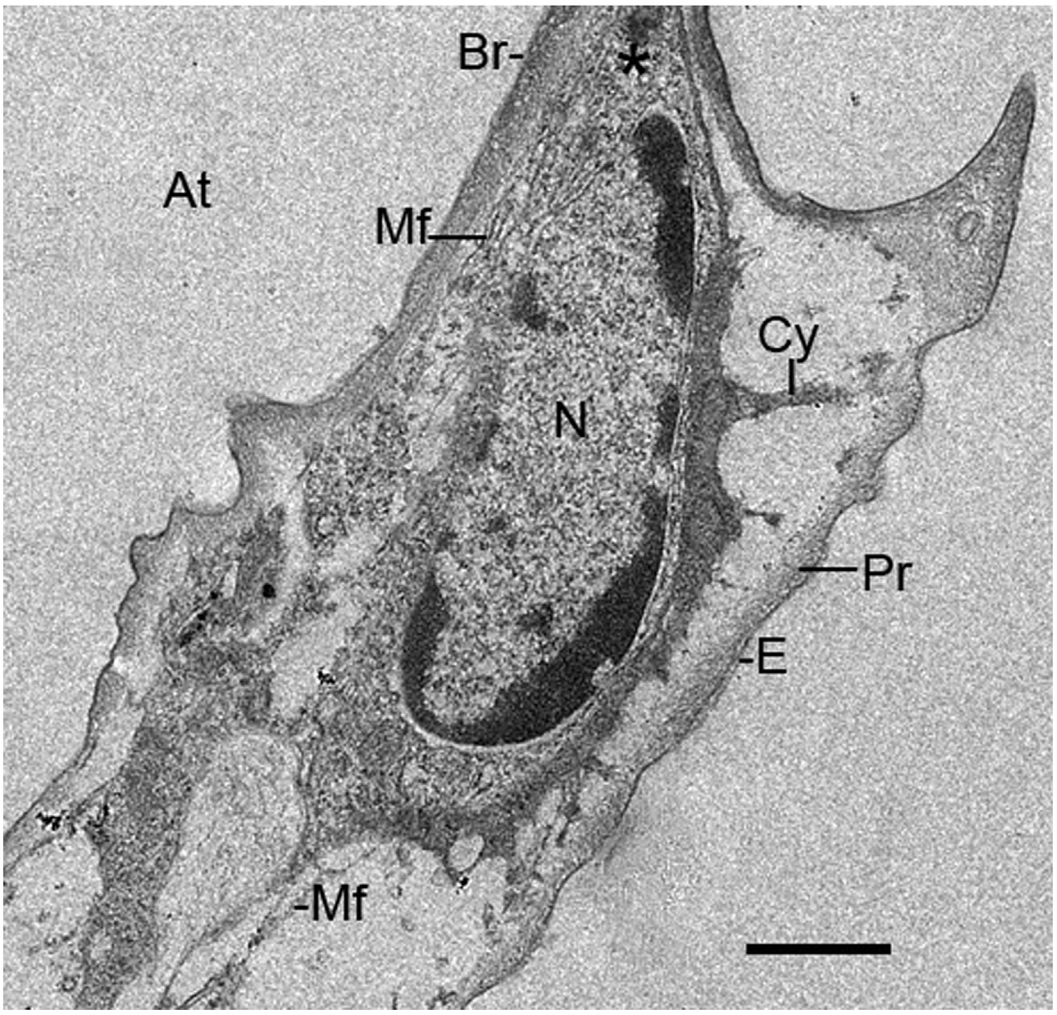



**Fig. 3.** Part of a long branching trabecula (Fig. 1A) with precursor cell (N, nucleus) in atrium (At) at posterior end of hemolymph channel. TEM. *Parasteatoda tepidariorum*, adult female. Cuticular branches (Br) result from cytoplasmic projections (asterisk) of parent cell. Common for these trabeculae, this cell with microfibers (Mf) and strands of cytoplasm (Cy) appears to be withdrawing and/or dying. The cuticular wall consists of an epicuticle (E) and an underlying procuticle (Pr). Scale, 1 μm.

cells (M) are present throughout the opisthosoma. As the animal molts and increases in size, the entapophyses may provide a continuing supply of mesenchyme cells among the developing organs and tissues. In the examples below, epithelial precursor cells producing lamellae appear to be derived from a row or cluster of mesenchyme cells at the periphery of the book lung chamber. These spider mesenchyme cells are irregular in shape and lack specialization or organization as is typical of mesenchyme cells described in other taxons (Lee et al., 2006).

*3.2.1. Mode I (coalescence of merocrine vesicle secretion)*

Lamellae produced by this process are commonly at the posterior parts of the book lung near the atrium and spiracle. As the spider increases in size, lamellae initially produced by mode I or mode II are both replaced in the molt by mode I lamellae with larger channel width and greater air flow. Some of the mode I sequence of development is enumerated below and is evident in Figs. 6A, B and 7.

1) Mesenchyme cells (M) migrate to the book lung site (Fig. 6A) where they apparently proliferate, undergo MET and begin producing rows of aligned precursor cells (PC). 2) Clear spaces (A, primordial air channels) begin to develop among the aligned cells, thus forming separate cell strands. These cell strands are the site of future hemolymph channels, i.e., lumina (Figs. 1A, 2B, 4 and 7) and space holders (pillar trabeculae, Figs. 1A, 4, 13 and 14) gradually develop among these cells. 3) In air chambers more distant from their site of origin, peg and branching trabeculae are formed at the ventral edge of each cell strand (V in Fig. 6B), and this becomes the ventral wall of the new air channel. 4) The strand of precursor cells also secretes a very thin layer of cuticle at the opposing, dorsal air channel wall (D in Figs. 6B and 7) as lumina continue developing for hemolymph within the cell strands. With this mode I process, lumina are usually slow to develop in the primordial hemolymph channels (Figs. 1B, 2B and 6A, B), but eventually there is a series of alternating air and hemolymph channels (Figs. 1A, 4 and 7) that are enlarged in successive molts.

The initial mesenchyme cells (M) that become book lung precursor cells (PC) in Fig. 6A are initially round or ovate, but striking changes occur in these cells as they become part of the book lung lamellae (Figs. 6A, B and 7). With their apparent start of MET, the mesenchyme cells become smaller precursor cells that align in rows (Fig. 6A). This process continues so that eventually, the precursor cells become very elongate and thin (C in Fig. 6B; C1 in Fig. 7) as they continue within the hemolymph channel, producing the cuticular wall and trabeculae of the ventral air channel wall (V) and the thin cuticle of the dorsal wall (D). Some cells within the

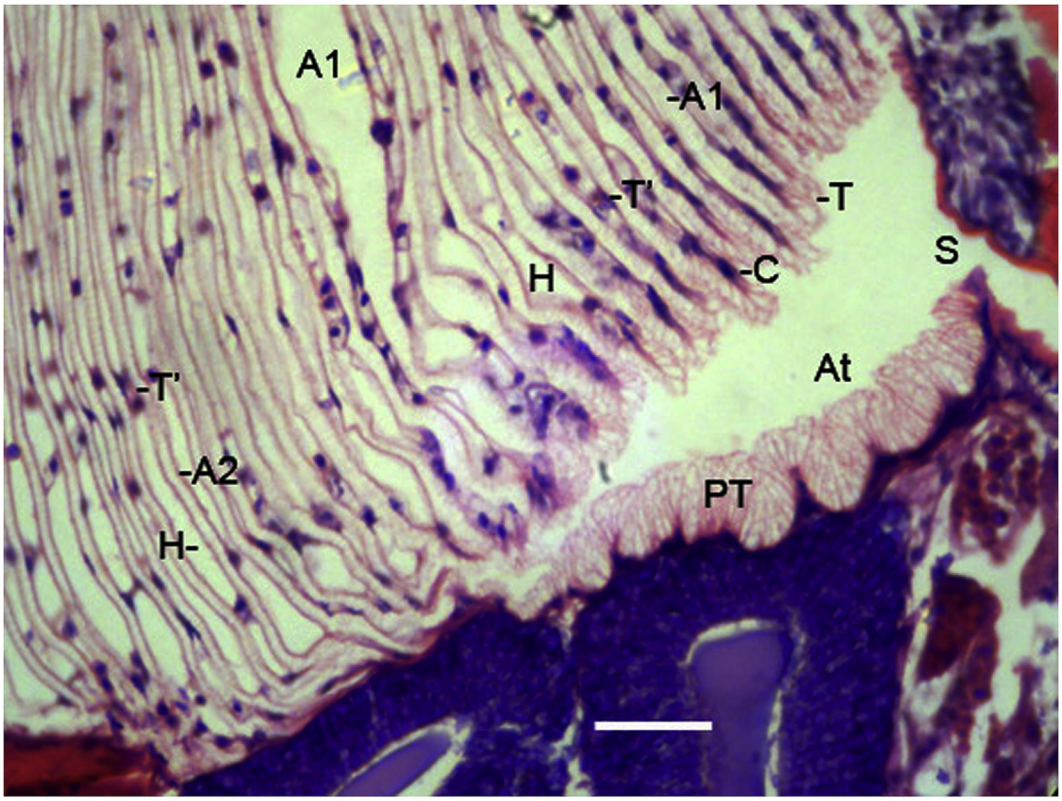



**Fig. 4.** Posterior end of book lung with air and hemolymph (H) channels produced at right by mode I and at left by mode II. LM. *Parasteatoda tepidariorum*, juvenile, para-saggital section. The older mode I air channels (A1) are irregular in size, and they open to the atrium (At) while the hemolymph channels are closed at their posterior end (Fig. 1A) by precursor cells (C) that secrete large cuticular trabeculae (T). The mode II air channels (A2) have bridging trabeculae, so these channels remain quite uniform in size throughout their length. PT, trabeculae at posterior atrial wall; S, spiracle; T′, pillar trabeculae. Scale, 40 μm.

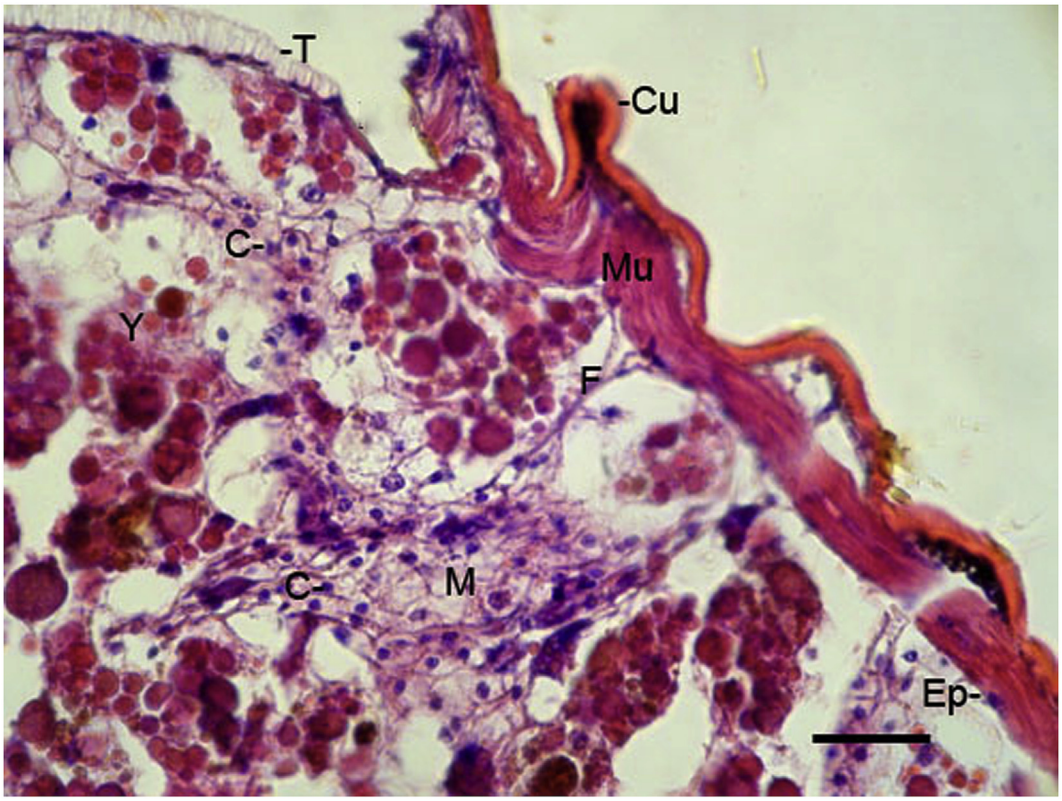



**Fig. 5.** Typical opisthosomal entapophyses with cells (C) and reticular fibers (F); the cells apparently migrated inward from EMT in peripheral epithelial cells (Ep). LM. *Parasteatoda tepidariorum*, juvenile, horizontal section. Larger mesenchyme cells (M) without specialization or organization appear to be associated with the entapophyses as though that is their origin. Cu, cuticle; Mu, muscle and/or muscle cell precursors; T, trabeculae of right book lung atrium; Y, yolk. Scale, 40 μm.

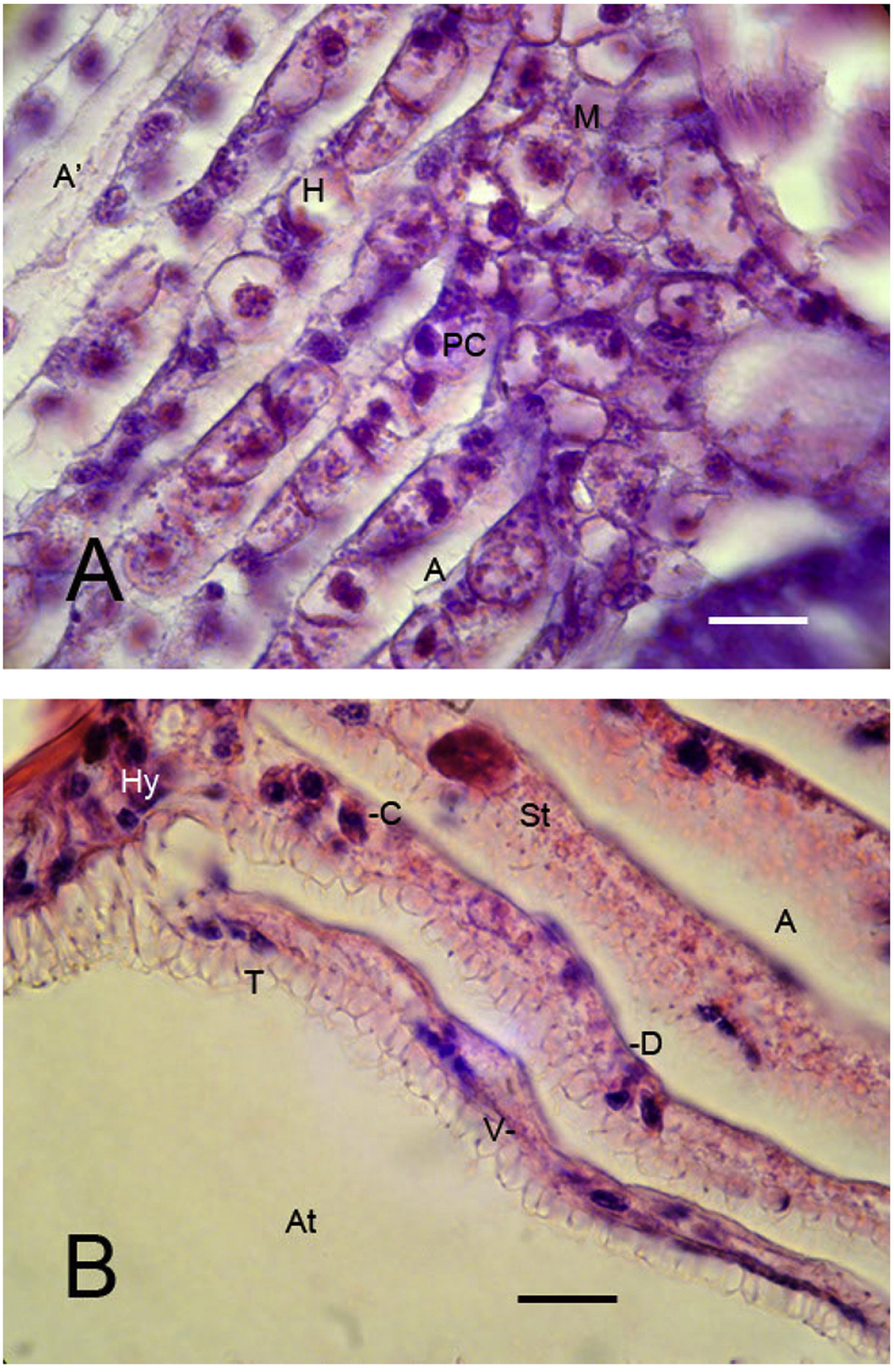



**Fig. 6.** Early development of air channels (A) produced by mode I from cells at book lung periphery. LMs. *Parasteatoda tepidariorum*, juveniles, horizontal sections. **A:** The book lung at this site has not yet developed an outer wall, so opisthosomal mesenchyme cells (M) can migrate into the area, undergo MET and become aligned book lung precursors (PC). The primordial hemolymph channels (H) consist of strands of precursor cells with little indication that a lumen will form among these cells. More advanced air channels (A′) have peg and branching cuticular trabeculae, barely evident at this magnification. **B:** Later stage. As usual, the air channels are irregular in size and shape, but they now have a clear lumen with a thin layer of cuticle on the dorsal (D) wall (Fig. 7) and cuticle with peg and branching trabeculae (T) on the opposing ventral (V) wall. The primordial hemolymph channels still consist of strands (St) of precursor cells (C) with little indication of a lumen. A strand of hypodermal (Hy) and/or mesenchyme cells at left may be a source of lamellar precursor cells. Scales, 20 μm.

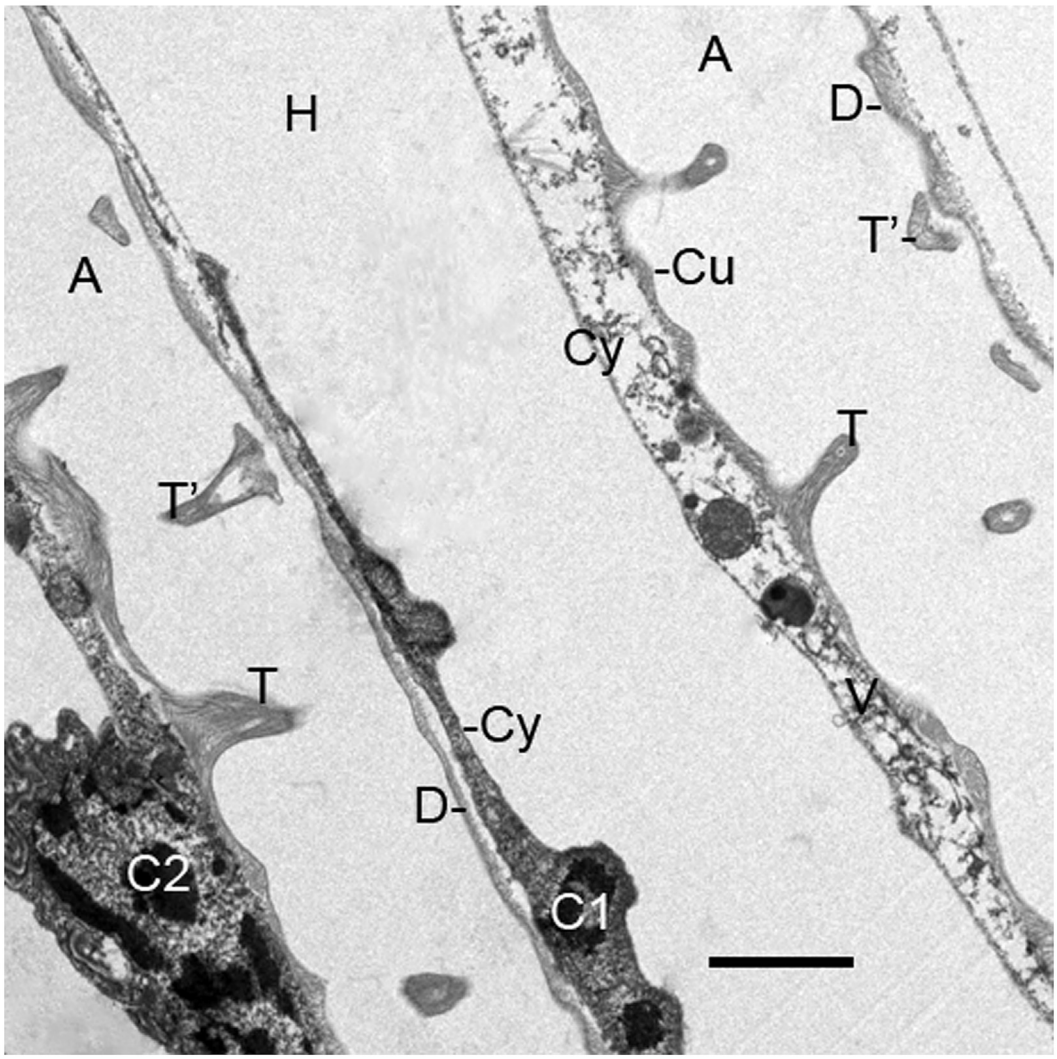



**Fig. 7.** A length of air (A) and hemolymph (H) channels from the older, posterior part of a book lung where the air channels were produced by mode I. TEM. *Parasteatoda tepidariorum*, juvenile. The ventral wall (V) of the air channels consists of a thin layer of cytoplasm (Cy) with attached cuticle (Cu) and peg trabeculae (T). Some trabeculae (T′) extend close to the opposed dorsal air channel wall (D) but do not connect with it. That wall also has a very thin layer of cuticle. Precursor cell bodies remain within the hemolymph channels, and most are very thin (C1) and have a thin, elongate layer of cytoplasm that produces the cuticle of the dorsal and ventral air channel walls. Precursor cells (C2) that remain large may become part of a pillar trabecula (Figs. 1A, 4, 13 and 14). Scale, 2.0 μm.

hemolymph channel remain fairly large (C2 in Fig. 7) and may increase in size as they become part of a pillar trabecula with a similar cell from the opposed channel wall (e.g., Fig. 14).

From the original strands of precursor cells in the primordial hemolymph channel (Fig. 6A and B), many cells seem to disappear as space develops for hemolymph. Cell debris, presumably from cell death, is common in these channels (e.g., Fig. 12). In Fig. 7 there are fibrous trabeculae (T) just starting to form, and there are the distal tips of peg trabeculae (T') that are close to the opposing dorsal wall but not connecting with it. This prevents the formation of bridging trabeculae and a channel that cannot become wider for increased passage of air.

### 3.2.2. *Mode II (alignment and merging of intracellular vesicles)*

Fig. 8A is an example of air channels being produced by this process from opisthosomal mesenchyme cells (M) that apparently migrated into the area. In forming the future hemolymph channel, the precursor cells may align very close end-to-end, resulting in a strand of cells (St) with little indication of the lumen that will eventually develop among these cells. The precursor cells may also align with substantial lumen between the cells so the hemolymph channel lumina (H) develop along with the air channels. Presumably this allows early perfusion of the lamellae with hemolymph. Near the origin of the hemolymph channels in Fig. 8A, there are some early pillar trabeculae (T) consisting of fused cells from opposed walls of the channel. It thus appears some precursor cells join into pairs as these channels are initially formed.

New mode II channels produced at the anterior, dorsal or medial aspects of the book lung may continue elongation to the atrium (e.g., Fig. 4) where they are later shed in the molt and replaced with channels made wider by mode I (Farley, 2016). Other mode II channels extend part way along the length or width of the book lung where they fuse with channels that were produced earlier by the mode I process (Fig. 8B). This alignment and fusion of new and old air and hemolymph channels is thought to have occurred in an example in a postembryo (Farley, 2015, Fig. 8), and it is a common feature in juvenile and adult book lungs as the book lungs are increased in size as the animal grows.

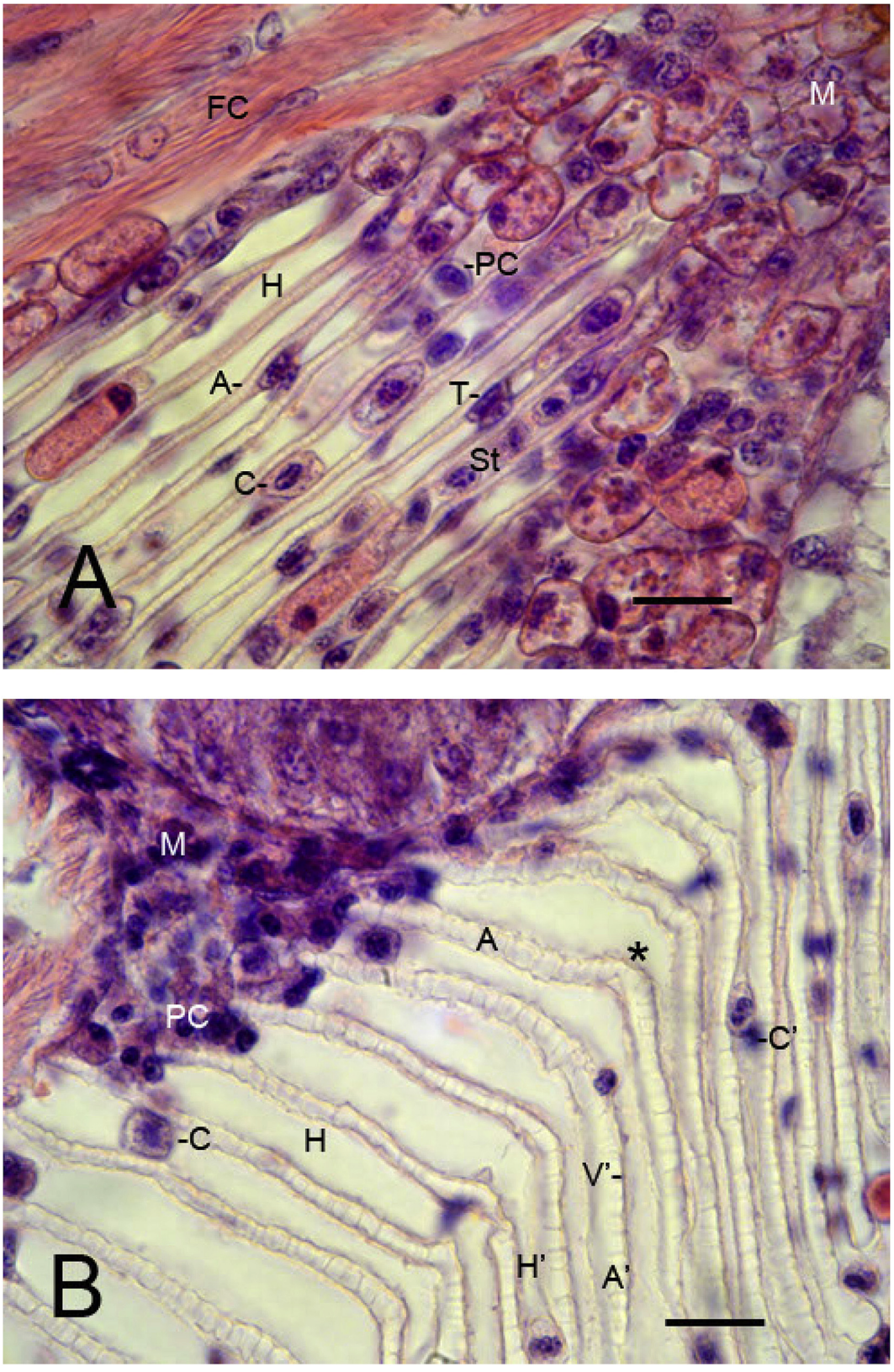



**Fig. 8.** The book lungs at these sites still lack a medial cuticular wall, so mesenchyme cells (M) from the opisthosoma can migrate into the area, undergo MET and become aligned precursor cells (PC) that produce new mode II air and hemolymph channels that fuse with the older mode I channels. LMs. *Parasteatoda tepidariorum*, juveniles, transverse sections. **A:** The new mode II air channels (A) are uniform in size with bridging trabeculae (not evident at this magnification). As the air channels are formed, some aligned precursor cells remain close together, forming a strand (St) of precursor cells as the site of a future hemolymph channel. Other precursor cells are separated and have a hemolymph channel (H) with a lumen that may be functional very early. Elongate, fibrous cells (FC), possibly muscle cell precursors, are often near the book lung site. T, primodial pillar trabecula. **B:** Alignment and end-to-end fusion of new mode II air channels (A) with older mode I air channels (A′). There is often a kink (asterisk) at the fusion site. The precursor cells (C) within mode II hemolymph channels (H) have produced new dorsal and ventral air channel walls that join with the older dorsal and ventral cuticle walls of air channels produced by precursor cells (C′) in the older hemolymph channels (H′). Scales, 20 μm.

In Fig. 8B, new mode II air channels (A) produced at the medial aspect of the book lung (top of figure) join with older air channels (A′, lower right) made larger by the mode I process, as described earlier (Farley, 2016). The walls of these mode I air channels were likely separated by earlier mode II air channels, but removal of the latter in a molt allowed the air channel to get increasingly large by mode I in successive molts. As usual, these mode I air channels are irregular in shape since they only have trabeculae attached to the ventral channel wall (V'). The new medial mode II air channels (A) are quite uniform in size with their many bridging trabeculae (barely evident at this magnification). The dorsal and ventral walls of the new air channels connect with the dorsal and ventral walls of the old air channels, and this results in continuity in the flow of air and hemolymph through channels made longer as a result of the fusion process.

### 3.2.3. *Cellular fine structure of mode II development*

Figs. 9–12 are electron micrographs of mode II channel formation in new-growth areas at the outer book lung wall near the anterior end of juvenile book lungs. The new lamellae originate from small clusters of mesenchyme and precursor cells at the periphery of the book lung. These precursor cells are initially round or oval in shape and crowded close together so it is difficult to discern cell boundaries. Fig. 9 is an example where the precursor cells are separated so the intracellular location of the aligned vesicles in cell C1 is clearly evident. Another cell (C2) in this figure has a cluster of small vesicles (V') that are in line with those in C1, so when the small C2 vesicles fuse together, the resulting large vesicle in that cell can merge with those in C1, forming a continuous longer air channel. This merging of small vesicles to make larger ones is a common feature of channel formation as well as alignment and fusion of vesicles in multiple cells to make longer and wider channels.

Within some precursor cells, the first indication of air and hemolymph channel formation may be numerous vesicles (V' in Figs. 9 and 10) and small clear spaces (H3 and asterisk in Fig. 11) that could presumably coalesce to become larger vesicles. Then as the large vesicles (V in Figs. 9–11) align and merge to become air channels, the fluid is evidently removed to allow for air passage. Channel clearance surely takes place within these developing air

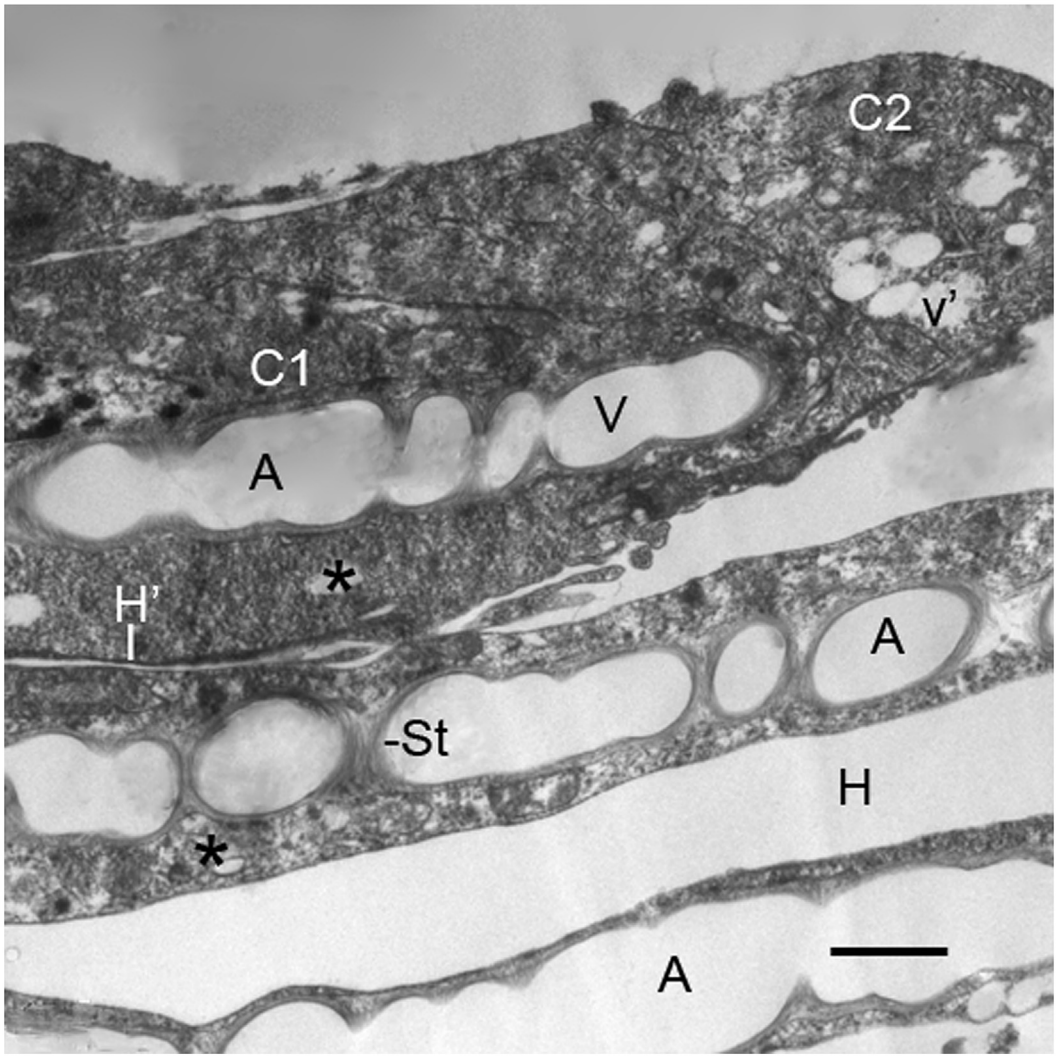



**Fig. 9.** The outer membrane of cell C1 is evident in this electron micrograph, so it shows the intracellular alignment and merging of vesicles (V) in the mode II formation of air channels (A) in a new-growth site at outer edge of a book lung. TEM. *Parasteatoda tepidariorum*, juvenile. The large, oval-shaped precursor cells (C1, C2) will become much thinner as they form new hemolymph (H) and air channels and continue the alternating pattern already present in older channels (below). Cell C2 has vesicles (V′) in alignment and about to coalesce and merge with those in C1. Hemolymph channel H′ consists of a thin line of clear space, possibly formed by mode III and vesicle secretion (mode I) since small vesicles (asterisks) are often in proximity. Strands (St) of cytoplasm and cuticle between air channel vesicles continue as bridging trabeculae. Scale, 1 μm.

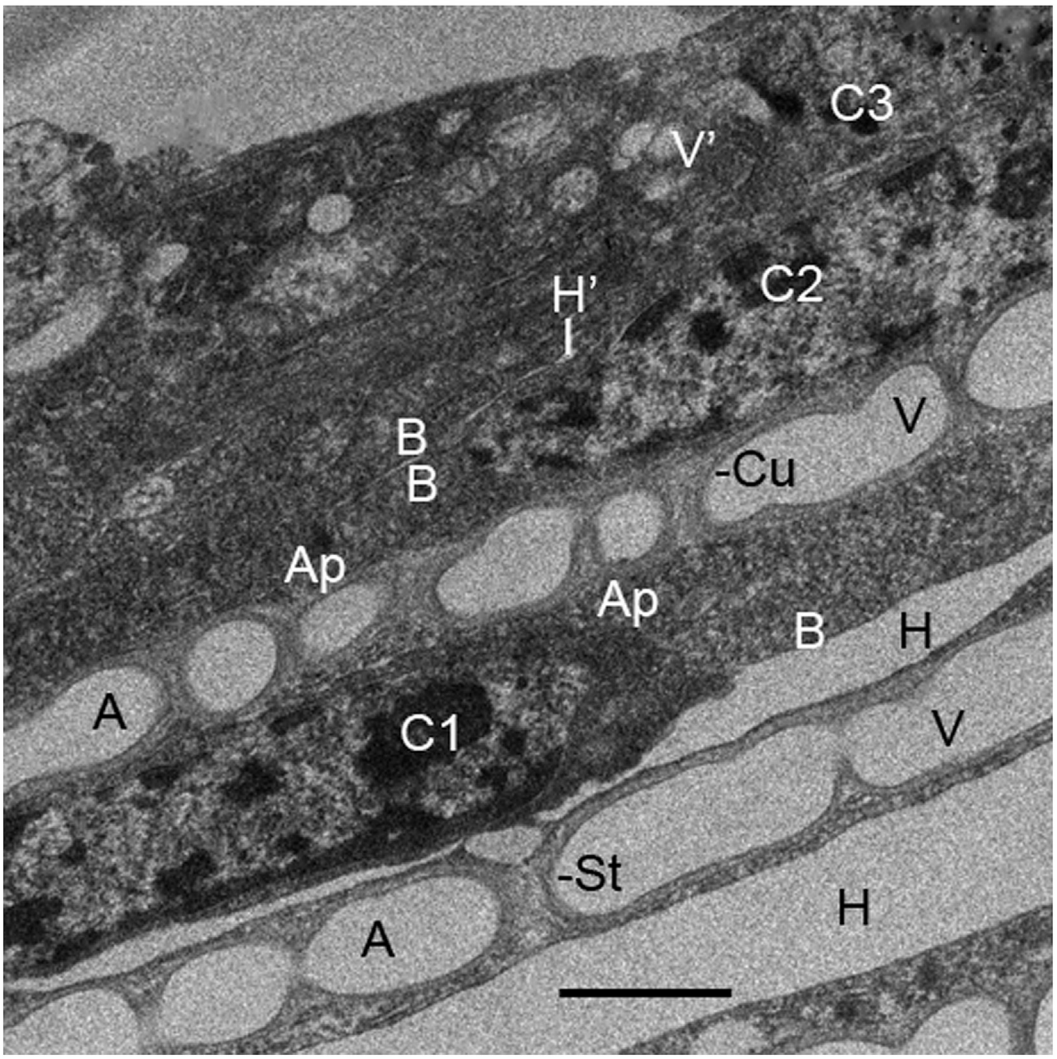



**Fig. 10.** As hypothesized for embryos earlier (Farley, 2016, Fig. 16) and as shown in Figs. 6A and 8A, mesenchyme cells in juveniles and adults also undergo MET and become epithelial precursor cells (C1-C3) that develop apicobasal polarity and continue the alternating pattern of air (A) and hemolymph (H) channels already present. TEM. *Parasteatoda tepidariorum*, juvenile, mode II lumen formation. The opposed basal surfaces (B) of precursor cells normally abuts the hemolymph channels, while the opposed apical surfaces (Ap) secrete the cuticle walls of the air channels. The air channels consist of aligned and fused vesicles (V). Both cells C1 and C2 appear to contribute to cuticularization (Cu) of an enclosed air channel, though it probably started inside one cell or the other. Cell C3 has vesicles (V′) that could presumably merge to form larger, aligned air channel vesicles. Apicobasal polarity has apparently been determined in this cell since its basal surface abuts H′, a new hemolymph channel barely evident as a thin line of clear space. The strands (St) of cytoplasm and cuticle between air channel vesicles continue as bridging trabeculae. Scale, 1 μm.

channels, but the process remains unknown. Fluid uptake of proteins and electrolyte by endocytosis is thought to occur in fruit fly and vertebrate tracheae (Tsarouhas et al., 2007; Behr, 2010).

In new-growth areas of the book lung, the hemolymph channel lumina often develop later than the air channels. The hemolymph channels may appear initially as lengths of opposed basal cell membrane and a thin, clear line among the aligned precursor cells (H' in Figs. 9 and 10; H2 in Fig. 11). This clear space between precursor cells is hypothesized to result from mode III, diffusion of fluid into the space in response to an osmotic gradient (Farley, 2015, 2016). There are often small vesicles (asterisks in Fig. 9) in close proximity to early hemolymph channels, so mode I is also likely involved, especially as the hemolymph channel gets larger. These primordial hemolymph channels gradually increase in size so that farther from the initial growth site, they are larger (Fig. 7, H in Figs. 9 and 10) and contain many pillar trabeculae (Figs. 1A, 4, 8A, 13 and 14).

Examination of the developing air and hemolymph channels in Figs. 10 and 11 show the new precursor cells (top of figures) are developing channels in a pattern that continues and coincides with that already established by the older thin and elongate precursor cells (bottom of figures). Thus, there appears to be a propagation of the alternating sequence of air and hemolymph channels from old to new channels.

The polarity pattern in these juvenile lamellae appears to be a continuation of that proposed earlier for embryos (Farley, 2016): the air channel lumina develop from the apical surface (Ap) of aligned and opposed precursor cells while the hemolymph channel lumina form at the basal surface (B) of these cells. As mesenchyme cells undergo MET and become lamellar precursor epithelial cells (Fig. 8A), the apicobasal polarity of the newly recruited cells and their resulting channel pattern is apparently induced by the polarity of the older precursor cells already present in the book lung (Figs. 10 and 11). The polarity of cell C3 in Fig. 10 and C4 in Fig. 11 has

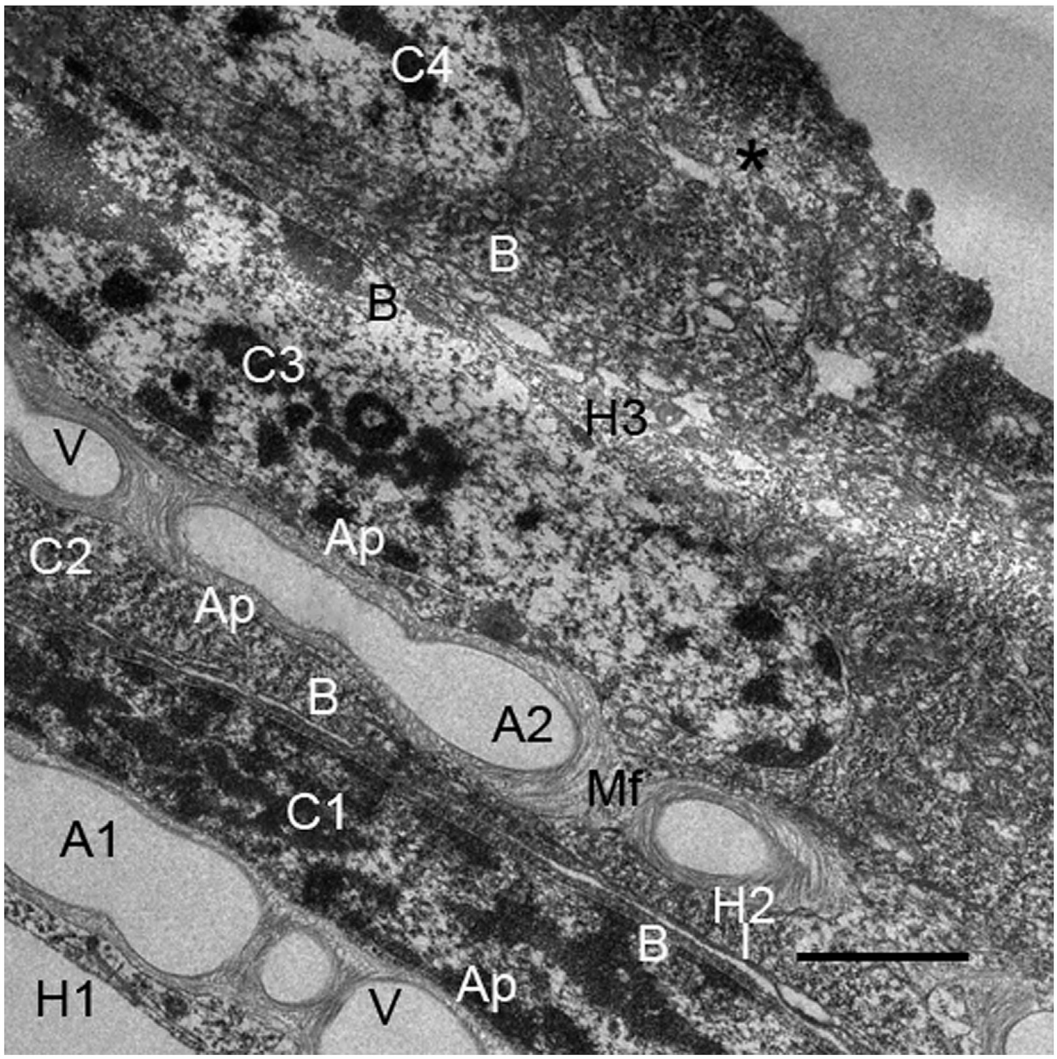



**Fig. 11.** The new-growth precursor cell (C4) is developing apicobasal polarity and lamellar channels that continue the alternating pattern of air (A1, A2) and hemolymph (H1, H2) channels already produced by precursor cells (C1-C3). TEM. *Parasteatoda tepidariorum*, juvenile, mode II air channel formation. As usual, the opposed basal surfaces (B) of precursor cells abuts the hemolymph channel (H2) while opposed apical surfaces (Ap) secrete cuticle for the wall of A2. C1 is contributing to cuticularization of channel A1; C2 and C3 appear to be contributing to channel A2. H2 consists of a length of opposed cell membrane and a thin clear line possibly formed by mode III. H3 consists of opposed cell membrane and many small clear spaces and vesicles that could presumably coalesce and extend the hemolymph channel. C4 does not yet have an air channel, but its polarity appears to be determined since its basal surface abuts the developing H3. C4 has clusters of small vesicles (asterisk) of uncertain future, but their location suggests the vesicles could merge to form a large air channel vesicle like those (V) in A1 and A2. Mf, cuticulin microfibers. Scale, 1.0 μm.

apparently been determined, although these new precursor cells only have clusters of small vesicles and clear spaces. The basal surface of these cells abuts developing hemolymph channels (H' in Fig. 10; H3 in Fig. 11).

In the development of mode II air channels, the morphology of the intracellular vesicles appears to give some indication of the intracellular pressure and forces applied against the vesicles as the parent cell undergoes changes in shape. These precursor cells become very thin and elongate as they become part of the book lung lamellae. In the examples in Figs. 9–11, the air channel vesicles (V) are shorter in the apical/basal axis of the parent cell as that cell becomes longer and thinner. In the more advanced cells C2 and C3 in Fig. 11, the air channel vesicles are shorter in their apical/basal axis, and that axis of the vesicles has fewer and more compacted cuticulin microfibers (Mf) (Merzendorfer, 2006; Moussian, 2010, 2013). Vesicles are longer in the elongating axis of the parent cells, and the microfibers are more abundant and diffuse as though there is less force in that direction. The alignment and merging of vesicles to form intracellular air channels may thus be a passive effect of changes in cell shape. The molecular basis for such intracellular forces and changes in shape are being studied in numerous model systems (see below).

In Fig. 9, the elongate vesicles (V) of a developing air channel are clearly within cell C1. As the parent cell thins and elongates and the air channel becomes cuticularized, the air channel gradually becomes extracellular with thin cell bodies and strands of cytoplasm on either side as in more developed air channels at the bottom of Figs. 9–11 and in the many advanced air channels in Fig. 12. Though very thin, these cells apparently retain the alternating polarity pattern: air channel cuticle secreted at the opposed apical surfaces and hemolymph channels at the basal surfaces. After the air channel cuticle walls are shed in the molt (Fig. 2B), new replacement cuticle walls are produced at the opposed apical cell surfaces.

The developing air channels have many cytoplasmic and cuticular strands (St) that are becoming bridging trabeculae at the new-growth site as the channels are initially produced by the mode II

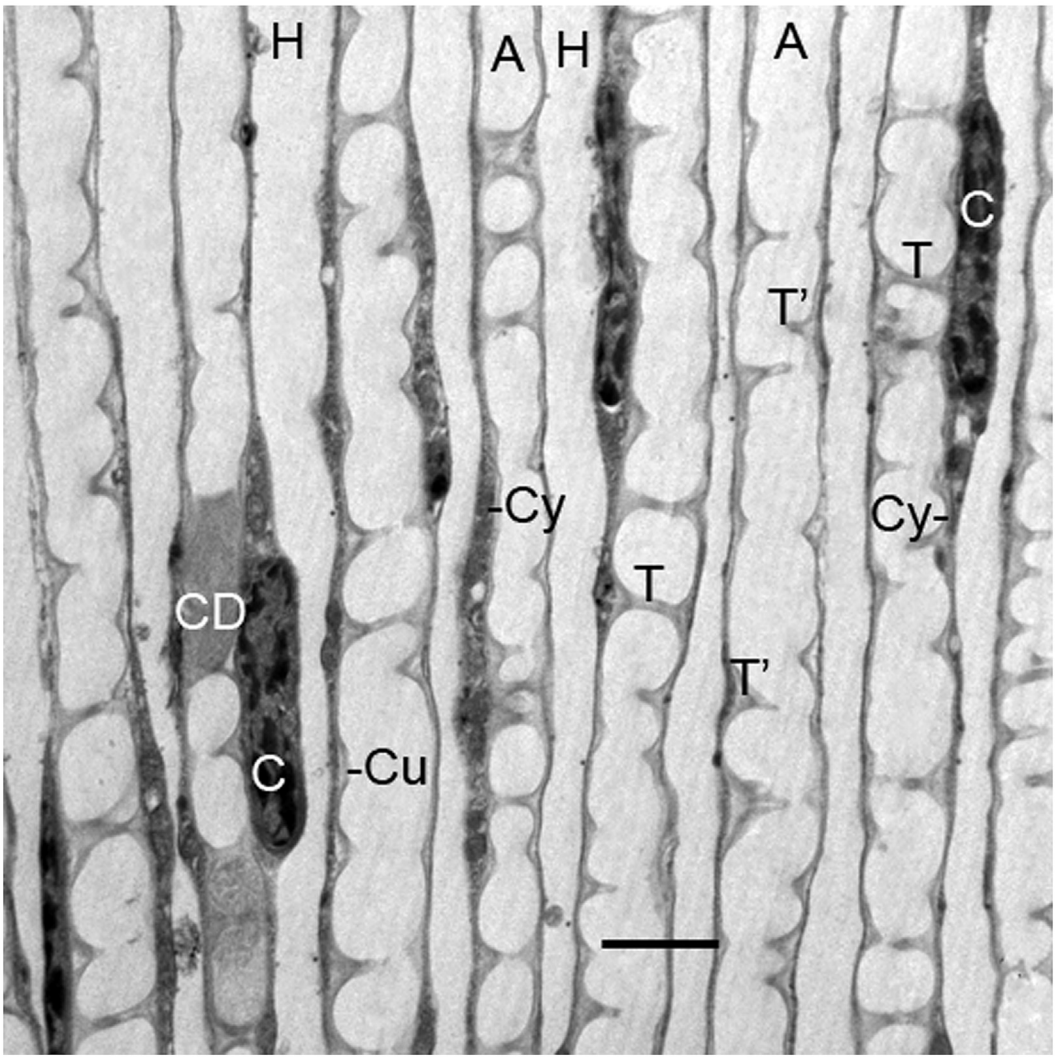



**Fig. 12.** With distance from site of origin, mode II air channels have bridging trabeculae (T) and fairly uniform size and structure, but trabeculae are less numerous and become thin strands of cuticle without the cytoplasm that was there on initial formation (Figs. 9–11). TEM. *Parasteatoda tepidariorum*, adult female. With bridging trabeculae so thin, only a fragment (T′) often appears in electron micrographs. A very thin layer of cuticle (Cu), thin strands of cytoplasm (Cy) and precursor cell bodies (C) form the opposed walls of air channels. These cells are in direct contact with contents of hemolymph channels (H). CD, cell debris. Scale, 2 μm.

process (Figs. 9 and 10). However, there appears to be increased merging and fusion of luminal vesicles (V) with distance from the channel site of origin, as evident in Fig. 12. The cuticular trabeculae (T) are thin and less abundant in this electron micrograph, reducing obstruction of air flow but still keeping the channel open. In histological and thin sections, the more distant mode II air channels have trabeculae that appear to be peg-like (T') since only a portion of the trabecula is present in the section. Closer examination shows there are portions of trabeculae from both walls of the air channel, as expected with bridging trabeculae produced by mode II as in Figs. 9–11.

*3.2.4. Development of pillar trabeculae in hemolymph channels*

The cell bodies of the channel precursor cells remain in the hemolymph channels, and some cells extend cytoplasmic processes across the channel to join with a cell on the opposite wall. These bridging pillar trabeculae are common in the embryo (Farley, 2015, 2016), and since there is no molting of the contents of the hemolymph channel, the trabeculae increase in number, size and complexity as the spider increases in age. There are often precursor cell bodies in close proximity as though they are migrating toward each other (Fig. 13), or they are exactly opposite each other (Fig. 14). The cells merge and begin forming a pillar trabecula consisting of two or three cells that span the channel. Specialized junctions (fascia adherens) are commonly seen at the points of contact between a cell process and cell body as in Fig. 13 or between opposed cell bodies (Fig. 14; Reisinger et al., 1990, 1991; Brunelli et al., 2015; Farley, 2015, 2016).

## 4. Discussion

*4.1. Mesenchyme cells*

In metazoan development, EMT occurs in gastrulation as ectodermal cells migrate inward and become mesoderm and endoderm (Lee et al., 2006). These cells are then available as a source of precursors cells for developing tissues and organs as reported for numerous model systems in vertebrates and invertebrates (Lee

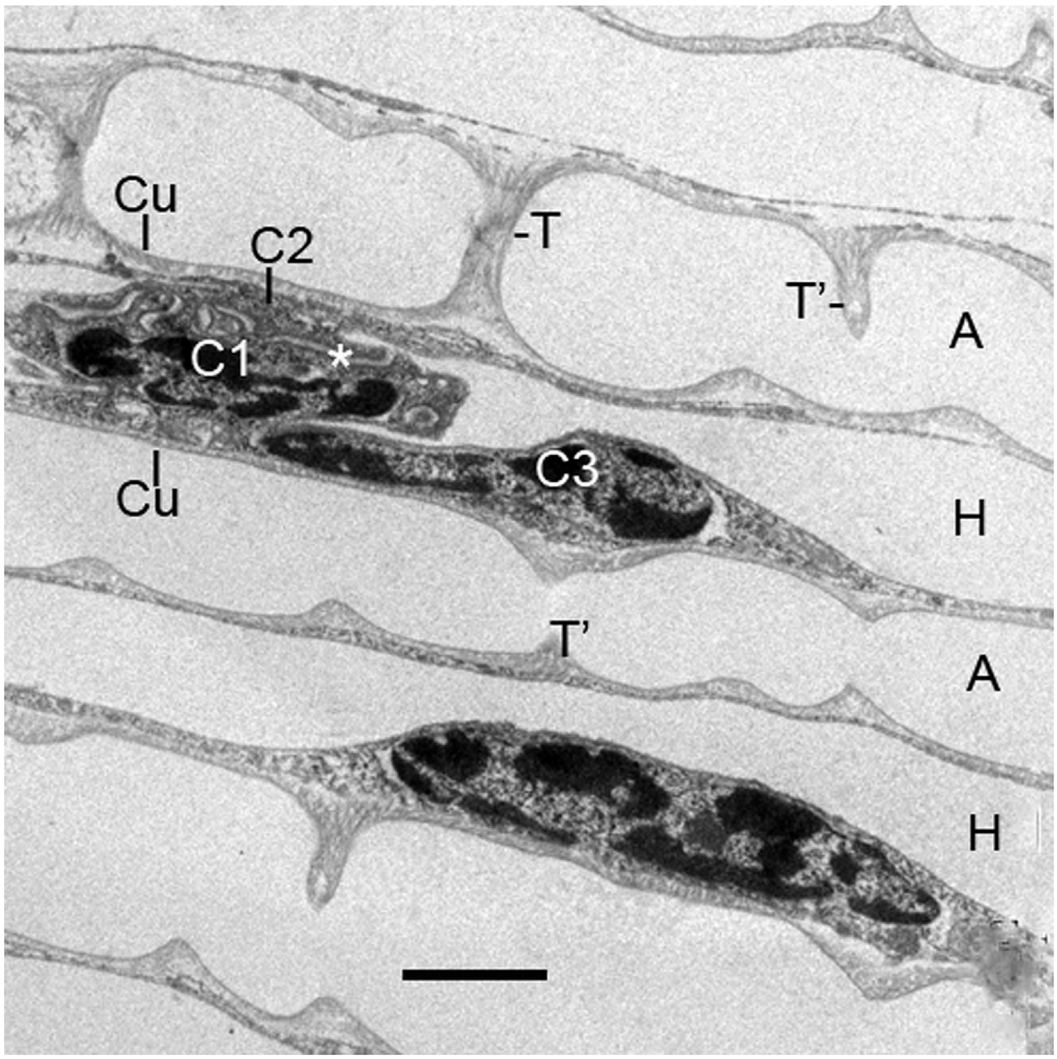



**Fig. 13.** Three precursor cells (C1-C3) join together in early formation of a pillar trabecula that spans a hemolymph channel (H). TEM. *Parasteatoda tepidariorum*, adult female, mode II air channel formation. C1 has formed a specialized junction (facia adherens, asterisk) with a small part of the thin strand of C2 cytoplasm. C3 appears to be migrating toward C1 as often seen among cells in hemolymph channels. These precursor cells with their thin layer of cytoplasm secrete the cuticle wall (Cu) and bridging trabeculae (T) of the air channels (A) and are in direct contact with the hemolymph. With distance from the site of channel origin, the cuticular trabeculae are sparse and thin and may only be partially evident (T′) in electron micrographs. Scale, 1 μm.

et al., 2006; Acloque et al., 2009; Thiery et al., 2009). In addition to its importance in morphogenesis, EMT is thought to occur in cancer and angiogenesis, so the molecular basis of EMT is now the focus of much research (Thiery, 2002; Lee et al., 2006; Ribatti, 2017).

There is a large and growing literature on mesenchyme and stem cells; their features and capabilities overlap, but details are well beyond the scope of this investigation. As in the present investigation, mesenchyme commonly refers to cells in the developmental (or tumorigenic) process that have undergone EMT in the early embryo or later stages. Mesenchyme cells have a capability for migration and differentiation into other types of cells (Lee et al., 2006; Bryant and Mostov, 2008; Lim and Thiery, 2012; Ribatti, 2017).

Stem cells are a broader class of cells that are relatively undifferentiated, and capable of self-renewal and production of differentiated daughter cells (Chen et al., 2017). There are different types of stem cells based mainly on origin and lineage. They may be uni/multi-potent, pluripotent or totipotent. Stem cells also undergo EMT and MET and in certain circumstances can become tumorigenic. So far, this and earlier studies (Farley, 2015, 2016) have only provided evidence that spider opisthosomal mesenchyme cells differentiate into book lung precursors, but further investigations will likely show other tissues and organs (e.g., tracheae) that result from mesenchyme cells.

### 4.2. Atrial exuvium

Presently unknown are the factors that trigger the withdrawal or death of cells at the posterior end of the hemolymph channels (Figs. 1B, 2A and 3). Possibly this is part of the tissue response to ecdysteroid molting hormones (Honda et al., 2017), and there may be some dehydration and loss of distal hemolymph channel cells as a result of air passage through the atrium. The atrial exuvia probably has a filtering function so long as it is present in the atrium.

Examination of the exuvia in histological sections shows the book lung exuvia is removed as a single unit, including the atrial

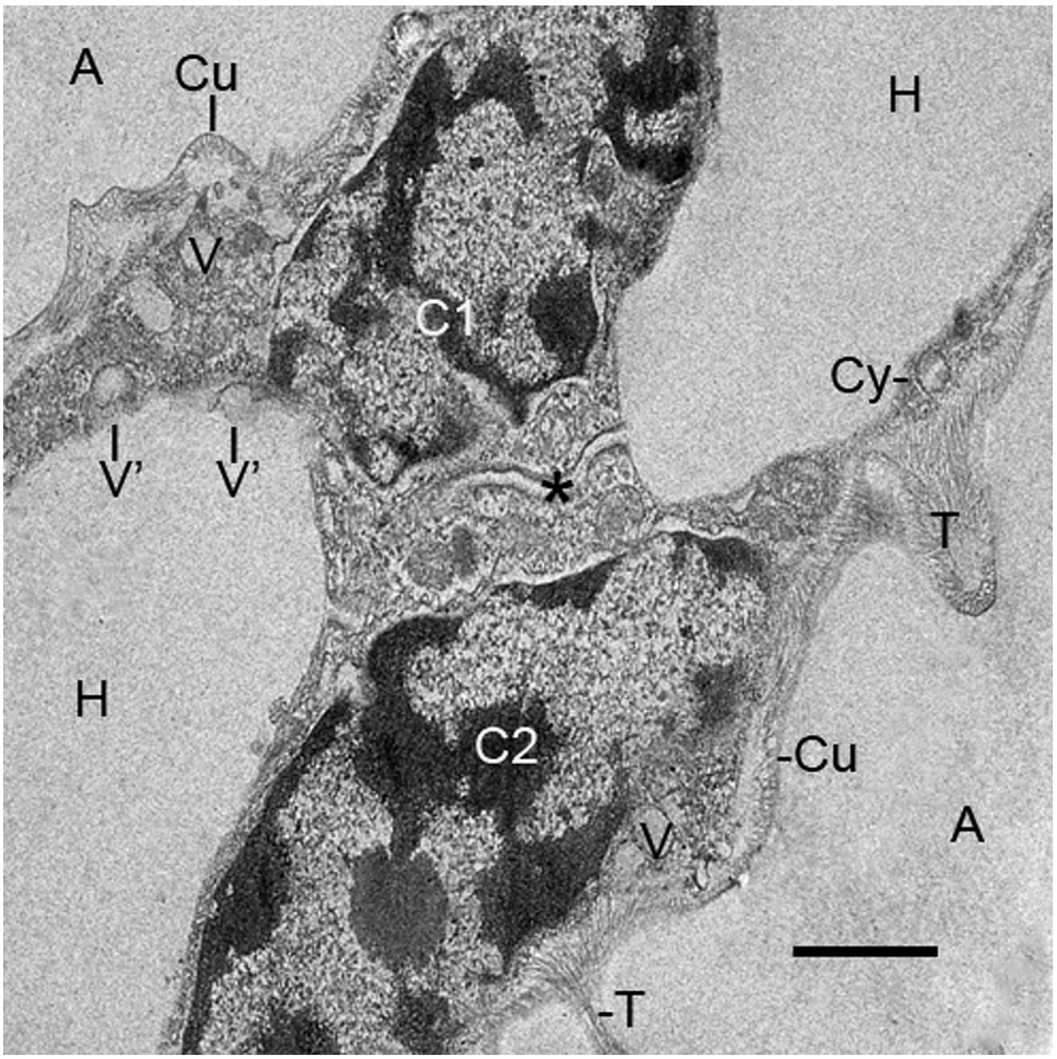



**Fig. 14.** Two precursor cells (C1, C2) span a hemolymph channel (H), forming a pillar trabecula. TEM. *P. tepidariorum*, adult female, mode II air channel formation. As usual, a specialized junction (fascia adherens, asterisk) is forming. These cells have vesicles (V) and thin strands of cytoplasm (Cy) that secrete the bridging trabeculae (T) and cuticle (Cu) that line the walls of the air channels (A). The hemolymph channel does not have cuticle, so C1 and C2 have direct contact with hemolymph. V', vesicles releasing contents. Scale, 1 μm.

trabeculae from the posterior and anterior atrial walls, the cuticle and trabeculae of the air channels and the outer cuticular wall of the book lung chamber (Farley, 2015). Complete removal of the book lung cuticle may not always occur successfully, however, since the cuticle must be pulled through the narrow spiracle to be fully shed.

### 4.3. Cell activity in lamellar development

#### 4.3.1. Mode I and II at differing locations in the book lung

The observations herein show there are substantial differences in the developmental sequence and resulting structure of the lamellae that are produced by these two processes. Their function and effectiveness are also likely to be affected, and this may account for the differences in location where these two modes are most likely to be found within the book lung.

In the many book lungs examined in embryos and early post-embryonic stages (Farley, 2015, 2016), the first air channels produced are usually mode I, and these remain at the posterior end of the book lung with channels increasing in size as the animal grows. Mode II air channels are more likely to be found farther anterior in the book lung as it is becoming larger in the postembryo and first instar. This pattern continues in juveniles and adults as observed in the present investigation.

In histological sections of the medial wall of juvenile and adult book lungs, there is often a transition from new mode II lamellae anterior in the book lung (Fig. 8A, B and 9–11) to new mode I lamellae near the atrium (Fig. 6A and B). Presumably, oxygen is more available and accessible in small embryo book lungs and also near the spiracle and atrium at the posterior end of larger juvenile and adult book lungs. This may be a factor causing new lamellae to be formed with the mode I process at the posterior end of the book lung. These air channels can become larger in size, thus providing a larger passageway for air to be funneled into the more distant channels of the book lung.

There are also likely to be differences in dehydration and perfusion of tissues with distance from the spiracle and atrium. In examining juvenile book lungs where mode I air channel formation

is in progress, it is common to see hemolymph channels that still consist of strands of precursor cells with little lumen formation (Figs. 1B, 2B and 6A, B) and presumably little perfusion with hemolymph. This may reduce fluid loss at the atrium, but also result in some death of distal lamellar cells as described above for atrial changes in the molt (Figs. 1B, 2A and 3). At new-growth sites more distant from the spiracle and atrium, the new air channels produced by mode II often have hemolymph channels with lumina (H in Fig. 8A) as the air channels are formed. Even when the hemolymph channels start as a thin clear line as in the electron micrographs of Figs. 9–11, other hemolymph channels are open at the bottom of these figures, a short distance from where new channels are forming. This suggests these hemolymph channels are effective soon after the air channels form as the precursor cells start to become thin and elongate.

The mode II process apparently allows for earlier hemolymph perfusion and distribution of oxygen from the new lamellae, and the air channels have bridging trabeculae (Figs. 8A, B and 9–13) that keep these air channels stable in size throughout their length (Figs. 4, 12 and 13). These features may be advantageous at sites distant from the oxygen source in the atrium. The new mode II channels are likely to be replaced in the next molt with larger mode I channels that can funnel more oxygen to even more distant sites as the animal and book lung grow and another new set of mode II lamellae is produced. These observations raise the hypothesis that structural and functional differences in mode I and II channels and differing oxygen and fluid conditions with distance from the spiracle and atrium determine whether the precursor cells use the mode I or II process in producing new channels.

#### 4.3.2. Induction of polarity

As noted in an earlier study (Farley, 2016) and observed herein, there apparently is induction of the polarity of mesenchyme cells by polarized book lung cells that have already produced alternating air and hemolymph channels. As unpolarized mesenchyme cells at the periphery of the book lung chamber undergo MET, the resulting epithelial precursor cells are soon polarized (Figs. 8A, B and 9–11). They are able to produce cuticle (air channels with trabeculae) at the apical surface and presumed nutrient transfer (hemolymph channel) at the basal surface. Also, the basal surface likely has vesicle secretion into developing hemolymph channels.

As a result of the apparent polarity induction of new cells by older ones, the new precursor cells begin producing new air and hemolymph channels that continue and coincide with the alternating sequence already present within the book lung (Figs. 9–11). This propagation of the alternating pattern enables the book lung to increase in size without disruption of air and hemolymph flow. Information about the external cues and cellular proteins and lipids that result in epithelial polarization and depolarization are provided in a review by Rodriguez-Boulan and Macara (2014).

#### 4.3.3. Intercellular alignment of polarity

As new mode II lamellae are produced at sites more distant from the spiracle and atrium, growth at these sites enlarges the book lungs in the anterior/posterior, horizontal and dorsal/ventral axes. The new channels fuse end-to-end with those already present (Fig. 8B), and then the cuticle from both the new and old channels is replaced in the molt by wider channels produced by mode I. This sequence is evident in electron micrographs of an embryo book lung (Farley, 2016) and is apparently repeated with each molt.

The fusion of new and old channels (Fig. 8B) depends on the continuity of the cellular polarity pattern through the site of fusion. The apical and basal ends of the new air channel cells apparently orient in the same direction as the apical and basal ends of the old air channel cells. This fusion process is evidence for intercellular alignment of polarity among new and old channel cells, thus maintaining continuity of air and hemolymph flow throughout the increased length and breadth of the book lung.

#### 4.3.4. Changes in cell shape

The molecular basis remains unknown for the many complex cell activities observed for book gill and book lung development in this and earlier studies (Farley, 2005, 2008, 2010, 2011, 2012, 2015, 2016). In numerous other investigations, many changes in cell shape and activity involve activation of an internal actomyosin network with forces transmitted to the cell cortex where there are adhesion molecules that secure the cells to each other (cadherins) and/or to the substrate (integrins; Munjal and Lecuit, 2014; Heller and Fuchs, 2015; Munjal et al., 2015; Han and de Rooij, 2016; Mège and Ishiyama, 2017). Since these features appear to be conserved among the various model systems, many of the cell activities (e.g., migration) and changes in shape (Figs. 6–14) observed herein probably involve the actomyosin network and interaction with cell adhesion molecules in the precursor cell membranes.

#### 4.3.5. Cell attraction and affinity

Results herein support the earlier hypothesis (Farley, 2016) that the planar tissue polarization (alternating air and hemolymph channels) seen in spider book lung development occurs if there is apical/apical and basal/basal attraction of the precursor cells. Based on research in other model systems, there may be proteins in the cells that repel each other within the same cell so there are different functions at the apical and basal ends (Adler, 2012; Heller and Fuchs, 2015; Adler and Nathans, 2016). Between cells, however, the cell-to-tissue sequence in book lung development is more likely based on apical/apical and basal/basal affinity (Figs. 9–11; Farley, 2016).

In the formation of pillar trabeculae, some precursor cells appear to migrate toward each other (Fig. 13), and there is sometimes regular spacing of these paired cells (Farley, 2016, Fig. 5B). This suggests some cell attraction and orientation over a distance, e.g., chemotaxis and a morphogen gradient as observed in tissues in other organisms (Iglesias and Devreotes, 2008; Stephens et al., 2008; Gilmour et al., 2017). Such a gradient and/or intercellular attraction and repulsion of cell membrane molecules (Adler and Nathans, 2016) may be involved in the affinities that result in air channels at the opposed apical surfaces of aligned cells and hemolymph channels at the cell basal surfaces.

As some cells from opposite walls in the hemolymph channel migrate together and form cell pairs (Figs. 1A, 4, 8A, 13 and 14), other cells have cytoplasmic processes that extend across the hemolymph channel and make contact with a cell body or process on the opposite wall (Fig. 13; Reisinger et al., 1990, 1991; Brunelli et al., 2015; Farley, 2015, 2016). This cross-channel merging of cell processes and somata is evidence of a strong affinity between the basal surfaces of some cells in the opposed rows of precursor cells.

The specialized junctions (fascia adherens) that form at the points of contact between the cell processes and the cell bodies (Figs. 13 and 14) are like those commonly found at sites of mechanical stress, e.g., cardiac muscle cells, tendon cells and muscle (Tucker et al., 2004; Brunelli et al., 2015; Balda and Matter, 2016. The stimulus is unknown for cells to form pillar trabecular space holders, but possibly it is physical force from widening and/or compression of the channel lumen. Physical force has been shown to stimulate cell activity, gene expression and morphogenesis in numerous other systems (Ramanujam et al., 2017).

Pillar trabeculae occur in embryos of the horseshoe crab, scorpions and spiders (Farley, 2010, 2011, 2012, 2015, 2016), and Scholtz and Kamenz (2006) reported these trabeculae in adult scorpions, Amblypygida (whip spiders), Uropygida (whip scorpions) and

mygalomorph spiders (tarantulas). Among other common features, they considered the pillar trabeculae are evidence for homology of arachnid book lungs.

## 5. Conclusions

The observations in this and earlier studies with LM, SEM and TEM (Farley, 2005, 2008, 2010, 2011, 2012, 2015, 2016) provide evidence that many different cellular activities are involved in the development of book gills and book lungs. The result is respiratory organs with no final tubular structures but many planar polarized layers, i.e., hemolymph channels alternating with air or water channels. The molecular basis of such transformations from cells to polarized tissue is being studied in numerous model systems (e.g., Zallen, 2007; Bryant and Mostov, 2008).

The spider species of this investigation has many advantages for studying development and evolution (McGregor et al., 2008; Hilbrant et al., 2012), and Farley (2011, 2015, 2016) suggested the relatively simple and repetitious developmental pattern of book lungs may be especially advantageous for studying cell-to-tissue morphogenesis. Since many cell activities for lamellar formation in embryos and early postembryonic stages continue in juveniles and adults, the larger size of the latter may make them preferable for some morphogenetic investigations as compared with the very small size of developing tissues in embryos.

## Acknowledgments

The comments and suggestions of the editor and reviewers are much appreciated. Special thanks to Evelyn E. Schwager for providing many specimens of *Parasteatoda tepidariorum* for starting our local colony. For technical assistance with sectioning and operation of the TEM, thanks to Mathias Rommelfanger, Staff Research Associate in the Central Facility for Advanced Microscopy and Microanalysis at the University of California, Riverside. This research was funded by intramural grants from the University of California.

## References

Acloque, H., Adams, M.S., Fishwick, K., Bronner-Fraser, M., Neito, M.A., 2009. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J. Clin. Invest. 119, 1438–1449.
Adler, P.N., 2012. The *frizzled/stan* pathway and planar cell polarity in the *Drosophila* wing. Curr. Top. Dev. Biol. 101, 1–31.
Adler, P.N., Nathans, J., 2016. The cellular compass. Sci. Am. 314, 66–71.
Andrew, D.J., Ewald, A.J., 2010. Morphogenesis of epithelial tubes: insights into tube formation, elongation, and elaboration. Dev. Biol. 341, 34–55.
Balda, M.S., Matter, K., 2016. Tight junctions as regulators of tissue remodelling. Curr. Opin. Cell Biol. 42, 94–101. www.sciencedirect.com.
Behr, M., 2010. Molecular aspects of respiratory and vascular tube development. Respir. Physiol. Neurobiol. 173S, 533–536.
Brunelli, E., Rizzo, P., Guardia, A., Coscarelli, F., Sesti, S., Tripepi, S., 2015. The ultrastructure of the book lungs of the Italian trap-door spider *Cteniza* sp. (Araneae, Mygalomorphae, Ctenizidae). Arthropod Struct. Dev. 44, 228–236. https://doi.org/10.1016/j.asd.2015.03.001.
Bryant, D.M., Mostov, K.E., 2008. From cells to organs: building polarized tissue. Mol. Cell Biol. 9, 887–901.
Chen, T., You, Y., Jiang, H., Wang, Z.Z., 2017. Epithelial-mesenchymal transition (EMT): a biological process in the development, stem cell differentiation, and tumorigenesis. J. Cell. Physiol. 232, 3261–3272.
Damen, W.G.M., Saridaki, T., Averof, M., 2002. Diverse adaptations of an ancestral gill: a common evolutionary origin for wings, breathing organs, and spinnerets. Curr. Biol. 12, 1711–1716.
Dunlop, J.A., 1998. The origin of tetrapulmonate book lungs and their significance for chelicerate phylogeny. In: Selden, P.A. (Ed.), Proceedings of the 17th European Colloquium of Arachnology: 1997; Edinburgh. British Arachnology Society, Burnham Beeches, Bucks, UK, pp. 9–16.
Dunlop, J.A., 2010. Geological history and phylogeny of Chelicerata. Arthropod Struct. Dev. 39, 124–142.
Dunlop, J.A., Lamsdell, J.C., 2017. Segmentation and tagmosis in chelicerata. Arthropod Struct. Dev. 46, 395–418.
Ellis, E.A., 2006. Solutions to the problem of substitution of ERL 4221 for vinyl cyclohexene dioxide in Spurr low viscosity embedding formulations. Microsc.Today 14, 32–33.
Farley, R.D., 2005. Developmental changes in the embryo, pronymph, and first molt of the scorpion *Centruroides vittatus* (Scorpiones: Buthidae). J. Morphol. 265, 1–27.
Farley, R.D., 2008. Development of respiratory structures in embryos and first and second instars of the bark scorpion, *Centruroides gracilis* (Scorpiones: Buthidae). J. Morphol. 269, 1134–1156.
Farley, R.D., 2010. Book gill development in embryos and first and second instars of the horseshoe crab *Limulus polyphemus* L. (Chelicerata, Xiphosura). Arthropod Struct. Dev. 39, 369–381.
Farley, R.D., 2011. The ultrastructure of book lung development in the bark scorpion *Centruroides gracilis* (Scorpiones: Buthidae). Front. Zool. 8 (18). http://www.frontiersinzoology.com/content/8/1/18.
Farley, R.D., 2012. Ultrastructure of book gill development in embryos and first instars of the horseshoe crab *Limulus polyphemus* L. (Chelicerata, Xiphosura). Front. Zool. 9 (4). http://www.frontiersinzoology.com/content/9/1/4.
Farley, R.D., 2015. Book lung development in the embryo, postembryo and first instar of the cobweb spider, *Parasteatoda tepidariorum* C. L. Koch, 1841 (Araneomorphae, Theridiidae). Arthropod Struct. Dev. 44, 355–377. https://doi.org/10.1016/j.asd.2015.04.006.
Farley, R.D., 2016. Book lung development in embryos of the cobweb spider, *Parasteatoda tepidariorum* C. L. Koch, 1841 (Araneomorphae, Theridiidae). Arthropod Struct. Dev. 45, 562–584. https://doi.org/10.1016/j.asd.2016.09.008.
Gilmour, D., Rembold, M., Leptin, M., 2017. From morphogen to morphogenesis and back. Nature 541, 311–320.
Han, M.K.L., de Rooij, J., 2016. Converging and unique mechanisms of mechanotransduction at adhesion sites. Trends Cell Biol. 26, 612–623.
Heller, E., Fuchs, E., 2015. Tissue patterning and cellular mechanics. J. Cell Biol. 211, 219–231. https://doi.org/10.1083/jcb.201506106.
Hilbrant, M., Damen, W.G.M., McGregor, A.P., 2012. Evolutionary crossroads in developmental biology: the spider *Parasteatoda tepidariorum*. Developement 139, 2655–2662.
Honda, Y., Ishiguro, W., Ogihara, M.H., Kataoka, H., Taylor, D., 2017. Identification and expression of nuclear receptor genes and ecdysteroid titers during nymphal development in the spider *Agelena silvatica*. Gen. Comp. Endocrinol. 247, 183–198.
Iglesias, P.A., Devreotes, P.N., 2008. Navigating through models of chemotaxis. Curr. Opin. Cell Biol. 26, 35–40.
Kamenz, C., Prendini, L., 2008. An atlas of book lung ultrastructure in the Order Scorpiones (Arachnida). Bull. Am. Mus. Nat. Hist. 316, 1–359.
Lankester, R., Benham, W.B.S., Beck, E.J., 1885. On the muscular and endoskeletal systems of *Limulus* and *Scorpio*; with some notes on the anatomy and generic characters of scorpions. Zool. Soc. Lond. Trans 11, 311–384.
Lee, J.M., Dedhar, S., Kalluri, R., Thompson, E.W., 2006. The epithelial- mesenchymal transition: new insights in signaling, development, and disease. J. Cell Biol. 172, 973–981.
Lim, J., Thiery, J.P., 2012. Epithelial-mesenchymal transitions: insights from development. Development 139, 3471–3486.
McGregor, A.P., Hilbrant, M., Pechmann, M., Schwager, E.E., Prpic, N.-M., Damen, W.G.M., 2008. *Cupiennius salei* and *Achaeranea tepidariorum*: spider models for investigating evolution and development. Bioessays 30, 487–498.
Mège, R.M., Ishiyama, H., 2017. Integration of cadherin adhesion and cytoskeleton at adherens junctions. Cold Spr. Harb. Persp. Biol 9.
Merzendorfer, H., 2006. Insect chitin synthases: a review. J. Comp. Physiol. B 176, 1–15.
Mittmann, B., Wolff, C., 2012. Embryonic development and staging of the cobweb spider *Parasteatoda tepi-dariorum* C. L. Koch, 1841 (syn.: Achaearanea tepidariorum; Araneomorphae; Theridiidae). Dev. Gene. Evol. 222, 189–216.
Miyashita, K., 1987. Development and egg sac production of *Achaearanea tepidariorum* (C.L Koch) (Araneae, Theridiidae) under long and short photoperiods. J. Arachnol. 15, 51–58.
Moussian, B., 2010. Recent advances in understanding mechanisms of insect cuticle differentiation. Insect Biochem. Mol. Biol. 40, 363–375.
Moussian, B., 2013. The apical plasma membrane of chitin-synthesizing epithelia. Insect Sci. 20, 139–146.
Munjal, A., Lecuit, T., 2014. Actomyosin networks and tissue morphogenesis. Development 141, 1789–1793.
Munjal, A., Philippe, J.-M., Munro, E., Lecuit, T., 2015. A self-organized biomechanical network drives shape changes during tissue morphogenesis. Nature 524, 351–368.
Pechmann, M., Khadjeh, S., Sprenger, F., Prpic, N.-M., 2010. Patterning mechanisms and morphological diversity of spider appendages and their importance for spider evolution. Arthropod Struct. Dev. 39, 453–467.
Purcell, W.F., 1909. Development and origin of the respiratory organs in Araneae. Q. J. Microsc. Sci. 54, 1–110.
Purcell, W.F., 1910. The phylogeny of tracheae in Araneae. Q. J. Microsc. Sci. 54, 519–563.
Ramanujam, R., Low, T.Y.F., Lim, Y.W., Motegi, F., 2017. Force patterning in mouse preimplantation embryos. Semin. Cell Dev. Biol. 71, 129–136.
Reisinger, P.W.M., Focke, P., Linzen, B., 1990. Lung morphology of the tarantula, *Eurypelma californicum* Ausserer, 1871 (Araneae: Theraphosidae). Bull. Br. Arachnol. Soc. 8, 165–179.

Reisinger, P.W.M., Tutter, I., Welsch, U., 1991. Fine structure of the gills of the horseshoe crabs *Limulus polyphemus* and *Tachypleus tridentatus* and of the booklungs of the spider *Eurypelma californicum*. Zool. Jahrb. Anat 121, 331–357.

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17, 208–212.

Ribatti, D., 2017. Epithelial-mesenchymal transition in morphogenesis, cancer progression and angiogenesis. Exp. Cell Res. 353, 1–5.

Rodiguez-Boulan, E., Macara, I.G., 2014. Organization and execution of the epithelial polarity programme. Nat. Rev. Mol. Cell Biol. 15, 225–242. https://doi.org/10.1038/nrm3775.

Schartau, W., Leidescher, T., 1983. Composition of the hemolymph of the tarantula *Eurypelma californicum*. J. Comp. Physiol. B 152, 73–78.

Scholtz, G., Kamenz, C., 2006. The book lungs of Scorpiones and Tetrapulmonata(Chelicerata, Arachnida) evidence for homology and a single terrestrialization event of a common arachnid ancestor. Zoology 109, 2–13.

Simonnet, F., Célérier, M.-L., Quéinnec, E., 2006. *Orthodenticle* and *empty spiracle*s genes are expressed in a segmental pattern in chelicerates. Dev. Gene. Evol. 216, 467–480.

Spurr, A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31–43.

Stephens, L., Milne, L., Hawkins, P., 2008. Moving towards better understanding of chemotaxis. Curr. Biol. 18, R485–R494.

Thiery, J.P., 2002. Epithelial-mesenchymal transitions in tumour progression. Nat. Resour. 2, 442–454.

Thiery, J.P., Acloque, H., Huang, R.Y.J., Nieto, M.A., 2009. Epithelial-mesenchymal transitions in development and disease. Cell 139, 871–890.

Tsarouhas, V., Senti, K.-A., Jayaram, S.A., Tiklová, K., Hemphälä, J., Adler, J., Samakovlis, C., 2007. Sequential pulses of apical epithelial secretion and endocytosis drive airway maturation in drosophila. Dev. Cell 13, 214–225.

Tucker, J.B., Mackie, J.B., Cottam, D.M., Rogers-Bald, M.M., Macintyre, J., Scarborough, J.A., Milner, M.J., 2004. Positioning and capture of cell surface-associated microtubules in epithelial tendon cells that differentiate in primary embryonic *Drosophila* cell cultures. Cell Motil. Cytoskel. 57, 175–185.

Wolff, C., Hilbrant, M., 2011. Embryonic development of the Central American wandering spider *Cupiennius salei*. Front. Zool. 8, 15. http://www.frontiersinzoology.com/content/8/1/15.

Zallen, J.A., 2007. Planar polarity and tissue morphogenesis. Cell 129, 1051–1063.