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FRONTAL FILAMENT MORPHOGENESIS IN THE SALMON LOUSE *LEPEOPHTHEIRUS SALMONIS*

Pablo Gonzalez-Alanis*, Glenda M. Wright, Stewart C. Johnson†, and John F. Burka

Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3

ABSTRACT: The objective of the present study was to understand how and when the frontal filament (FF) in the salmon louse *Lepeophtheirus salmonis* is produced by examining the sequence of morphological changes leading to FF production in the copepodid and early chalimus stages. Atlantic salmon (*Salmo salar*) were heavily infested with newly molted copepodids. Sea lice were sampled prior to infestation and at 1, 2, 3, 4, 5, 6, 7, 8, and 9 days postinfestation. FF morphogenesis from newly molted copepodid to chalimus II, i.e., through 2 molts, was studied using high-resolution light microscopy of serial transverse and sagittal resin sections. Three groups of cells, identified as A, B, and C, are thought to be involved in the production of the secretions (S1 and S2) that form the filament material. The amount and shape of S1 and S2 and their association with B- and C-group cells, respectively, changed with the molt cycle. The following scenario for FF development is proposed: the first secretion to form after the molt for both copepodid and chalimus stages is S1, and it is formed by B-group cells and becomes the basal plate of the external FF. C-group cells produce S2 during mid-intermolt to premolt stage. The S2 becomes the stem of the external FF. In premolt larvae, S1 and S2 were contained within a cuticle-lined invagination that had a form similar to that of the extruded filament. The axial duct present in both copepodid and chalimus originates from the A-group cells and probably carries a secretion used to attach the filament to the host. This study provides strong evidence that *L. salmonis* produces a new filament with each molt, creating the possibility of using a sea lice control method based on interference with filament production more feasible.

The attachment organ used during the chalimus stages of siphonostomatoid parasites of fish is the frontal filament (FF), previously known as the mediofrontal, median sucker, cordon frontal (Wilson, 1905), and attachment filament (Oldewage and Van As, 1989). The FF of *Lepeophtheirus salmonis* (Caligidae) is considered to be an integral part of the body of the chalimus (Pike et al., 1993). It consists of a stem that stains differently from the basal plate (Bron et al., 1991; Johnson and Albright, 1992). The stem is formed of densely packed fibers and contains an axial duct that runs through its center (Bron et al., 1991; Johnson and Albright, 1992; Pike et al., 1993). Covering the stem is the external lamina that is continuous with the cuticle of the chalimus (Bron et al., 1991; Johnson and Albright, 1992; Pike et al., 1993).

The process of FF production in *L. salmonis* is poorly understood. The presence of both precursor and fully formed filaments within the copepodid stage of *Caligus elongatus* have been described by Pike et al. (1993). Bron et al. (1991) reported that there was no sign of any preformed filaments in any sections of copepodid or chalimus stages of *L. salmonis,* but speculated that reservoirs of secreted material seen in the copepodid were filament precursors. Later, Johnson and Albright (1992) reported the presence of fully formed FF (stem and basal plate) in the anterior cephalothorax of 2 chalima that were in the process of molting.

Leopeophtheirus salmonis is one of the most serious pathogens of sea-farmed salmonids (Johnson and Albright, 1992). The FF could be a potential weak link in the developmental cycle of *L. salmonis* at which control methods might be directed (Pike et al., 1993), and ultimately it may be possible to effectively prevent the copepodid from establishing itself on the host if it is possible to interfere with the formation or attachment of the FF. Development of such a strategy would be helped greatly by a chemical analysis of the FF and an understanding of its morphology, mode of production, and development in the different stages of *L. salmonis*.

The objective of the present research was to improve our knowledge of the morphology and development of the FF in *L. salmonis*. We describe the morphology of the FF, the tissues involved in its formation, and the timing of its production in relation to the molt cycle in the copepodid through chalimus II stage. Results of this study will form the basis for future research into methods of preventing the attachment of the chalimus stages, providing improved therapeutic management strategies for sea lice control in salmonid aquaculture.

MATERIALS AND METHODS

Sea lice collection and culture in the laboratory

One-hundred and twelve ovigerous sea lice *L. salmonis* were collected from Atlantic salmon (*Salmo salar*) at aquaculture sites in the Bay of Fundy. Egg strings were excised and placed in 45-L containers provided with flow-through, filtered seawater at a temperature of 10 ± 1 C. Circulation within the containers was maintained by gentle aeration. The container drains were covered with a 100-μm Nitex mesh screen to prevent loss of larvae. Nauplii were allowed to hatch and were reared to the infective copepodid stage. The larval stage of development was determined by examining random samples under a dissection microscope and comparing larvae to descriptions of developmental stages reported by Johnson and Albright (1991).

Fish infection and sample collection

Thirty-two, post-smolt Atlantic salmon (100–150 g) were acclimatized and maintained in a 1,500-L tank with flow-through, filtered, UV-treated seawater for 2 mo prior to the onset of the experiment. Water temperature was measured every 40 min using a temperature logger, and the overall average temperature observed was 12.38 ± 0.50 C. The fish were fed twice daily with pelleted dry commercial salmon feed (Corey Feeds, Fredericton, New Brunswick, Canada), and they were all assessed to be in good health.

Fish were infected by placing approximately 1,120 newly molted copepodids into the tank (production estimated from 112 ovigerous sea

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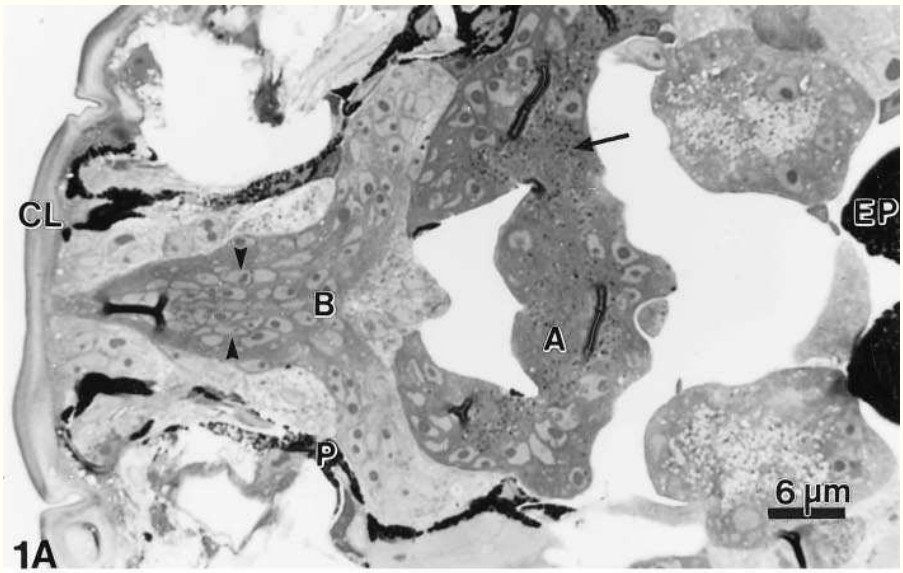
* Present address: Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia, "Dr. Norberto Treviño Zapata" Carretera Victoria- Mante Km. 5 A.P. No. 263, C.P. 87000, Cd. Victoria Tamaulipas, México.

† Institute for Marine Biosciences, National Research Council, 1411 Oxford St., Halifax, Nova Scotia, Canada B3H 3Z1.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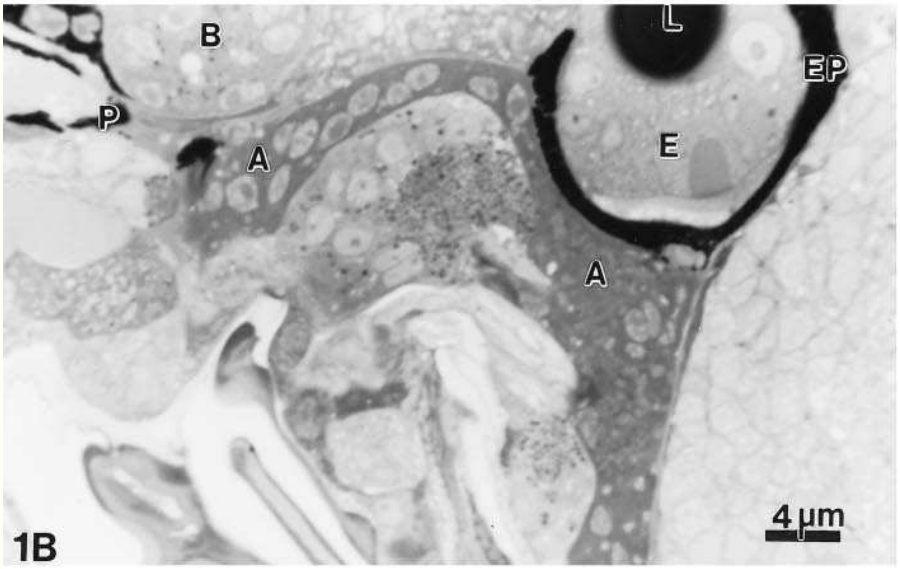
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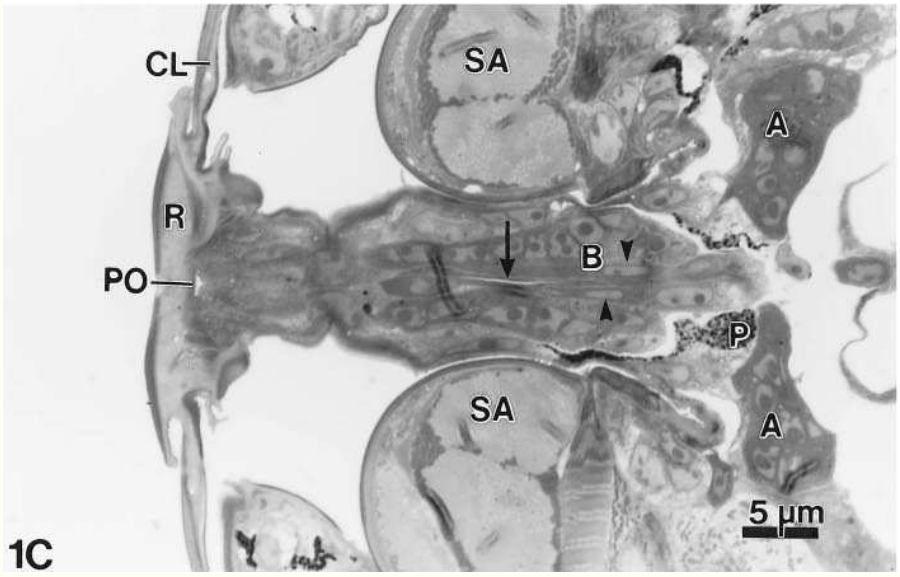
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TABLE I. Summary of the components involved in frontal filament formation during development of *Lepeophtheirus salmonis* indicating the presence (+) or absence (−) of secretion 1 (S1), secretion 2 (S2), and frontal filament (FF) in intermolt (INT), newly molted (NM), or premolt (PRE) larvae.

Days postinfection	S1	S2	FF	Stage
0, 1	−	−	−	NM, copepodid
2	+ (4 of 5)	−	−	INT, copepodid
3	+	+	−	INT, copepodid
4	+	+	−	PRE, copepodid
4	−	−	+	NM, chalimus I
5, 6	+	−	+	INT, chalimus I
7	+	+	+	INT, chalimus I
8	+	+	+	INT, chalimus I
8	+	+	+	PRE, chalimus I
9	+	+	+	PRE, chalimus I
9	−	−	+	NM, chalimus II

lice females with fully formed egg strings), reducing the water flow and eliminating the light for approximately 14 hr. Salmon lice were sampled from 3 fish at the same time each day from day 1 to day 10 postinfection. Fish were caught in a dipnet, withdrawn from the tank, and killed by a single blow to the head. The body surfaces of the fish were examined under a dissection microscope, and at least 20 sea lice from each fish were removed by excising the integument of the fish to which they were attached.

For an accurate assessment of the development rate as a function of temperature-growth, degree-day (DD) values were calculated for each day postinfection (dpi) using the following formula: DDPI (degree dpi) $= \Sigma_{N=1}^{10} T_N$, where N = number of dpi and T = average temperature over 24 hr.

Fixation, embedding, and analysis of samples

For high-resolution light microscopy, 45 sea lice per day were fixed at room temperature in a solution of 3% glutaraldehyde in Millonig's (Millonig and Marinozzi, 1968) phosphate buffer (pH 7.4), containing 4% NaCl (1,100–1,200 mosmol) for 1 hr, then transferred to 4 C overnight. This was followed by 2 rinses (10 min each) with fresh buffer at room temperature. After rinsing, the samples were postfixed in 2% osmium tetroxide in Millonig's phosphate buffer for 2 hr at 4 C, washed twice (10 min each) with fresh phosphate buffer, followed by a 10-min wash in distilled water. Samples were dehydrated through a graded ethanol series, infiltrated, and embedded in Spurr's (1969) low-viscosity embedding medium.

The following numbers of sea lice were serially sectioned for analysis: never on fish (NOF) (n = 5), 1 dpi (n = 5), 2 dpi (n = 5), 3 dpi (n = 5), 4 dpi (n = 8), 5 dpi (n = 5), 6 dpi (n = 5), 7 dpi (n = 5), 8 dpi (n = 6), 9 dpi (n = 7), 10 dpi (n = 5). For each day sampled, horizontal and sagittal serial sections (0.5 μm thick) were cut. Sections were mounted on clean glass slides using standard techniques, and all sections were stained with 1% toluidine blue in 1% sodium borate. A minimum of 5 sets of slides was examined for each day using brightfield microscopy.

The histological examination focused on the FF and the tissues within the anterior portions of larvae. At least 5 individuals were examined for each sampling day. The number of samples examined was based on time considerations and the fact that the level of development of the FF was very similar between individuals collected on the same day. The designation of cell types involved with filament production and of FF material within the body was based on the original description by Bron et al. (1991).

RESULTS

Never on fish and 1 dpi (11.1 DDPI)

At 0 dpi and 1 dpi, the 2 major cell groups believed to be involved with filament production were evident, but there was no evidence of an external FF or internal FF material (Table I). The A group appeared to be larger than the B group. Cells of the A group (A cells) stained more intensely than those of the B group (B cells) (Fig. 1A). In horizontal medial section, the A group of cells was H-shaped having 2 lateral lobes connected medially, anterior to the eyes. Anteriorly the lateral lobes of A-group cells extended beneath group B (Fig. 1B) and posteriorly the lateral lobes extended to the eyes. Some A cells in the medial region of group A contained densely stained granules (Fig. 1A).

In horizontal medial sections, the B group was horseshoe-shaped and was partially surrounded by cuticular pigment cells. The left and right portions of the B group were lateral to the anterior lobes of the A group (Fig. 1A). Ventrally the anterior medial portion of the B group narrowed to a triangular shape. Cells of the B group appeared more abundant than those in group A and were organized in rows. No granulated material was present in B group cells (Fig. 1A).

A duct passed anteriorly through the ventral portion of the B-cell group. Cells that surrounded the duct were elongate and lay parallel to the long axis of the duct (Fig. 1C). The anterior portion of the duct extended to a small pore through the cuticle at the anterior margin of the cephalothorax in the vicinity of the rostrum (Fig. 1C). The origin of this duct could not be determined.

Two days postinfection (22.2 DDPI)

Internal FF material (S1), based on the designation by Bron et al. (1991), was first observed at 2 dpi (Fig. 2A, Table I). Four of the 5 copepodids examined contained small amounts of S1 material that was located within the anterior dorsal region of the B-group cells (Fig. 2A). B cells anterior to S1 formed a thin layer. B cells posterior to S1 had microvilli-like structures present on their surface where they made contact with the S1 material.

The 2 cell groups, A and B, were well defined and appeared similar in structure and staining characteristics to those seen at 0 and 1 dpi (Fig. 2A). The duct described at 0 dpi was easier to resolve at 2 dpi. Cells that surrounded the duct were elongate and laid parallel to the long axis of the duct (Fig. 2B), but the

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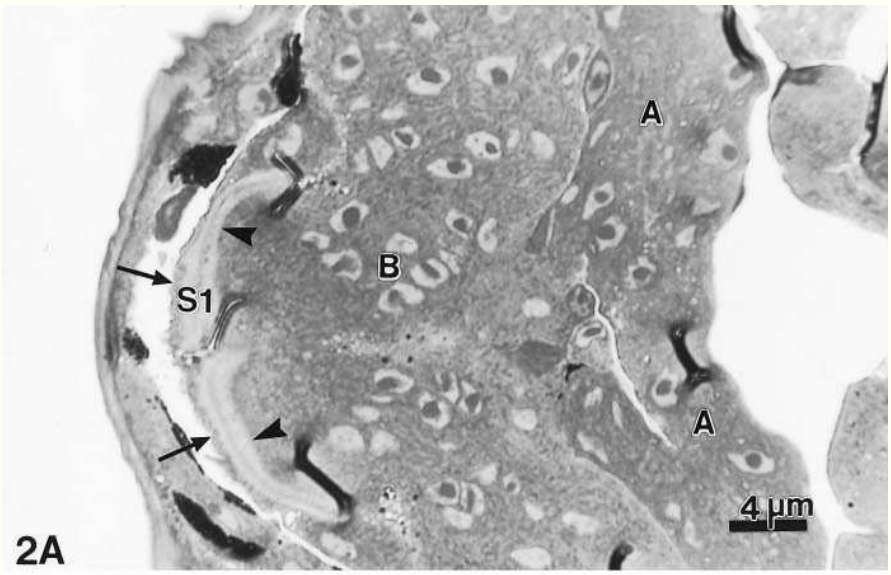
FIGURE 1. **A–C.** Copepodid, never on fish (0 DDPI). **A.** Horizontal medial section of the anterior cephalothorax showing the cell groups A and B involved in FF production. A-group cells are dark staining and granulated (arrow); B-group cells are lighter staining and appear organized in rows (arrowheads). **B.** Sagittal section of the anterior cephalothorax shows the A group (A) extending anteriorly beneath the B group (B) and ventral to the eye (E). **C.** Horizontal ventral section of the anterior cephalothorax. The lumen of a duct (arrow) surrounded by elongate cells (arrowheads) passes anteriorly through the B group (B). Nuclei of the B-group cells are aligned along the long axis of the duct. This duct opens to the environment through a small pore (PO) in the cuticle (CL). EP, eye pigment; L, lens; P, pigment; SA, second antenna base; R, rostrum.



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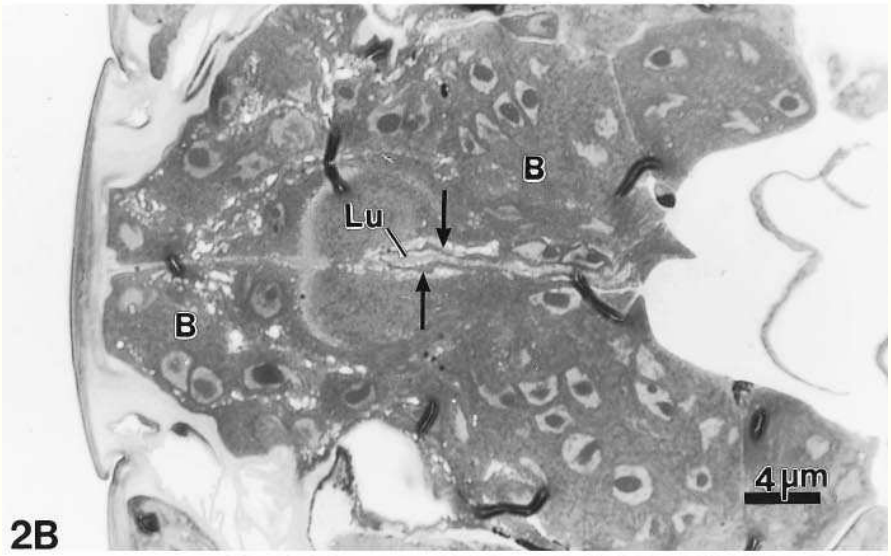

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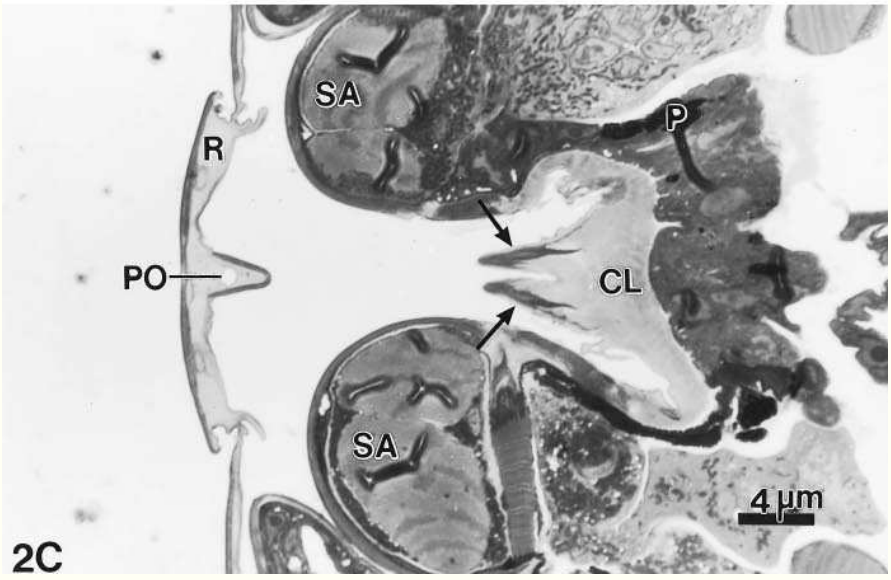

2C

duct origin could still not be distinguished. The anterior portion of the duct appeared as a pore that extended through the cuticle (Fig. 2C). A portion of the external cuticle extended ventrally from the pore and passed posteriorly between the second antennae forming a cuplike structure at the midpoint of the body (Fig. 2C).

Three days postinfection (34.8 DDPI)

The S1 and a second type of internal filament material (S2), based on the designation of Bron et al. (1991), were present in all 5 copepodids examined at 3 dpi (Fig. 3A–C, Table I). There was variation in the amount and shape of the S1 and S2 material among the copepodids at 3 dpi. Three of the larvae sampled had S1 and S2 separated from one another by B-group cells (Fig. 3A, B) and 2 animals had S1 and S2 in contact with each other (Fig. 3C). When continuous, S1 and S2 presented an interface medially between the 2 secretions (Fig. 3C). The S1 always appeared larger in volume than S2 and had an internal structure consisting of numerous laminations that varied in orientation with development. The S1 was surrounded by B-group cells, and both B group and S1 changed shape as the S1 enlarged (Fig. 3A–C). In lateral section, S1 appeared crescent-shaped, and its laminations were also crescent-shaped. The B-group cells immediately posterior to S1 conformed to the shape of the S1 and had microvilli-like structures where they contacted S1 (Fig. 3A). As the S1 enlarged, its shape became more cuboidal (Fig. 3B) and then triangular (Fig. 3C). In this later stage of filament development, laminations in the S1 material were crescent-shaped anteriorly and were vertical and parallel to each other posteriorly (Fig. 3C).

A third group of cells, the C group, could be defined in all 5 larvae at 3 dpi (Fig. 3A–C). The C group of cells was the most ventral. However, unlike A group and B group, the boundaries between this group and surrounding cells were difficult to define (Fig. 3A–C). The C group was identified by its ventral location and the dark staining cytoplasmic extensions of the C-cells at their boundary with the S2 material (Fig. 3A–C).

The A cells remained well defined and appeared similar in structure and staining characteristics to those seen at earlier times. The microvilli-like structures of the B cells, where they contacted the posterior surface of the S1, initially appeared more developed than those seen at 2 dpi. However, as the amount of S1 material increased, a reduction in their microvilli-like structures was evident (Fig. 3B, C). The duct seen at earlier times could now be traced between the S1 and the S2, passing anteriorly through the ventral portion of the B group and anterior through the cuticle.

Four days postinfection (47.5 DDPI)

At this stage of infection, a proportion (25%) of the copepodids had completed their molt to the first chalimus stage. Because of this, 8 larvae instead of 5 were examined at 4 dpi. Six of 8 individuals examined were at the late copepodid stage, as identified by the lack of an external FF. Two of the individuals were chalimus I, as they each had an external FF. The shape of the recently molted chalimus I was more slender and elongate than those at the late copepodid stage.

Late copepodid: All of the late-stage copepodids had completely formed internal FF within their anterior cephalothorax and were apparently about to molt into the chalimus I stage as evidenced by having 2 cuticles surrounding their bodies (Fig. 4A). The duct and its associated pore appeared to be the same as described for earlier stages (Fig. 4A). At this stage, both the S1 and S2 were contained within an hourglass-shaped pocket lined with new cuticle that varied in thickness (Fig. 4A). The S2 was restricted by a narrowing of the cuticle-lined pocket. C cells were present at the posterior ventral region of the pocket and B-group cells surrounded the pocket. Cuticle covered the C cells except where they extended into the S2 material. In horizontal section the S1 showed laminations arranged perpendicular to the long axis of the body. The S2 appeared to contain densely stained fibrous material and long cytoplasmic extensions from the C cells (Fig. 4A). The appearance of the A group was similar to that seen in earlier stages. Cells of the B group no longer contacted the S1 due to the presence of the new cuticle (Fig. 4A).

Chalimus: Examination of the 2 recently molted chalimus I revealed an extruded FF that consisted of a stem and a distal basal plate (Fig. 4B). The basal plate stained pale and adhered to the fish skin surface, adopting its irregular shape. The basal plate was attached to the stem that consisted of 2 regions: a distal area containing a dark-staining filamentous material and a proximal one that consisted of a pale-staining material (Fig. 4B). Within the now extruded FF, there was a lumen from the proximal region of the stem to the basal plate (Fig. 4B).

The proximal portion of the stem was continuous with the inner wall of the external cuticle of the anterior cephalothorax (Fig. 4B, C). The external surface of the stem was delineated by a layer of material that was similar in appearance to and continuous with the external surface of the cuticle. This surface covering extended to the basal plate. The material at the proximal end of the stem was in contact with cells, presumably C-group cells (Fig. 4B, C).

In these recently molted larvae, there was no second cuticle beneath the external cuticle and no evidence of S1 or S2 secretory material (Table I). The B cells surrounded an empty cavity. B cells dorsal to the cavity appeared narrow and long. Ventral to the cavity, the B cells were vacuolated and had microvilli-like structures at the surface of the cavity (Fig. 5A).

The duct appeared similar to that seen in earlier stages, and its origin could be traced to a region close to group-A cells near the eyes (Fig. 5B). From this region of origin, the duct coursed anteriorly and ventral to the B group, continuing ventrally to-

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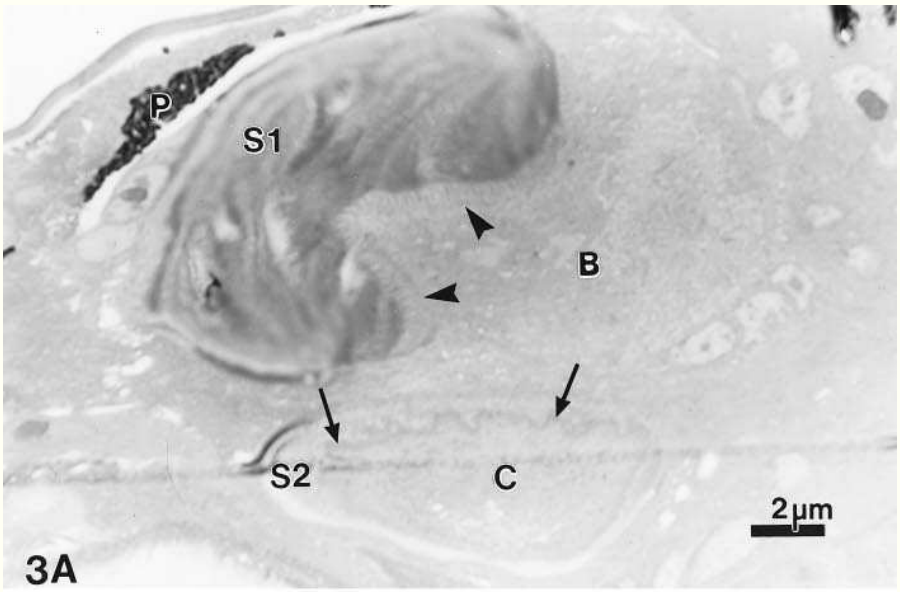
FIGURE 2. **A–C.** Copepodid, 2 dpi (22.2 DDPI). **A.** Horizontal dorsal section of the anterior cephalothorax. Newly formed filament material (S1) is surrounded by group-B cells (B). Anterior to S1 the B cells are elongate (arrows); posteriorly the B cells in contact with S1 have microvilli-like structures (arrowheads). **B.** Horizontal ventral section of the anterior cephalothorax shows the duct lined with elongate cells (arrows) passing through B group (B). **C.** Horizontal ventral section of the anterior cephalothorax shows a pore (PO) through the external cuticle (CL). Arrows indicate the cuplike structure; A, A-group cells; Lu, lumen; P, pigment; R, rostrum; SA, second antenna base.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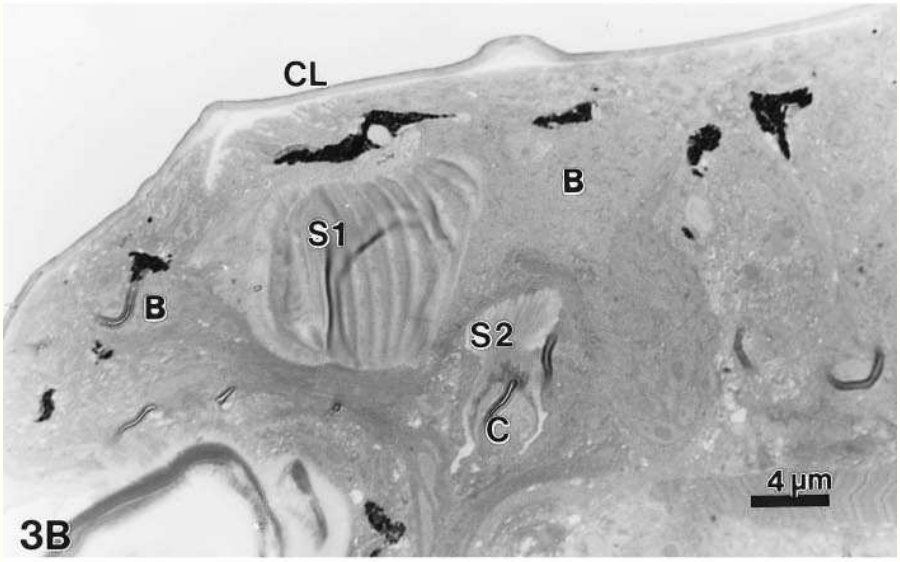
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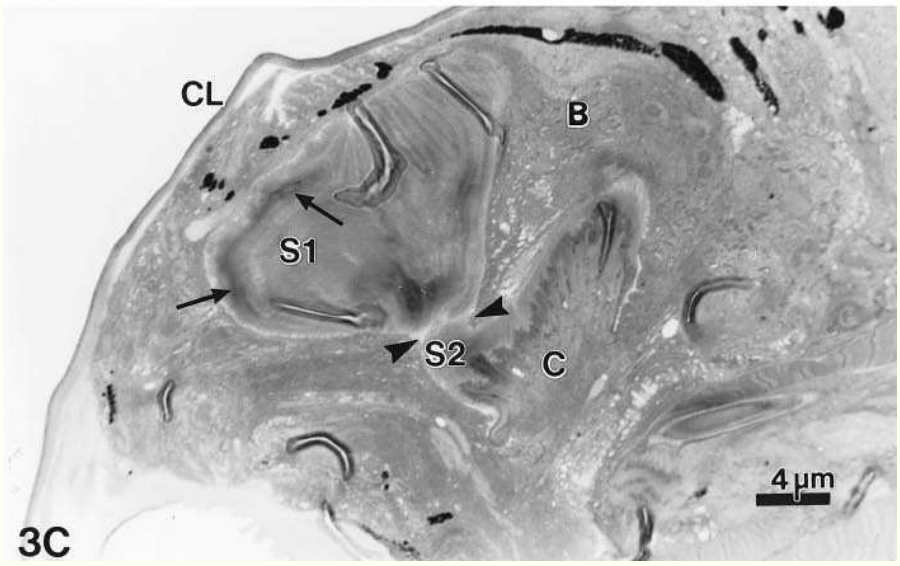
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ward the stem of the extruded filament (Fig. 5A, B). No pore was seen through the cuticle at the anterior margin of the cephalothorax.

Five, 6, 7, and 8 dpi

All salmon lice examined at 5 (n = 5), 6 (n = 5), 7 (n = 5), and 8 (n = 6) dpi showed evidence of an external FF and, therefore, had molted to the first chalimus stage. These newly molted chalima were more elongate and slender than intermolt and premolt chalimus I.

The cell groups and secretory material in all samples at 5 dpi (58.4 DDPI) and 6 dpi (68.7 DDPI) appeared similar to those observed at 2 dpi. Neither C-group cells nor S2 material were distinguishable. In newly molted chalima, a small amount of S1 material was found in contact with microvilli-like structures on the anterior dorsal region of the B-group cells (Fig. 5C). The 2 cell groups, A and B, were well defined. The B group appeared to be partially surrounding the S1, forming a thin layer at the anterior end (Fig. 5C).

Salmon lice at 5 dpi differed from those at 2 dpi as follows: at 5 dpi the origin of the duct could be traced close to group-A cells; the pore observed in earlier stages extending through the cuticle at the anterior portion of the duct was not seen; cell groups A and B had large spaces between and appeared more distant from each other, and the external FF was present (not shown).

All the individuals sampled at 7 dpi (78.9 DDPI) showed similarities with copepodids in the intermolt stage at 3 dpi. All the groups of cells (A, B, and C) and the internal filament material (S1 and S2) were seen at different stages of development. Two different degrees of S1 and S2 development were evident. Early in its development, the S1 was not in contact with S2 material. Lamina of the S1 were evident, and the B cells have long microvilli-like processes where they contact the S1. Later in its development the S1 was in contact with the S2 material. Lamination of the S1 was crescent-shaped anteriorly and had a parallel orientation posteriorly. The microvilli-like processes of the B-group cells were completely lost. At 7 dpi, 4 larvae of 5 had the S1 in contact with the S2.

Larvae at 3 and 7 dpi did not have a newly formed second cuticle. However, the S1 and S2 were present within an hourglass-shaped pocket (Fig. 6A). The 7 dpi larvae differed from those at 3 dpi in that all the groups of cells and secretions associated with the FF production were more apparent, as were the duct and its origin (Fig. 6A, B).

Five of 6 chalimus I examined at 8 dpi (90.36 DDPI) showed similar characteristics to the late copepodid stage at 4 dpi. These individuals were near to molting (premolt) as evidenced by the 2 surrounding layers of cuticle with the innermost cuticle layer (new cuticle) having abundant folds. In these individuals, the S1 and S2 were in full contact with each other, forming a new FF. One individual examined was at an earlier stage in development, having the S1 not in contact with the S2, similar to that seen in 7 dpi larvae.

In the 5 premolt larvae, B-group cells surrounded the hourglass-shaped pocket but had lost most of their contact with other surrounding tissues. A large portion of the S1 material projected out from the pocket and was in contact with the proximal portion of the stem of the external FF. The origin of the duct within cells of the A group was more distinctive.

Nine days postinfection (101.5 DDPI)

At this stage of infection, 2 of 7 chalima I examined were ready to molt (premolt) and showed characteristics similar to the late copepodid stage at 4 dpi and premolt chalimus I at 8 dpi. The other 5 had completed molting to the second chalimus stage. The shape of the anterior cephalothorax in the recently molted larvae was more slender, seeming more elongate than a premolt larva. The 2 premolt individuals had completely formed FF contained within cuticle-lined hourglass-shaped pockets in the anterior cephalothorax (Fig. 6C). They could be identified as being about to molt as they had 2 layers of cuticle. The inner new cuticle demonstrated abundant folding (Fig. 6C). The duct was evident and clearly traceable from the basal plate to the A-cell group and appeared to be the same as described for the earlier stages. In the recently molted larvae, there was no second cuticle beneath the external cuticle and no evidence of the S1 or S2.

DISCUSSION

Several studies of copepods within the Caligidae have described the structure of the FF and its mode of formation (Bron et al., 1991; Johnson and Albright, 1992; Piasecki and MacKinnon, 1993; Pike et al., 1993). However, the present study is the first to provide detailed information on the timing and cell groups involved in the formation of the FF in the salmon louse *L. salmonis*. Observations have been used to modify the diagrammatic representations of the FF-producing structures in the chalimus stage of *L. salmonis* previously reported by Bron et al. (1991). Modifications were made with respect to the location of the groups of cells (A, B, and C) involved in filament production, the positioning of the filament material (S1 and S2) within the anterior cephalothorax, and the position of the duct (Fig. 7).

Results suggest that *L. salmonis* has a duct involved in FF extrusion and 3 cell groups responsible for FF production and attachment. The 2 materials (S1 and S2) that make up the FF are produced and stored within the anterior cephalothorax until the molt. The extruded FF consists of a basal plate and a stem.

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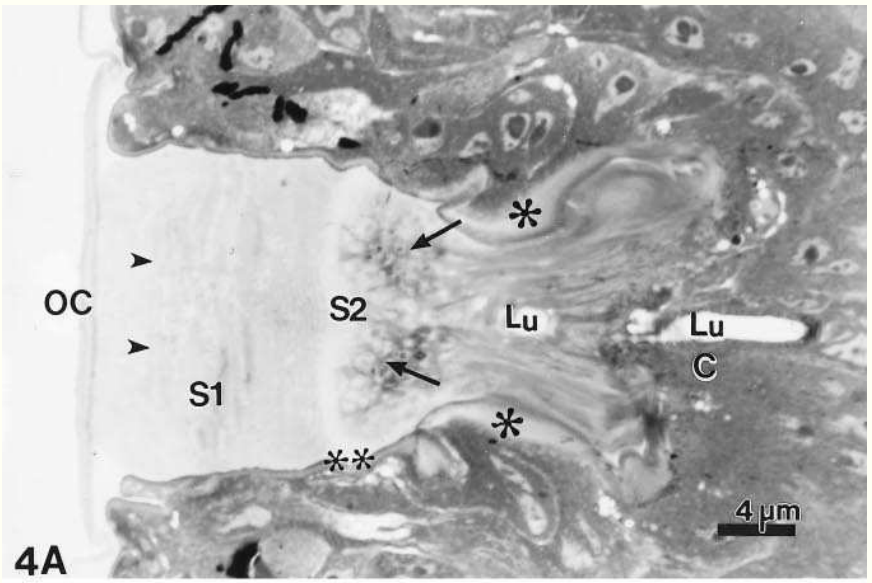
FIGURE 3. **A–C.** Copepodid, 3 dpi (34.8 DDPI). Sagittal sections of the anterior cephalothorax. Note the variation in the amount and shape of the S1 and S2 material with development of the internal filament. **A.** The S1 material is crescent-shaped with laminations. The shape of the S1 material conforms to the convex surface of the B group (B) that have microvilli-like structures (arrowheads). Densely stained cytoplasmic extensions (arrows) of C cells (C) contact the S2. **B.** The S1 material is within a more cuboidal-shaped area, and the surface of the B group at the posterior end of the S1 is flat. **C.** The S1 and S2 are in contact with one another (arrowheads). The S1 material is in a triangular-shaped area. Lamellae within S1 anteriorly are C-shaped (arrows); posteriorly the lamellae are parallel to the surface of the B group. CL, external cuticle; P, pigment.



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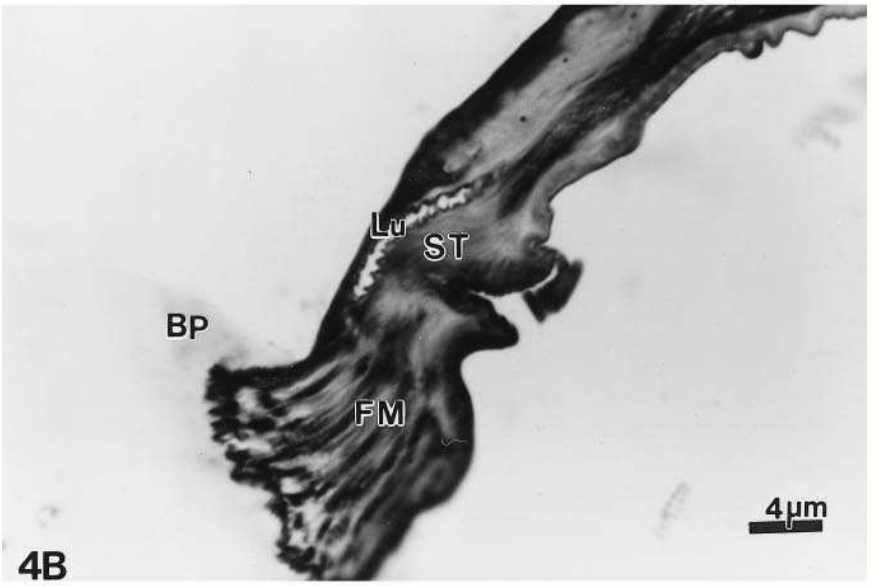
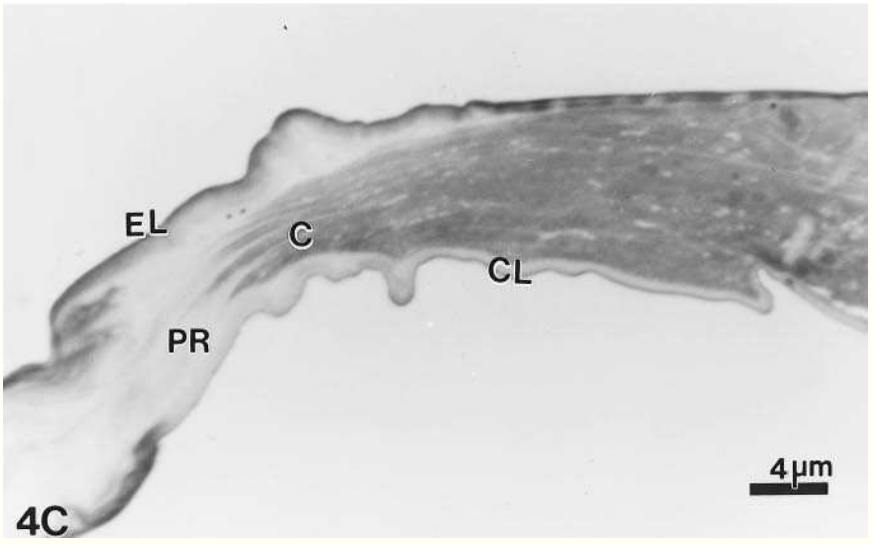


4B



4C

The morphological evidence suggests that the basal plate is composed of S1 and the stem of S2.

Several differences in morphology and histological characteristics of the A, B, and C cell groups as originally described by Bron et al. (1991) were identified in the present study. In newly molted copepodids, the location and the differentiation of staining properties of the cell groups A and B were similar to that reported in the copepodid and chalimus stages (Bron et al., 1991). However, in contrast to descriptions by Bron et al. (1991), there was no evidence of any encapsulation of the cell groups by squamous cells.

The A group of cells was observed in the present study to be the largest group through development to chalimus II and identified to be the origin of the axial duct, as first described by Bron et al. (1991). However, the collecting ducts previously reported by Bron et al. (1991) were never observed in the present study. It is possible that Bron et al. (1991) misinterpreted the presence of these ducts due to the limited degree of resolution in the paraffin sections they used.

The location and appearance of the B-group cells were similar to the description made by Bron et al. (1991). However, no granulated material was observed within the B-group cells. Results from the present investigation suggest that cells of the B group produce the internal filament material S1 as discrete layers that accumulated with time to form a laminated pattern. A striated pattern in the internal filament has been reported for *C. elongatus* (Piasecki and MacKinnon, 1993); however, the arrangement of layers are different from those observed in *L. salmonis*. The B-group cells had microvilli-like structures extending into the S1 while S1 was being produced. Because B cells in the copepodid stage contain no microvilli prior to the appearance of S1, it is possible that B cells modify their cellular surface to form the microvilli-like structures when actively secreting S1 material. Although many cells containing microvilli have an absorptive function, secretory surfaces of epithelial cells are also known to be characterized by microvilli (Patt and Patt, 1969). Microvilli-like structures have been reported to be associated with cuticle formation in crustaceans (Horst et al., 1993) and more specifically in some parasitic copepods (Bresciani, 1986). The microvillar surface of the B cells appears to be lost prior to molting when secretion apparently concluded. Electron microscopy studies are needed to investigate further the role of B cells in S1 production.

The B group changed in morphology as S1 enlarged during development, as reported in FF formation for larval parasitic copepods in general (Shields, 1967) and for *C. elongatus* in particular (Piasecki and MacKinnon, 1993). In the present study, changes within the S1 laminated pattern were observed, and the laminations followed changes in the shape of the B cells involved in production.

The C group of cells were the most difficult to define. The C group was identified both by its ventral location, as described by Bron et al. (1991), as well as by the presence of dark-staining cytoplasmic extensions where these cells contact the S2 (present study). Cells specialized for absorption and secretion have modifications observed in their plasma membrane (Junqueira et al., 1998) either microvilli as presented by the B cells or cytoplasmic extensions as presented by the C cells. Because the C group did not present any distinctive staining properties prior to the formation of S2, they could not be distinguished early in the molt cycle of both the copepodid and chalimus stages in the present study. The presence of secreted material (S2) beside the C group has been described by Bron et al. (1991). The long, dark-staining cytoplasmic extensions of C cells into the S2 material observed in the present study suggest that the cells of the C group are responsible for the production of S2 filament material. The cytoplasmic extensions may be a mechanism for increasing the secretory area of the C cells, or a mechanism by which C cells can align the S2 material to give it its characteristic filamentous-looking structure, or both. The differences in the appearance of the S1 and S2 in both internal and extruded filaments support the view that these 2 secretions are produced by different cell types, using different mechanisms of production.

The filamentous or membranous material in the distal portion of the stem may originate from the C cells. The C group was observed to lose apical portions of the cytoplasmic extensions with the extrusion of the FF. This suggests that the finger-like extensions of the C cells are torn from the cells and become part of the stem of the FF during extrusion. However, in contrast with the description provided by Johnson and Albright (1991), there is no evidence of entire C cells within the S2 material or external FF because nuclei were not observed. After a period, the C cells started another secretory cycle.

The formation of the FF within the cephalothorax appears closely linked to the molt cycle of *L. salmonis* with a similar pattern of development seen in each of the copepodid and chalimus I stages studied. Early in the molt cycle, both the copepodid and chalimus I were observed to have formed only S1. Later in the intermolt period, both S1 and S2 were present in different degrees of development. Initially, S1 and S2 are separated from each other. In the later stages of the intermolt period, S1 and S2 are in contact with each other. Immediately preceding the molt, an internal FF formed by the fusion of S1 and S2 is present within a cuticle-lined pocket.

The continued presence of fibrous material in contact with S2 suggested that these become the stem of the FF. The laminar nature, similar staining characteristics, and the proximal position of S1 (with respect to S2) suggest that S1 becomes the basal plate as the external FF is formed. In contrast, Bron et al.

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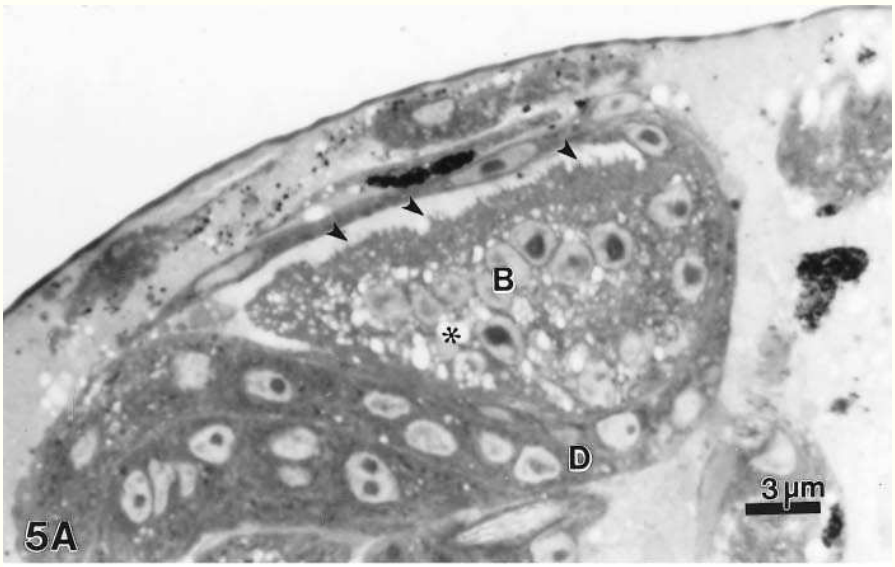
FIGURE 4. **A.** Late-copepodid, 4 dpi (47.5 DDPI). Horizontal medial section of the anterior cephalothorax shows a completely formed internal FF (S1 and S2) contained within a cuticle-lined hourglass-shaped pocket. Note the thick (*) and thin (**) regions of the new cuticle lining the pocket. Long cytoplasmic processes (arrows) extend from C-group cells (C) at the posterior end of the pocket into the S2. A lumen (Lu) passes through C group and S2. Arrowheads indicate the laminated pattern of S1. **B, C.** Newly molted chalimus I FF, 4 dpi (47.5 DDPI). **B.** Sagittal section of the newly formed external FF. Note the light staining of the basal plate (BP) in relation to the stem (ST); the presence of fibrous material (FM) in the distal end of the stem; and presence of a lumen (Lu) dorsal through the stem. **C.** Sagittal section of the proximal region (PR) of a newly formed external FF shows a pale-staining stem in contact with cytoplasmic processes of C-group cells (C). The surface of the stem is delineated by an external lamina (EL) continuous with the external cuticle layer (CL). B, B-group cells; OC, old cuticle.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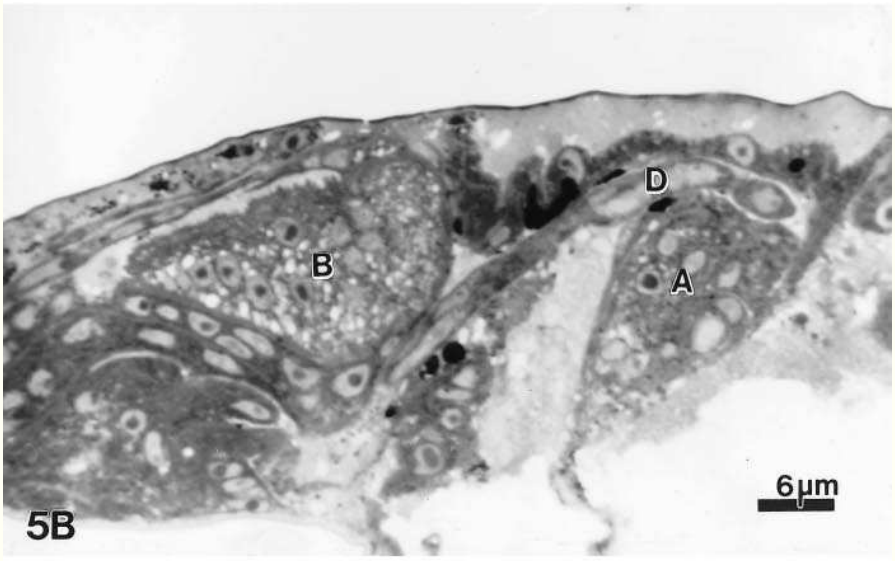
*
D
3 µm
5A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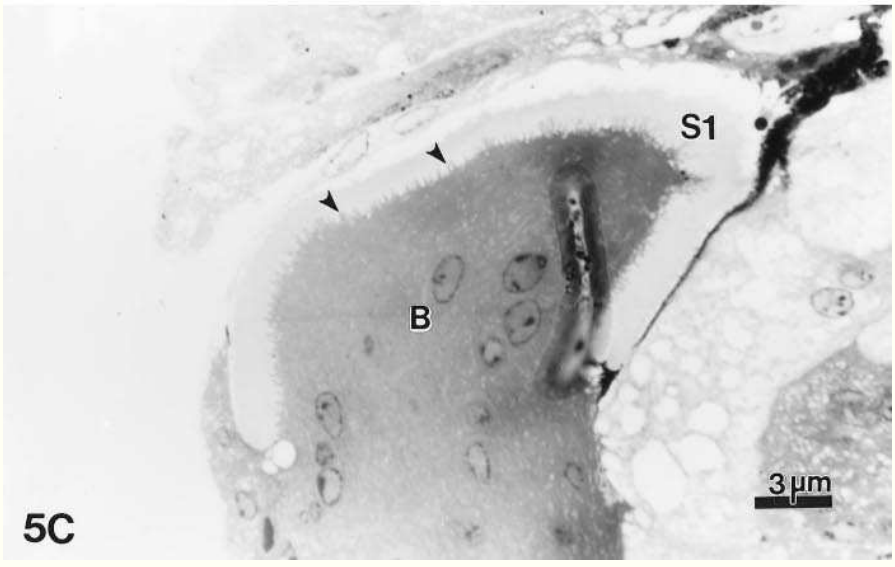
S1
B
3 µm
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(1991) reported no relationship between S1 and S2. The present study indicates that both secretions are contained within a cuticle-lined pocket forming the external filament, similar to an earlier description (Johnson and Albright, 1992).

Whether the groups of cells involved in the FF production have another function besides those described in the present study remains unknown. Electron microscopy studies are needed to elucidate if these cell groups produce just 1 type of material or several different materials during development.

In the present study, a duct that originated in the A group and passed anteriorly through the ventral portion of the B group was identified. In the copepodid stage, this duct was seen to open to the outside via a small pore through the cuticle in the vicinity of the rostrum. However, in the chalimus I stage, there was no evidence of an external pore, and the duct terminated near the proximal end of the FF stem. It appeared that the stem of the FF covered this pore.

This duct is homologous to the main axial duct described by Bron et al. (1991). The fact that the duct identified in the present study emerged near to the rostrum as a pore suggested that the pore may function as an application opening for some filament-associated material. There was evidence that the duct is coupled to this pore. The present study suggests that the pore allows a material produced by the A-group cells to be released into the external environment. Because the pore is connected to the duct and the duct to the A-group cells, it is possible that the duct carries a gluey secretion produced by the A group to the pore, and this is applied to the host. This gluey secretion would then fix the basal plate (S1) to the host. It is possible that this secretion is the gluey material suggested by Johnson and Albright (1992) to bind the FF to the epidermis of the fish. Bron et al. (1991) concluded that the basal plate material is secreted via the axial duct from the A cells. Results from the present study show no evidence that the basal plate is formed in this way.

The present results suggest that the adhesive and the basal plate described by Pike et al. (1993) are the basal plate and the distal stem, respectively. It is probable that either the basal plate attaches to the host integument by changing its properties or, more likely, by using a secretion carried by the axial duct to act directly as a glue.

Within the extruded FF, there was a lumen from the proximal region of the stem to the basal plate. This lumen was not observed to run through the center of the stem as described previously (Bron et al., 1991) but tended to be oriented dorsally in most of the stem and then ventrally at the proximal end to join the indiscernible pore. The lumen may not have any function and may be formed within the stem either by the pore or by the duct while the filament is extruded. While molting, the salmon louse is contracting its body to shed the old cuticle (Antensrud, 1990). Therefore, this lumen may be created in the stem at the same time the new FF is extruding.

Heegaard (1947) described an attachment mechanism for *Caligus curtus* beginning with a drop of secretion emerging from the mouth of a gland that may be pressed out of a reservoir where the secretion has been gradually amassed. Maxillipeds carry the secretion from the mouth of the gland and press it down into a previously drilled lesion on the host to form a disklike secretion. The rest of the secretion is then drawn out into a long, thin thread to secure the attachment. It is speculated that a similar process occurs in *L. salmonis* when it is molting; the attachment may begin with a drop of A-group secretion emerging from the pore and being applied to the host surface. The internal filament material (S1) forming the basal plate would then be pressed down on this drop of secretion that is now firmly glued to the host. The larva then begins to back away, and the S2 and the cuticle pocket are drawn away to form the stem of the filament that may also include some C-group cellular material. The stem may actually have stretched from the cephalothorax of the larvae, as suggested by the elongate shape of the anterior cephalothorax observed in newly molted larvae. However, further studies on molting behavior would be useful to elucidate the molting process in *L. salmonis* and its relation with FF formation.

The presence of the axial duct and the pore at the newly molted copepodid stage prior to any other structure involved in the FF production suggests that other secretions are probably being applied through the duct and the pore from the A group.

Different mixtures of fibrous materials at the distal region may provide elasticity and flexibility to the stem to facilitate feeding around the basal plate while maintaining permanent contact with the substrate (i.e., the host) in ways similar to those that have been described for the byssus of *Mytilus edulis* (Benedict and Waite, 1986).

C cells extending from the cephalothorax into the proximal region of the stem in newly molted larvae support the suggestion that the filament material is pulled out as a mode of extrusion prior to hardening of the new cuticle (Johnson and Albright, 1992). The fact that a large portion of the S1 material projected out from the cuticle pocket in premolt (8 dpi) chalimus I and was in contact with the proximal portion of the external stem supports the concept that the FF will be lost and replaced at the molt. Thus, the current study suggests that the FF is extruded at the molt to chalimus I and is replaced at the molt to chalimus II. Based on this, it is possible that the FF in *L. salmonis* is replaced every molt during subsequent phases up to chalimus IV, as previously suggested by Johnson and Albright (1992). Further studies on FF at chalimus III and IV are necessary.

The present study provides evidence that the cuticle-lined pocket covering the S1 and S2 produced in premolt is extruded together with the secretions at the molt to form most of the external lamina of the stem of the FF after extrusion. Similar descriptions have been made for other Caligidae species (Hee-

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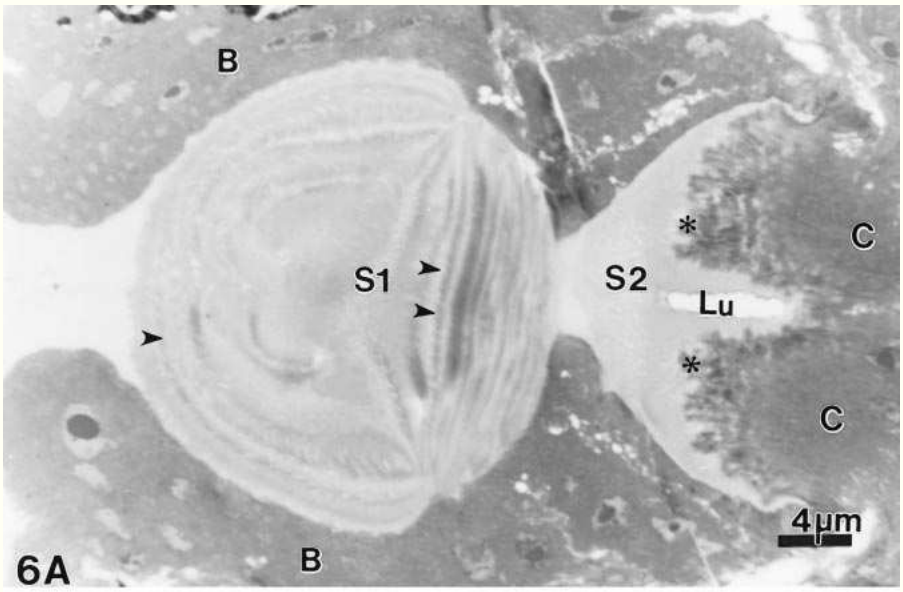
FIGURE 5. **A, B.** Newly molted chalimus I, 4 dpi (47.5 DDPI). Sagittal sections of anterior cephalothorax. **A.** The B-group cells appear vacuolized (*). Microvilli-like structures (arrowheads) are present on the surface of the B-group cells (B). Note the absence of filament material. Cells covering the duct (D) pass under the B cells. **B.** The origin of the duct (D) is in close proximity to group-A cells (A). The duct passes ventral to the B group (B) toward the anterior of the cephalothorax. **C.** Chalimus I, 5 dpi (58.4 DDPI). Sagittal section of the anterior cephalothorax. Note the early development of filament material (S1) partially surrounding the B group (B). Microvilli-like structures (arrowheads) from cells of the B group extend into the S1 material.



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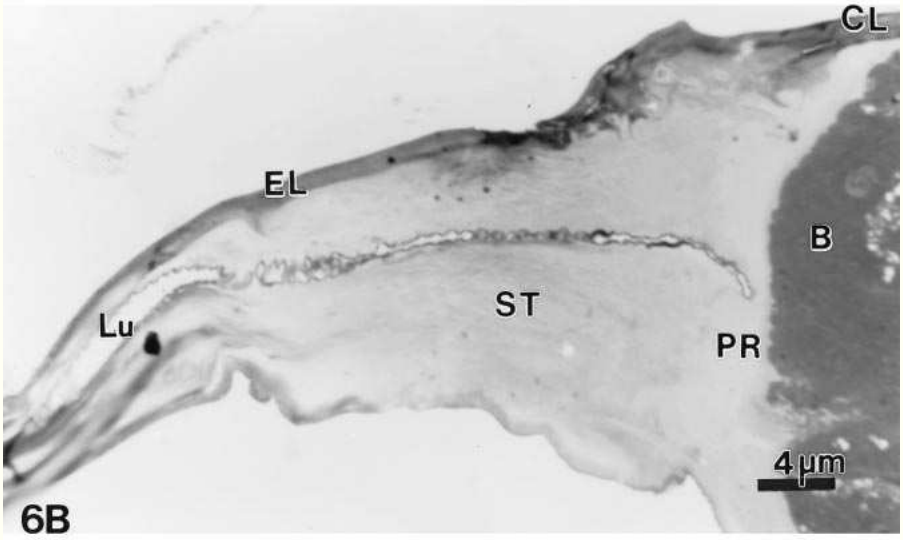

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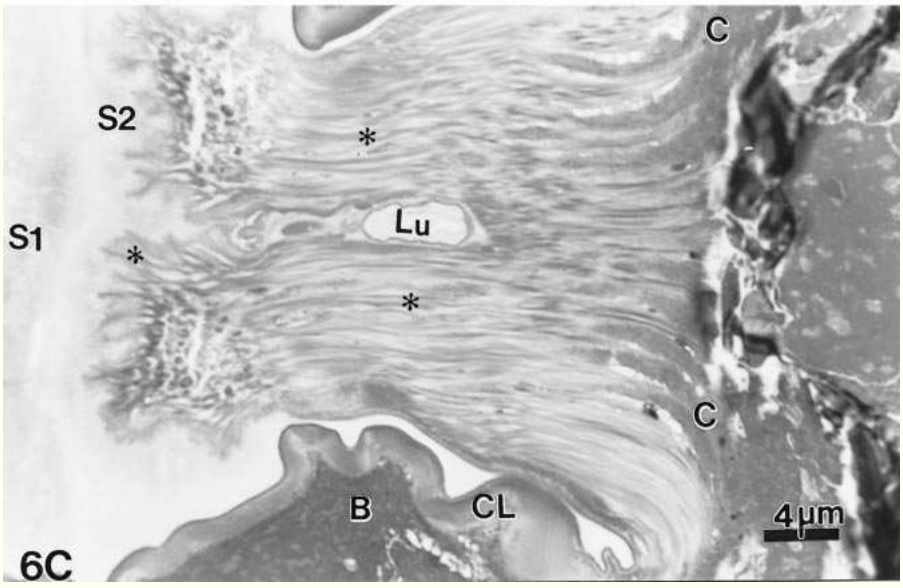

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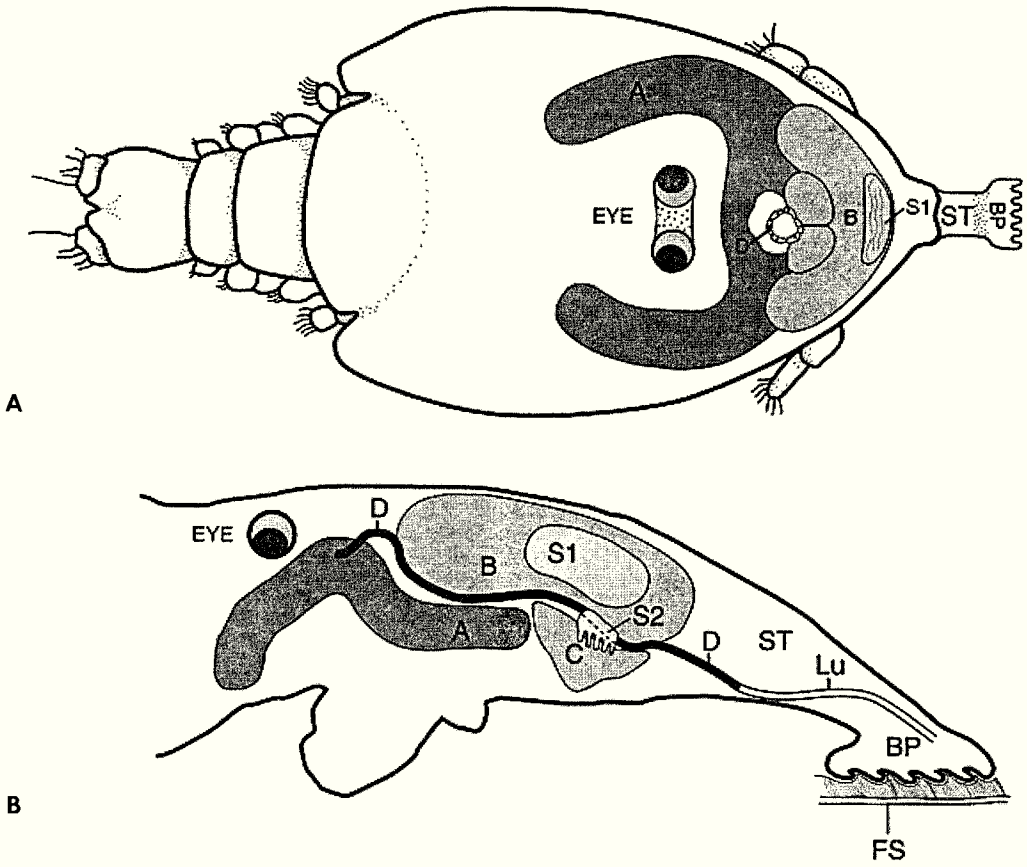

FIGURE 7. **A, B.** Diagram showing the arrangement of cell groups A, B, and C and internal filament material S1, S2, and duct (D) in chalimus at 7 dpi. **A.** Dorsal view. **B.** Sagittal view. BP, basal plate; FF, frontal filament; FS, fish scale.

gard, 1947; Kabata, 1972; Piasecki and MacKinnon, 1993). The external lamina is formed simultaneously with the external filament and not during formation of the stem, as described by Pike et al. (1993).

Based on our results, the FF appears to be a good target for the development of new methods and/or therapies aimed at interfering with its formation or function, thereby negating the ability of chalima to attach to salmon.

ACKNOWLEDGMENTS

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FIGURE 6. **A, B.** Late chalimus I, 7 dpi (78.9 DDPI). **A.** Horizontal medial section of anterior cephalothorax shows a completely formed internal FF (S1 and S2) in an hourglass-shaped pocket without any cuticle lining. Note the long cytoplasmic extensions (*) from the C cells (C) into the S2. A lumen (Lu) passes through the C group and S2. Arrowheads indicate laminations of S1. **B.** Sagittal section of external FF. Proximal region (PR) of the stem (ST) is in contact with the B group (B). The external lamina (EL) of the stem is in continuum with the cuticle (CL) and a lumen (Lu) extends through the stem. **C.** Late chalimus I, 9 dpi (101.5 DDPI). Horizontal section of anterior cephalothorax shows the thick cuticle (CL) lining the hourglass-shaped pocket, and long extensions (*) from the C cells in contact with S2.

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