Comparative Ultrastructures between Different Types of Silk Gland in Insect

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The silk glands give protection during growth and metamorphosis throughout the life cycle of silkworms correlating their habitat and behavior. Molecular silk proteins are synthesized in the silk gland cells (Akai, 1971), and the glands can be classified into four types: labial gland, tarsal gland, Malpighian tubules and accessory gland (Sefnal and Akai, 1990). Many orders of insects are involved in silk production (Table 1). The Indian tasar silkworm, *Antheraea mylitta*, makes a complete, large and hard cocoon with thick filaments from the labial silk gland for protection during their larval-pupal diapause (Akai *et al.*, 1992). The webspinner, *Oligotoma japonica* Okajima, on the other hand, spines the finest filament from the tarsus silk gland and makes a silken tunnel which it anchors to the substrate (Nagashima *et al.*, 1991). The labial silk gland cells usually hypertrophy without cell division during the larval stage, especially in cocoon making insects. In *Bombyx mori* the DNA content in each cell at the mature stage is increased by endomitosis to more than 1,000,000 times that of the embryonic stage (Akai, 1984). In contrast, the silk gland of the webspinner can be differentiated by having apocytes.

In this short communication we will compare the ultrastructural characteristics of the largest labial gland that of the Indian tasar silkworm, and the tarsus silk gland of the webspinner.

Test animals used were the Daba, an ecorace of the Indian tasar silkworm, *A. mylitta*. The larvae were reared on fresh leaves in Asan (*Terminalia tomentos*) and Arjun (*T. arjuna*) fields in Ranchi, India. Female matured larvae weighting about 47 gm were dissected and the pair of silk glands was immediately fixed in a prefixative solution of 2% paraformaldehyde and 2.5% glutaraldehyde (Nagashima *et al.*, 1991). After postfixation by 1% OsO_4 solution and dehydration with ethanol, these materials were embedded in Epon 812. Golden thin sections were cut by a Reichert OMU2 microtome, and observed with TOPCON LEM-2000 and JEM 100-CX electron microscope. For scanning electron microscopy (SEM), cocoon filaments were dehydrated in an ethanol /*t*-butyl alcohol series, and freeze-dried. Specimens were examined with a HITACHI S-2400 SEM.

Also were used adult males of the webspinner, *O. japonica* Okajima captured on Ishigaki Island of Okinawa Prefecture. Specimens were observed by TEM and SEM following the above procedures.

Table 1 Silk-producing organs in insect.

LABIAL GLAND:	
Orthoptera (Gryllacrididae)	Psocoptera Thysanoptera (Aeolothripidae)
Trichoptera Lepidoptera	Hymenoptera Diptera (Nematocera, Orthorrhapha)
TARSAL GLAND:	
Embioptera Diptera (Empididae)	
MALPIGHIAN TUBES:	
Coleoptera (Carabidae, Curculionidae)	Neuroptera (Chrisopidae, Myrmeleontidae)
ACCESSORY SEX GLAND:	
Coleoptera (Hydrophilidae)	Neuroptera (Chrysopidae, Berothidae, Mantispidae)

Labial silk gland of the Indian tasar silkworm

In the posterior silk gland of this insect, rough endoplasmic reticulum (er) and Golgi complexes develop greatly in the cytoplasm during the last larval instar (Fig. 1A), as is true in *Bombyx mori*, which has been studied in detail by electron microscope (Akai, 1984). In basal region the cell surface is covered with a thick basement membrane, and conspicuous infoldings are visible. Numerous mitochondria and small lysosomes are densely distributed in this area. In mature larvae, many mitochondria in the central area degenerate to autophagosomes and are released into the lumen (Fig. 1B). Characteristic profiles of Golgi vacuoles containing dark masses of fibroins are seen in the cytoplasm. Fibroin globules are concentrated in apical regions. Extremely developed bundles of microfilaments stand in a row and prominent ahead of the lumen in the longitudinal section (Fig. 1C). Masses of fibroin fibers secreted by exocytosis congregate directory on the main fibroin column which occupies most of the gland lumen, because there is no cuticular intima on the apical surface.

The fibroin column contains numerous round vacuoles (or voids) with diameters of various sizes. These seem to be the origins of the canals in the cocoon filament as this filament is the same as that in *Antheraea yamamai* (Akai *et al.*, 1989). Each cocoon filament is composed of fibroin filament covered with sericin layers which are more than 15 deniers thick (Fig. 3B). SEM observation shows the filaments to be round and flat in appearance (Fig. 3B), with numerous Ca crystals originated from Malpighian tubules adhering to the surface.

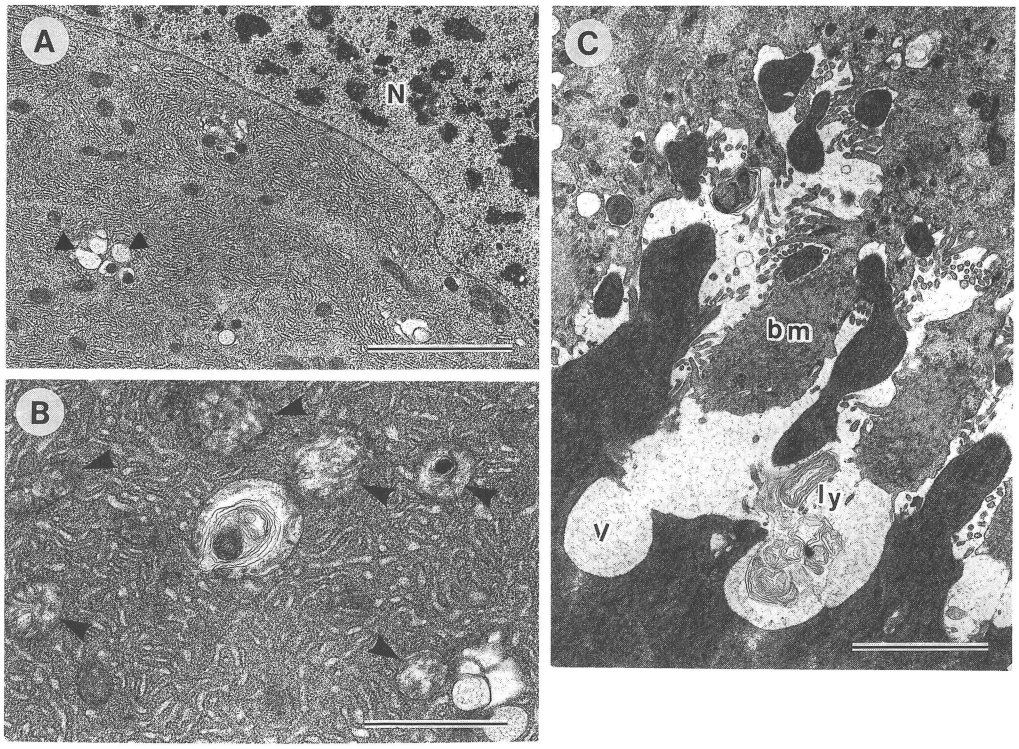

Fig. 1 Portions of the silk gland cell from the posterior silk gland of *Antheraea mylitta*. A. Nuclear and adjacent cytoplasmic area containing cellular organelles. Er is fairly well developed throughout the cytoplasmic area. Golgi vacuoles contain dense materials or diffused fibroin fibers (arrow heads). Scale=3μm. N: nucleus. B. Some of the mitochondria (arrow heads) are beginning to degenerate into autophagosomes. Scale=1.5μm. C. Apical cytoplasmic area. Bundles of microfilament (bm) develop and prominent ahead of the lumen, and dark fibroin masses are secreted and congregate on the main fibroin column in the lumen. Residual lysosomal materials (ly) are taken into the vacuoles (v). Scale=3μm.

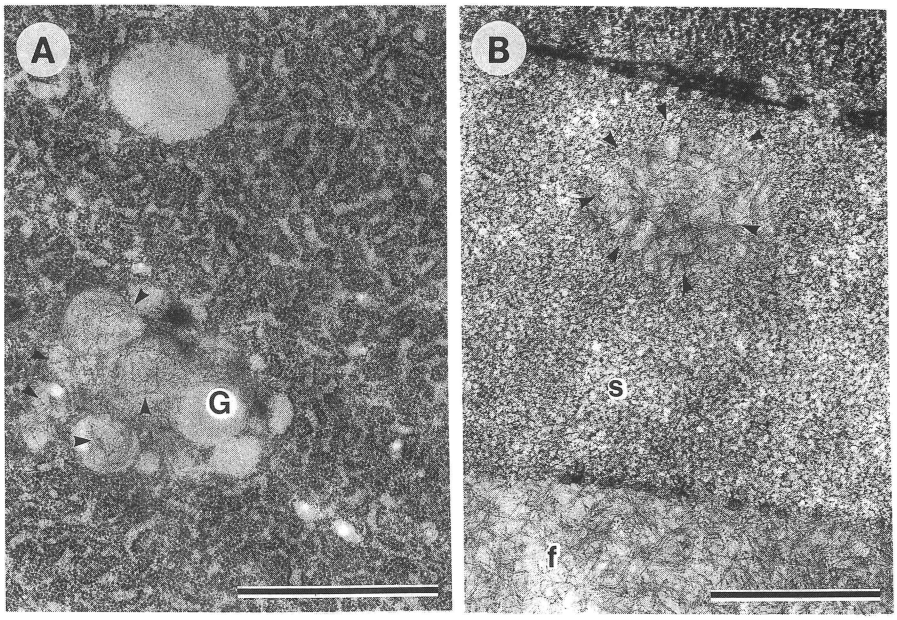

Fig. 2 Parts of silk gland from the tarsus silk gland of the webspinner, *Oligotoma japonica* Okajima. A. Er and Golgi complex (G). Some Golgi vacuoles contain materials (arrow heads). Scale = 1 μm. B. Sericin and fibroin fibers in the lumen. A sericin layer (s) composed of sericin fibers lies between the apical cell surface and the central fibroin mass. The fibroin fibers (f) are in the central part of lumen and are sometimes found in the sericin layer (arrow heads). Scale=1μm.

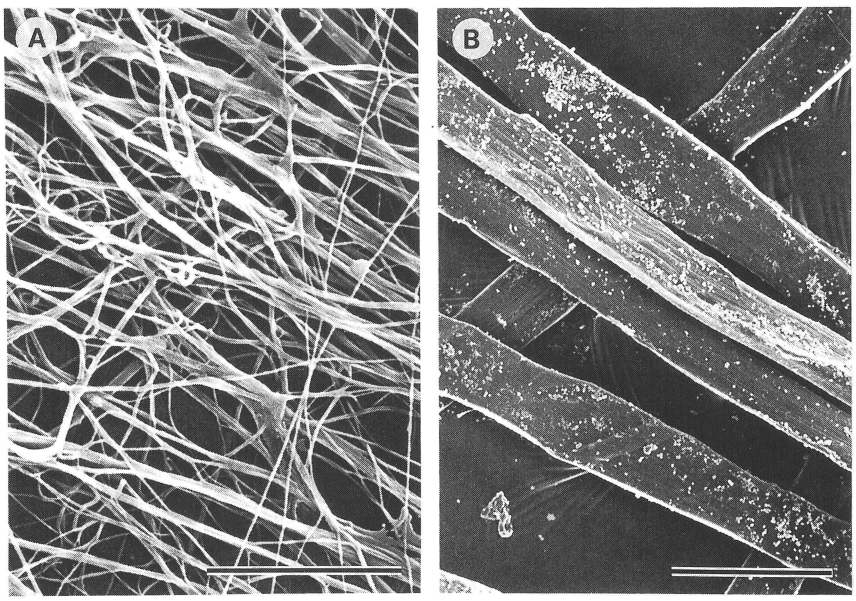

Fig. 3 SEM photographs of spun silk filament. A. Silk filaments spun by webspinner. Size of the filament is fine and varies in thickness. Scale=5μm. B. Cocoon filaments spun by Indian tasar silkworm. Each filament is fairly uniform and much thicker than that of the webspinner. Ca crystals are scattered on the filaments. Scale=100μm.

Tarsus silk glands of webspinner

Glands are located on the ventral surface of the 1st tarsal segment of the forelegs and have many cuticular processes which are connected with the glandular chambers inside. There are about 100 of these silk glands, and each glandular chamber is a globular form bounded by one large multinuclear cell showing the typical apocytes.

Both fibroin and sericin globules are produced through the er and Golgi complex which are well developed (Fig. 2A). Matured Golgi vacuoles contain much fibrous fibroin and this released into the gland cavity by exocytosis. A layer of sericin which is densely packed with sericin fibers and an amorphous concentration of fibroin fibers is detected in the central region of the lumen.

The spun filaments of webspinner vary in thickness, are tangled together and branch to many parts of the filaments (Fig. 3A), indicating that each filament was spun independently by spinnerets of many different sizes and then attached to each other.

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