# INTRODUCTION

Insect silk is chiefly used for the production of luxurious textiles but it seems to contain a host of compounds suitable for other practical applications. Two silk properties are particularly interesting: (1) silk resistance to the attack by microorganisms and omnivores (mites, some insects, etc.), and (2) silk compatibility with mammalian immune system. The purpose of this theses is to contribute to the identification of silk components responsible for both properties. The silk resistance to microbial challenge (point 1) was attributed to the presence of small proteins that inhibit bacterial proteinases such as subtilisin and fungal proteinases represented by proteinase K (Nirmala et al., 2002a, b). However, it was not excluded, and actually never tested, if the silk does not contain some of the antibacterial and antifungal peptides that occur in insect hemolymph. Therefore, one subject of the theses was to find out if genes encoding three selected antimicrobial proteins (hemolin, galleriomycin, and the mucin-like peptide) are expressed in the silk glands and if their expression can be stimulated by bacterial challenge. The results of this research, which are presented in chapters 2 and 3, provided positive answers to both questions. It seems that silk endurance to microbes rests on a mix of compounds that are deposited in the cocoon silk but are absent or less abundant in the silk produced by young larvae. Some of these components may also be responsible for limited silk attractiveness to animals that should be eager to devour such proteinaceous meal. Chapter 4 demonstrates that silk produced by the feeding larvae of *Galleria mellonella* is readily eaten by the larvae and thereby recycled, whereas cocoon silk deters feeding.

Silk immunotolerance (point 2) seems to be limited to fibroin and possibly only to some sericin fractions (see Introduction in chapter 1). Sericin extracts have been commercialized as additives to the cell culture media and preparations of both native and recombinant sericins were tested as scaffolds guiding cell proliferation during tissue reconstruction (cf. Sehnal, 2008). Absence of a method for reproducible fractionation of sericins from the cocoon silk is the major obstacle of such studies. Chapter 5 describes small contribution to the solution of this problem.

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# RESULTS

**Publications:**

Shaik A.H. & Sehnal F. (2009) Hemolin expression in the silk glands of *Galleria mellonella* in response to bacterial challenge and prior to cell disintegration. *J. Insect Physiol.* 55: 781-787.

Shaik A.H. & Sehnal F. (2009) Antimicrobial defensin-like gallerimycin and a mucin-like peptide are expressed in the silk glands of *Galleria mellonella* (to be submitted to the *Journal of Invertebrate Pathology* before defence of the PhD thesis).

Shaik A.H. & Sehnal F. (2009) Silk recycling and feeding-deterrent properties of the cocoon silk (to be submitted to the *Journal of Insect Biotechnology and Sericology* before defence of the PhD thesis).

**Presentations:**

Haq A.S. and Sehnal F. (2005) Induction of hemolin gene expression in selected tissues of *Galleria mellonella*, poster presented at IVth International Conference on Arthropods Chemical, Physiological And Environmental Aspects, University Of Wroclaw, Faculty Of Chemistry, Zakopane, September 18-23, 2005. Page no:20.

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# Hemolin expression in the silk glands of *Galleria mellonella* in response to bacterial challenge and prior to cell disintegration

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Hemolin, a member of the immunoglobulin protein superfamily, functions in Lepidoptera as an opsonin in defence against potential pathogens and seems to play a role in tissue morphogenesis. We show that *hemolin* gene is expressed in several organs of *Galleria mellonella* larvae, including the nervous system and the silk glands. The expression in the silk glands of the wandering larvae and their isolated abdomens is enhanced within 6 h after an injection of bacteria, lipopolysacharides, or peptidoglycans. The magnitude of silk gland response to bacterial challenge is similar to that seen in the fat body. A profound rise of hemolin expression without bacterial inoculation occurs in the silk glands of isolated abdomens when they are induced to pupate by a topical application of 20-hydroxyecdysone (20E). The induction of pupation is associated with silk gland programming for disintegration by apoptosis and phagocytosis. Administration of a juvenile hormone agonist prevents pupation and abolishes the stimulatory 20E effect on the hemolin expression. Hemolin protein can be immunodetected in the silk glands as well as in the spun-out cocoon silk. The results suggest that silk glands are a component of the insect immune system and that hemolin may mark the apoptic cells for the elimination by hemocytes.

# A defensin-like and a mucin-like immunopeptides are expressed in the silk glands of *Galleria mellonella*

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Insects have three major defence lines against infection: integument, digestive tract, and haemolymph. Most investigations focused on the immune mechanisms in the haemolymph and identified antimicrobial peptides classified into four structural categories: (1) α-helical cecropin-like peptides; (2) cysteine-rich peptides (defensins and related peptides); (3) proline-rich peptides (e.g., drosocin); (4) glycine-rich peptides (attacins, sarcotoxin II, and diptericins). Most of these peptides are synthesized mainly by the fat body in response to microbial infection or body injury at an early stage of the immune response. Some antimicrobial peptides are also expressed in the midgut, integument, or blood cells. This is also true for hemolin that is characterized as opsonin facilitating recognition of foreign bodies by the hemocytes. However, in the larvae of the waxmoth *Galleria mellonella* we found hemolin expression also in the silk glands and detected deposition of the hemolin protein in the silk. This surprising finding encouraged us to investigate whether genes encoding other immunopeptides are also expressed in the silk glands. We selected a defensin-like peptide galiomycin and a unique mucin-like peptide.

Transcripts of the defensin-like antimicrobial peptide galiomicin and of a mucin-like protein that is produced during blood clotting occur in the silk glands of the post-feeding, cocoon-spinning larvae. The amounts of transcripts are enhanced in larvae injected with dry bacteria. By contrast, no transcripts can be detected in the silk glands of feeding larvae that spin temporal protective tubes. The transcript of galiomicin, but not of the mucin-like protein, is also produced in the fat body cells.

# Silk recycling and feeding-deterrent properties of the cocoon silk

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Larvae of *G. mellonella* feed in nature on the honeycombs that contain large proportion of beeswax, some honey, cast exuviae of immature bee stages, and residues of pollen. Larvae obtain from their food large amounts of energy but are poorly supplied with protein. Under laboratory conditions the larvae develop well on various artificial diets in which the wax is replaced with carbohydrates. However, if the amount of protein in the diet drops below a certain threshold, the larvae stop spinning, apparently because they lack essential amino acids needed for the production of silk proteins such as heavy chain fibroin. For the larvae it would be obviously advantageous to consume silk of the narrow abandoned tubes and recycle the amino acids. This possibility was addressed by the present study in parallel with another intriguing question that concerned possible roles of seroins, proteinase inhibitors, and other proteins that are specifically deposited in the cocoon silk. It has been proposed that these ingredients protect cocoons against microorganisms and possibly also against mites, insects, and other omnivorous animals. To answer both questions, we fed larvae with raw or processed silk and followed their feeding rate and silk production.

Silk produced by the feeding larvae of *Galleria mellonella* is consumed by the larvae in addition to the standard diet that is sufficient to support normal development. Replacement of dry milk in the diet slows down food consumption and usually also silk production. Silk from the cocoons of *G. mellonella* or *B. mori*, and in particular the sericin fraction from the cocoon silk, have much stronger feeding-deterrent effect than the silk produced by feeding larvae. The results indicate that *G. mellonella* larvae recycle silk that they produce during feeding and deposit feeding-deterrent ingredient(s) into the cocoon silk. The silk of *B. mori* cocoons is also less readily consumed than the silk produced prior to cocoon spinning.

# CONCLUSIONS

Hemolin, a member of immunoglobulin super family plays a vital role in the part of defense. We show that *hemolin* gene is expressed in several organs of *Galleria mellonella* larvae, including the nervous system and the silk glands. The magnitude of silk gland response to bacterial challenge is similar to that seen in the fat body. We also observe the induction of hemolin gene by topical application of 20-hydroxyecdysone (20E). Administration of a juvenile hormone agonist prevents pupation and abolishes the stimulatory 20E effect on hemolin expression. Hemolin protein can be immunodetected in the silk glands as well as in the spun-out cocoon silk. The results suggest that silk glands are a component of the insect immune system and that hemolin may mark the apoptotic cells for the elimination by hemocytes.

Genes encoding defensin-like galiomicin and a mucin-like protein are also expressed in the silk glands and the contents of corresponding transcripts in both middle and posterior silk gland sections are enhanced after injection of dry *Micrococcus luteus.* Primers specific for the mucin-like cDNA amplify in the silk gland RNA at least 7 products of which one corresponds to the desired fragment, the identity of the remainder is unknown. No transcript of either of the two genes can be detected in the feeding larvae of the penultimate or last larval instar, suggesting that both galiomicin and the mucin-like proteins may play roles in cocoon protection. Galiomicin, but not of the mucin-like protein, is also expressed in the fat body.

Not only galiomicin and the mucin-like protein, but also proteinase inhibitors and seroins are expressed in the silk glands only at the time of cocoon spinning. Possible reason for such developmental gene expression pattern is that the corresponding gene products could interfere with the role of silk produced in *G. mellonella* during the feeding period. Chapter 4 of the thesis demonstrates that larval silk is at least partly consumed by the larvae. Cocoon silk, and in particular its sericin fraction, is less readily accepted and reduces the food consumption rate as well the rate of silk production. We conclude that the silk produced during the feeding period is recycled, allowing larvae to replenish their pool of essential amino acids. Since cocoon silk cannot be recycled by emerged moths, it contains feeding-deterrent compounds that protect it against any animal.

# FUTURE PERSPECTIVES

The work on galiomicin and mucin-like protein has been done at the level of transcript detection. We do not know how much protein is produced and what its fate is. It could be used within the silk gland cells, secreted into the hemolymph, or deposited in the cocoon silk. Preparation of antisera is considered to distinguish between these possibilities. The role of mucin-like proteins deserves special attention because the array of tentative transcripts indicates occurrence of several related proteins. At least some of these transcripts must be analyzed in detail.

The work on silk recycling should be completed by identification of the feeding-deterrent and digestion-curbing silk components. They could be of practical significance. However, completion of such a study would require a number of years and will be probably undertaken in future.