Proc. Arthropod. Embryol. Soc. Jpn. 42, 31–32 (2007)

ISSN 1341–1527

Development of Embryonic Appendages in the Sawfly, *Athalia rosae ruficornis* Jakovlev (Hymenoptera): Morphological Observation and Cloning of a *Distal-less* Orthologue*

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Many insects have abdominal appendages in their larval stages. In larvae of Lepidoptera and the symphytan Hymenoptera, these abdominal appendages, known as prolegs, are well developed. Recent developmental biologies revealed differences in abdominal appendicular formation between these two orders by analyzing the distribution of Distal-less protein, which is one of the key factors to regulate distal elaboration. The prolegs of Lepidoptera originate from the whole limb (Panganiban *et al.*, 1994), while those of Hymenoptera consist only of the proximal part, lacking the distal portion (Suzuki and Palopoli, 2001). It is of interest to clarify whether the prolegs of Lepidoptera and Hymenoptera are direct homologues, and whether those of Hymenoptera are serial homologues with thoracic legs. Genetic regulation of abdominal appendicular formation among Hymenoptera is another issue. Most species belonging to the primitive suborder Symphyta have prolegs, while the higher apocritan species lack them. Both morphological and molecular analyses of embryonic appendicular development give cues to elucidate the above questions.

We used a symphytan hymenopteran, *Athalia rosae ruficornis*, as a model species, and demonstrated that one of the essential genes for appendicular development, *decapentaplegic (dpp)*, was expressed in all appendicular primordia including prolegs (Yamamoto *et al.*, 2004). This suggested that the prolegs had serially homologous traits with other appendages, but further detailed morphological observations and molecular dissections must be required. In the present study, we observed embryonic appendicular development by SEM, and then cloned a *Dll* orthologue of *A.rosae* for molecular analyses.

We described the outline of appendicular development. In the gnathal regions, the rudiments of appendages appear at 24 h of embryogenesis (24 h after egg activation). The maxillary and labial primordia started elongating and segmenting immediately after their appearance. The proximal part of the maxillary primordium formed two swellings extending ventrolaterally. The inner and outer swellings were the lacinia and galea, respectively. The proximal part of the labial primordium formed a single swelling, or the glossa and paraglossa complex. The distal parts of the maxilla and labium elongated as the palpi. The mandibular primordium was enlarged without elongation, lacking a structure comparable to the palp. The thoracic leg primordia also appeared at 24 h. Then these primordia were elongated and segmented into the proximal coxopodite and distal telopodite. At 64 h, the primordia were divided into the coxa, trochanter, femur, tibia, tarsus and pretarsus. Appendicular formation was delayed in the abdomen, and small proleg buds appeared on all 11 abdominal segments at 38 h. The proleg buds retained in the second to eighth abdominal segments were further elongated, without formation of a distal portion.

We then cloned a *Dll* orthologue of *A. rosae*. An initial clone was obtained by degenerate PCR using a cDNA library constructed from 72 h embryos. Full-length cDNA (1,698 bp) was obtained by RACE (Rapid Amplification of cDNA Ends) methods. The cDNA encoded 379 amino acids in a single open reading frame. The deduced amino acid sequence aligned well with known insect Dlls. Polyclonal antibodies were raised against oligopeptides corresponding to the homeodo-

* Abstract of paper read at the 42nd Annual Meeting of the Arthropodan Embryological Society of Japan, June 1–2, 2006 (Tsuchiyu, Fukushima).

main, and N- and C-terminal regions, respectively. We are ready for spatiotemporal analyses of *Dll* gene expression and Dll protein distribution during embryonic appendicular development.

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