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Does *abd-A* Suppress Proleg Development in the Silkworm, *Bombyx mori*?*

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The basic body plan of insects comprises six thoracic legs, four wings and an abdomen lacking appendages. The underlying molecular mechanism of the evolution of limb-less abdomen of insects is believed to be the acquisition of the limb-suppressing function of *Ultrabithorax* (*Ubx*) and *abdominal-A* (*abd-A*) which are expressed in the abdominal segments (Vachon *et al.*, 1992; Palopoli and Patel, 1998). The larvae of the order Lepidoptera and suborder Symphyta of Hymenoptera often bare abdominal appendages called prolegs. In lepidopteran larvae, prolegs typically occur on the four abdominal segments from the third to sixth. Warren *et al*. (1994) suggested that circular clearings of *abd-A* expression at the tip of the proleg primordia during embryogenesis of a butterfly lead to the permissive expression of *Distal-less* (*Dll*) which results in the development of prolegs on the segments in question.

Since a large number of mutant stocks are available, the silkworm, *Bombyx mori* has long been used as an experimental animal for developmental genetics (Tazima, 1964). There are at least 30 different mutants that belong to pseudoallele group *E* (Ueno *et al*., 1995), which covers the genomic region of bithorax complex (Yasukochi *et al*., 2004) and many of the mutations in this region show anomalies in proleg development. Using one of such mutant lines, E^{Mu}, we have shown that proleg development occurs in segments where *abd-A* expresses and *Abdominal-B* (*Abd-B*) does not express. Furthermore, embryonic RNAi experiments strongly suggested that *Abd-B* is a proleg suppressor in the posterior abdomen of *Bombyx* (Tomita and Kikuchi, 2009).

The knockdown of *abd-A* resulted in the total loss of prolegs. In the same line E^{Ca} mutant that lacks genomic *abd-A* region also shows similar phenotype (Ueno *et al.*, 1992). These results strongly suggest that *abd-A* plays a maintenance, rather than a suppressive role in proleg development in *Bombyx*. To further investigate the role of *abd-A*, we injected both dsRNAs for *abd-A* and *Abd-B* to knock-down both genes simultaneously. The double-knockdown embryos showed almost the same phenotype to the *abd-A* depleted embryos. These embryos lost their prolegs and small remnants of leg primordia are often observed in A1–10. Since these remnants are never found in A8–10 of *abd-A* RNAi embryos, these additional occurrences may be due to the lack of *Abd-B* in these segments. This result suggests that the extra prolegs observed in A7–9 in E^{Mu} and A7–10 in *Abd-B* RNAi embryos developed by the ectopic *abd-A* expression in the relevant segments under the mutant background or RNAi effect, that supports the hypothesis that *abd-A* promotes and is indispensable to the proper proleg development.

We investigated detailed expression pattern of Abd-A protein at and around the proleg primordia by immunocytochemistry. Through stages 18–22 proleg primordia appear and prune in the segments that lack prolegs in larval stages. In the proleg baring segments, leg primordia are established and begin outgrowth. During this period there were basically no detectable differences in Abd-A expression between proleg baring (A3, A4) and proleg lacking (A2) segments. Abd-A expression was always present in the cells at the very tip of the primordia and no clearance of the expression was observed. The expression of *Dll* at stage 19 was assessed by *in situ* hybridization and weak but distinct expression was detected at the tips of the proleg primordia.

These results raise the possibility that *abd-A* does not suppress *Dll* expression and these two genes cooperate together in proleg development in *Bombyx* embryo. The abdominal segments expressing *Ubx* (A1 and A2) or *Abd-B* (A7–10) do not develop prolegs and only the segments in between have prolegs. E^{N} mutant which lacks both *Ubx* and *abd-A* genomic region develops thoracic leg-like appendages in A1–6 (Ueno *et al*., 1992) suggesting *abd-A* specifies the appendages as prolegs and plays instructive role in proleg development. This may provoke the next question: when and how this regulatory system evolved? Since caterpillars lose prolegs during pupal metamorphosis, the basic body plan, limb-less abdomen, is conserved in Lepidoptera.

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Thus the molecular mechanisms underlying the development of prolegs should be small modifications on the limb-suppressing system. Considering the fact that the proleg development in sawfly does not depend on *Dll* expression (Yamamoto *et al*., 2004) and hemimetabolous insects don't develop prolegs, this hypothetical modification should be specific to lepidopteran linage.

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