17. Sigogneau-Russell, D., Découverte du premier mammifère tribosphénique du Mésozoïque africain. *C. R. Acad. Sci. Paris*, 1991, **313**, 1635–1640.
18. Luo, Z.-X., Cifelli, R. L. and Kielan-Jaworowska, Z., Dual origin of tribosphenic mammals. *Nature*, 2001, **409**, 53–57.
19. Besse, J. and Courtillot, V., Paleogeographic maps of the continents bordering the Indian Ocean since the Early Jurassic. *J. Geophys. Res.*, 1988, **93**, 791–807.
20. Sahni, A., Kumar, K., Hartenberger, J.-L., Jaeger, J.-J. Rage, J.-C., Sudre, J. and Vianey-Liaud, M., Microvertébrés nouveaux des Trapps du Deccan Inde: mise en évidence d'une voie de communication terrestre probable entre la Laurasie et l'Inde à la limite Crétacé-Tertiaire. *Bull. Soc. Geol, Fr.*, 1982, **14**, 1093–1099.
21. Prasad, G. V. R. and Rage, J.-C., A discoglossid frog in the Late Cretaceous (Maastrichtian) of India. Further, evidence for a terrestrial route between India and Laurasia by the latest Cretaceous. *C. R. Acad. Sci. Paris*, 1991, **313**, 273–278.
22. Prasad, G. V. R. and Rage, J.-C., Amphibians and squamates from the Maastrichtian of Naskal (India). *Cretaceous Res.*, 1995, **16**, 95–107.
23. Thewissen, J. G. M. and McKenna, M. C., Paleobiogeography of Indo-Pakistan: a response to Briggs, Patterson, and Owen. *Syst. Biol.*, 1992, **41**, 248–251.
24. McKenna, M. C., The mobile Indian raft: A reply to Rage and Jaeger. *Syst. Biol.*, 1995, **44**, 265–271.
25. Jaeger, J.-J., Courtillot, V. and Tapponnier, P., Paleontological view of the ages of the Deccan Traps, the Cretaceous/Tertiary boundary, and the India/Asia collision. *Geology*, 1989, **17**, 316–319.
26. Rage, J.-C., Le peuplement animal de Madagascar: Une composante venue de Laurasie est-elle envisageable? *Biogéogr. Madagascar*, 1996, 27–35.
27. Sahni, A., Rana, R. S. and Prasad, G. V. R., New evidence for palaeobiogeographic intercontinental Gondwana relationships based on Late Cretaceous–Earliest Palaeocene coastal fauna from peninsular India. *Gondwana Six, Geophys. Monogr.*, 1987, **41**, 207–218.
28. Sahni, A. and Bajpai, S., Eurasiatic elements in the Upper Cretaceous nonmarine biotas of peninsular India. *Cretaceous Res.*, 1991, **12**, 177–183.
29. Cooper, A., Lalueza-Fox, C., Anderson, S., Rambaut, A., Austin, J. and Ward, R., Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature*, 2001, **409**, 704–707.
30. Macey, J. R. *et al.*, Evaluating trans-Tethys migration: an example using acrodont lizard phylogenetics. *Syst. Biol.*, 2000, **49**, 233–256.
31. Bossuyt, F. and Milinkovitch, M. C., Amphibians as indicators of early Tertiary 'out of India' dispersal of vertebrates. *Science*, 2001, **292**, 93–95.
32. Murphy, W. J., Eizirik, E., Johnson, W. E., Zhang, Y. P., Ryder, O. A. and O'Brien, S. J., Molecular phylogenetics and the origin of placental mammals. *Nature*, 2002, **409**, 614–618.

ACKNOWLEDGEMENTS. We thank the anonymous referee for his valuable suggestions on the manuscript. This work was supported by grants from the Department of Science and Technology and University Grants Commission to A.K. and G.V.R.P. respectively. O.V. thanks the Geological Society of India for L. Rama Rao Research Grant.

Received 5 December 2003; revised accepted 3 March 2004

# Developmental and hormonal regulation of actin and tubulin in the central nervous system of silkworm, *Bombyx mori* during postembryonic development†

**A. Shanavas[1], Abul Arif[2], Ch. R. K. Murthy[2] and Aparna Dutta-Gupta[2,*]**

[1]Dr Reddy's Research Foundation, Miyapur, Hyderabad 500 050, India
[2]Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India.

**We investigated changes in the synthesis and content of the cytoskeletal proteins to understand their role in ganglionic fusion and nerve-cord shortening during metamorphosis in insects. SDS–PAGE and [$^{35}$S]-methionine incorporation studies revealed high protein synthesis in the central nervous system (CNS) of *Bombyx mori*, during the late-last instar larval and late-pupal stages of development, and actin and tubulin were the two major proteins synthesized. Western analysis revealed high β-tubulin in CNS during the larval stages. It declined at the pupal stage, which might be due to the resorption of the interganglionic connectives. A specific β-tubulin protein band was expressed during the pupal stages, when endogenous 20-hydroxyecdysone (20E) titre was reported to be high. Based on the similarity to an earlier report on the developmental expression of tubulin in *Drosophila*, we speculate this as a β3 isoform of β-tubulin. The increased accumulation of actin in the CNS during pupal stages suggests an active role for microfilaments in nerve-cord shortening. Synthesis of actin as well as tubulin was stimulated by 20E.**

HOLOMETABOLOUS insect metamorphosis is accompanied with neurogenesis, programmed cell death and reorganization of larval neurons to perform new functions in the adult central nervous system (CNS). During the transformation of larval CNS to that of adult, drastic reduction occurs in the length of the nerve cord and in the number of ganglia[1,2]. Active participation of the cytoskeletal components in cellular movements, extension of neurites, coiling or looping of axons, and resorption of axonic material in the CNS has been reported[3,4]. However, there is hardly any information on the role of cytoskeletal elements in the ganglionic fusion and nerve-cord shortening process during metamorphosis.

The mechanical explanation for changes in cell shape and motility during metamorphosis in insects lies in the assembly and movement of the cytoskeleton component.

*For correspondence. (e-mail: apdgsl@uohyd.ernet.in)
†A. S., A. A. and A. D. G. dedicate the paper to Ch. R. K. M., who passed away recently.

Two major structural elements involved in these functions are tubulin-containing microtubules and actin-containing microfilaments. Tubulin is one of the most widespread classes of multiple family proteins, the polymer consists of heterodimer of α and β-tubulins and has been widely characterzied from several species[5]. In the nervous system, microtubules are abundant and form the major structural component of axons[6]. On the other hand, actin is a highly conserved protein, representing a class of ubiquitous gene families. In non-muscle cells, it is a major component of microfilaments, which are implicated in cytoskeletal morphology, cell motility and phagocytosis[7].

The growth of the nervous system and organization of its synaptic field in insects occur mainly during the postembryonic development, which consists of growth punctuated by a series of moults followed by metamorphosis[1,8] that are initiated and coordinated by morphogenetic hormones, ecdysone and juvenile hormones[8,9]. Previous studies demonstrated the hormonal regulation of ganglionic migration in wax moth *Galleria mellonela*, and 20E was more pronounced in its effect on nerve-cord shortening compared to α-ecdysone[10]. 20-Hydroxyecdysone has also been demonstrated to initiate ganglionic migration, which resulted in fusion in *Manduca sexta*[11]. The present study focuses on the changes in the content and synthesis of actin and tubulin in the CNS of *Bombyx mori* during postembryonic development. We also studied the hormonal regulation of expression of these proteins.

In the present study, third-instar larvae of silkworm, *B. mori* (pure Mysore strain, India) were obtained from local breeding centre and reared in an insect culture room at 26 ± 1°C, 70 ± 5% relative humidity and 14 : 10 h light-dark period on fresh mulberry leaves. Staging of insects was based on their age after the fourth ecdysis[12]. One to two-day-old last-instar larvae were designated as early-last instar (ELI), 5 to 6-day-old as mid-last instar (MLI), 9 to 10-day-old as late-last instar (LLI). Larvae collected after spinning were designated as prepupa (PP), 1-day-old prepupa as early-prepupa (EPP), 2-day-old as mid-prepupa (MPP), 3-day-old as late-prepupa (LPP), 4 to 6-h-old pupa as white pupa (WP), 1 to 2-day-old pupa as early-pupa (EP), 4 to 5-day-old as mid-pupa (MP) and 9 to 10-day-old as late-pupa (LP) and freshly emerged moths (< 12 h old) as adult (A).

For morphometric studies, to determine the changes in the length of the nervous system, CNS from insects of different stages was dissected and placed on a glass microslide carefully, to prevent the stretching of ventral nerve cord. The length of CNS was measured under a binocular macroscope using an ocular micrometer, calibrated with a stage micrometer.

The tissue samples were prepared by rapidly dissecting intact CNS (brain + ventral nerve cord) in ice-cold insect Ringer (130 mM NaCl, 5 mM KCl, 0.1 mM $CaCl_2$) containing 1 mM phenylmethylsulphonylfluoride (PMSF) and frozen in liquid nitrogen. The frozen tissue was homogenized (4 CNS/50 µl) in 10 mM Tris buffer (pH 7.1), containing 0.1% Triton X-100 and 1 mM PMSF, using all-glass microhomogeniser (Kontes) and centrifuged at 1000 *g* for 5 min at 4°C to remove large debris. An aliquot of the supernatant was used for protein estimation[13]. To resolve CNS proteins, electrophoresis was carried out using 10% Tris-glycine SDS–PAGE[14] and proteins were visualized by silver-staining[15]. For Western blotting, the resolved proteins were transferred to nitrocellulose membrane[16] and immunodetection was carried out using rat monoclonal antibody to actin and β-tubulin (Boehringer Mannheim). The crossreactivity of these antibodies was detected using anti-rat alkaline phosphatase conjugated secondary antibody followed by processing with substrates of alkaline phosphatase (nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate). The mass of polypeptides was determined by co-electrophoresing protein markers followed by analysis on UVP gel documentation system. The quantitation of immunoblots was done with a computerized laser-scanning densitometer using Image-Quant software.

For *in vitro* studies on protein synthesis, intact CNS was dissected under aseptic conditions from different developmental stages of insects. The tissues were cultured in TC-100 insect culture medium (2 CNS/100 µl) for 12 h in the presence of 20 µCi of [$^{35}$S]-methionine (1000 Ci/mmol from BRIT, Mumbai) at 25°C under sterile conditions. For studying the effect of hormones on protein synthesis, the dissected CNS was incubated in TC-100 culture medium (JRH Biosciences, Inc., USA) initially for 4 h to deplete the endogenous hormones[17] and treated either with JH I ($7 \times 10^{-7}$ M) or with 20E ($5 \times 10^{-6}$ M) for four hours[18,19]. Subsequently, [$^{35}$S]-methionine (20 µCi) was added and incubation was carried out for another 4 h. At the end of the incubation period, tissue was removed, rinsed extensively and homogenized as mentioned earlier. Radioactivity in the samples was quantified after TCA precipitation, and fluorography[20] of the electrophoretically separated proteins using 2,5-diphenyloxazole in dimethylsuphoxide was carried out for the detection of radiolabelled polypeptides.

The statistical analysis was performed by one-way ANOVA followed by comparisons of means by Student–Newman–Keuls multiple comparison test using the Sigma Stat software (Jandel Corporation). $P < 0.05$ was defined as the criterion for statistical significance. The data are represented as mean ± SD.

Morphometric studies (Figure 1) revealed that the length of CNS increased gradually during larval development and reached to its maximum in the LLI larvae and remained more or less the same in EP. Thereafter, it declined gradually through the PP and pupal stages and reached its minimum in 10–12-h-old adult. However, the process of shortening was more pronounced during the PP stage. Protein content of the nervous system was found to be low in the ELI larvae, it increased gradually through

MLI, LLI larval, PP and pupal stages and reached a high value in LP and remained more or less the same in 10–12-h-old adult (Figure 2). Studies with [$^{35}$S]-methionine revealed fairly high protein synthesis in the CNS of MLI larvae among the larval stages and in LP during the pupal stage (Figure 3 *a*). Adult CNS showed the lowest rate of protein synthesis among all the stages used in the present study (Figure 3 *a*). Fluorographic analysis also indicated high degree of [$^{35}$S]-methionine incorporation into the CNS proteins during MLI larval and LP stages (Figure 3 *b*), especially into two major polypeptides of 45 kDa and 55 kDa.

SDS–PAGE analysis of the CNS proteins from various developmental stages revealed a quantitative change in the 45 kDa actin protein (Figure 4 *a*). This was further confirmed with immunoblotting studies using monoclonal actin antibody (Figure 4 *b*). Quantitative analysis of the immunoblot revealed that the actin content in CNS remained more or less the same during the larval stage, but it significantly increased from EP and reached a peak value at adult stage (Figure 4 *c*).

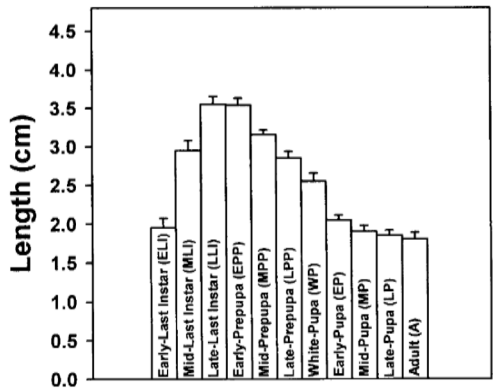


**Figure 1.** Changes in total length of CNS of *B. mori* during different stages of postembryonic development. Values are mean ± SD of 12 independent measurements.

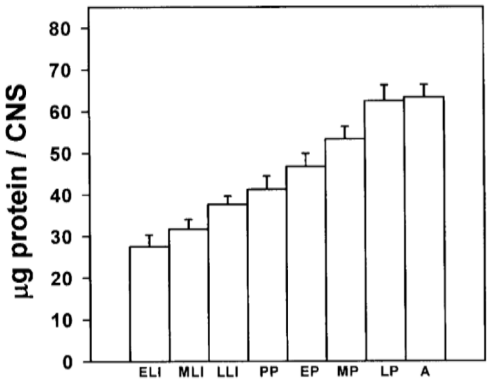


**Figure 2.** Changes in total protein content of *B. mori* CNS during various stages of postembryonic development. Values are mean ± SD of 10 independent determinations.

Studies on quantitative as well as qualitative changes in β-tubulin protein expression (Figure 5 *a–c*) showed the presence of large amount of β-tubulin in the CNS of ELI larvae (Figure 5 *b*, lane 1), which increased and reached its maximum in LLI larvae (Figure 5 *b*, lane 2). Thereafter, the content declined during PP (Figure 5 *b*, lane 3) and pupal stages of development (Figure 5 *b*, lanes 4–6). However, it increased once again and adult CNS showed a higher tubulin content (Figure 5 *b*, lane 7). An interesting observation was the expression of a new β-tubulin protein band migrating just above the major β-tubulin band in the samples from the pupal stages (Figure 5 *b*). The expression of this protein begins in the EP CNS (Figure 5 *b*, lane 4) and reaches a maximum in MP (Figure 5 *b*, lane 5) and then decreases in LP (Figure 5 *b*, lane 6). This protein was found to be absent in larval and adult stages.

The study to check the effect of morphogenetic hormones 20-hydroxyecdysone (20E) and juvenile hormone (JH) revealed the stimulatory role of 20E on the synthesis of cytoskeletal proteins actin and tubulin (Figure 6, lane 2). In addition, synthesis of a few other polypeptides was also stimulated by 20E treatment. Short-term JH treatment (8 h) in this experiment did not exert any significant effect on the synthesis of proteins (Figure 6, lane 3).

In the CNS of holometabolous insects, postembryonic development is characterized by profound increase in the size of ventral ganglia and length of interganglionic connectives during the larval stages. This corresponds to the increase in body length of the larvae. During metamorphosis, there is a reduction in the body length, which is accompanied by a reduction of interganglionic connectives followed by fusion of ganglia. Thus, there is reduction in the number of ganglia and length of the connectives between the ganglia undergoing fusion. This is well demonstrated in *G. mellonela*[10]. In *M. sexta*, ecdysteroid fluctuations have been demonstrated to regulate the ganglionic fusion during metamorphosis[11]. The present study also revealed an increase in the length of the nervous system at larval stage, which remained stable at EP and thereafter declined gradually through the PP and pupal stage and was minimal in 10–12-h-old adult. Our study shows that there is a gradual but continuous increase in the total protein content of CNS and high rate of protein synthesis in MLI larvae among the larval stages and in LP during the pupal period, with high degree of radiolabel incorporation into two major polypeptides with masses of 45 and 55 kDa. These polypeptides were identified as actin (45 kDa) and tubulin (55 kDa) using monoclonal antibodies. Interestingly, adult CNS showed the lowest rate of protein synthesis among the stages studied.

Expression of genes for cytoplasmic actin was shown to play an active role in cell motility, migration, neurogenesis, and axonal sprouting and outgrowth[7,21]. A precise developmental regulation of cytoplasmic actin gene expression in *B. mori* has been reported[22]. The genes are

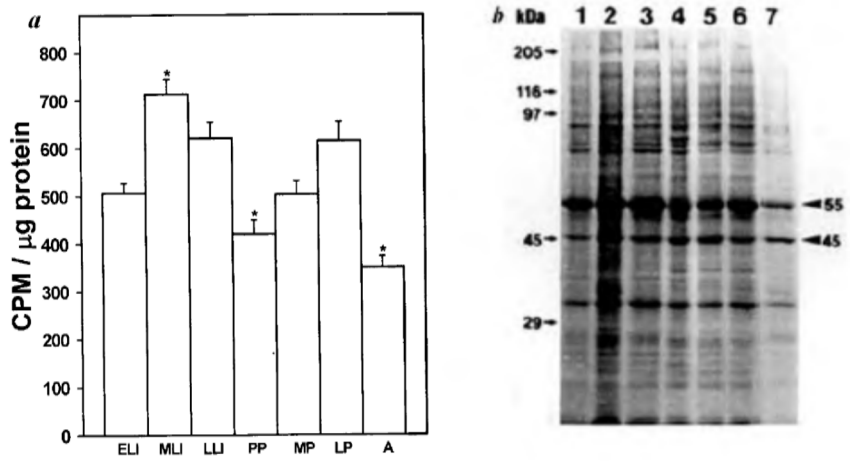


**Figure 3.** Changes in *in vitro* [$^{35}$S]-methionine labelled CNS polypeptides at different stages of insect development. Radiolabelling of intact CNS was done in TC-100 insect culture medium (2 CNS/100 µl) for 12 h in the presence of 20 µCi [$^{35}$S]-methionine at 25°C under sterile conditions. ***a***, Graphical representation as CPM/µg protein. ***b***, Fluorograph of [$^{35}$S]-methionine-labelled proteins. Lane 1, ELI; lane 2, MLI; lane 3, LLI; lane 4, PP; lane 5, MP; lane 6, LP and lane 7, A.*$P < 0.05$.

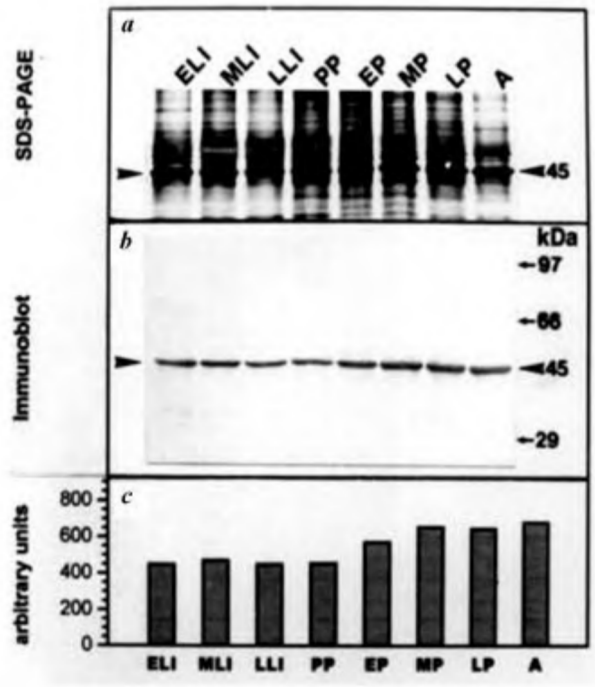


**Figure 4.** Developmental profile of actin (arrow head 45 kDa) in CNS of *B. mori*. (***a***) SDS–PAGE, (***b***) immunoblot probed with mouse monoclonal antibody to actin and (***c***) quantitative representation of data obtained by laser scanning densitometry of immunoblot. Ten µg protein was loaded in each lane.

principally expressed in embryo, silk gland and gut of larva and whole body of pupa. In the present study, a marked increase was observed in the β-actin content of CNS during pupal development compared to the larval stages; this might be due to the addition of microfilaments during the reorganization and development of the adult nervous system.

Microtubules are known to play an active role in the generation and orientation of the growing axons[4]. Reports also suggest that microtubule polymerization or extension alone can drive axonal growth, because neurites continued to grow in the presence of cytochalasin B, an actin-depolymerizing drug[23]. Further, a direct correlation was demonstrated between longitudinal growths of axons in developing neurons and that of high levels of tubulin expression[24]. In the light of these results, increase in β-tubulin content observed in the CNS of *B. mori* from ELI to LLI larval stage, can be correlated to the dramatic increase in the length of the interganglionic connectives during the last-instar larval growth. Similarly, decrease in β-tubulin content during pupation might be due to resorption of some of the connectives between the ventral ganglia undergoing fusion during this period. Based on earlier reports on the characterization of β-tubulin isoforms β1 and β3 and their pattern of expression in *Drosophila*, we suggest that the specific tubulin isoform detected in the pupal CNS of *B. mori* is probably a β3 tubulin[6,25]. Expression of this probable isoform began in the EP stage and reached a maximum in MP and declined in LP, before disappearing in the adult. A similar pattern in β3 tubulin content was earlier observed with the whole-body extract during pupal development in *Drosophila*[26]. Based on the β3-tubulin expression in *Drosophila*, Kimble *et al.*[25] proposed that this isoform may be important for the assembly and functioning of cytoplasmic microtubule arrays in cells undergoing rapid changes in shape and/or tissue organization. Specific expression of this isoform in the CNS of *B. mori* seen in the present study during pupation, a stage characterized by drastic reorganization and remodelling, lends support to this proposal.

Studies on *Drosophila* cell lines and tissues from other insects demonstrated that actin gene expression is regu-

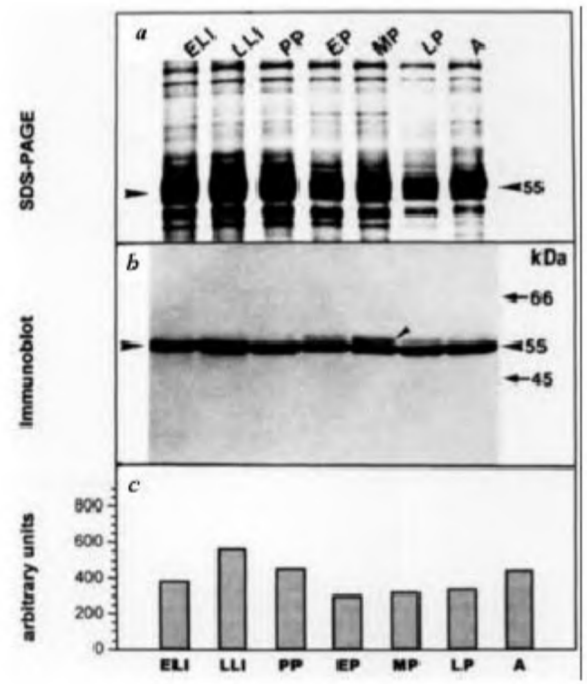


**Figure 5.** Developmental profile of β-tubulin (arrow head 55 kDa) in CNS of *B. mori*. (***a***) SDS–PAGE, (***b***) immunoblot probed with mouse monoclonal antibody to β-tubulin and (*c*) quantitative representation of data obtained by laser scanning densitometry of the immunoblot. Ten μg protein was loaded in each lane. Note the presence (arrow head) of a new isoform of tubulin in EP, MP and LP stages.

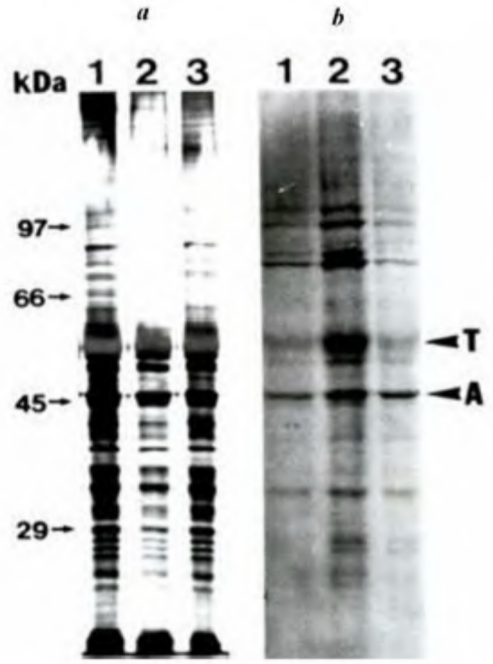


**Figure 6.** Effect of hormones on CNS protein synthesis *in vitro*. ***a***, SDS–PAGE; ***b***, Fluorograph. Lane 1, Control; lane 2, 20E treated and lane 3, JH-I treated. Arrowhead T indicates tubulin band and arrowhead A indicates actin band.

lated/modulated by the ecdysteroid[22,27]. When exposed to 20E, *Drosophila* cell cultures rapidly undergo cell-shape changes, acquire motility and aggregate. This 20E-induced morphological transformation was accompanied by a five-fold increase in rate of cytoplasmic actin synthesis and a two-fold increase in actin content[28]. Our observation of increased actin synthesis in the cultured CNS of *B. mori* in response to 20E treatment as well as the increase in actin content of CNS during pupal development, which is induced by the major reported pupal peak[19] of 20E, clearly suggests that cytoplasmic actin genes are transcriptionally regulated by ecdysteroids.

Studies on the hormonal regulation of β-tubulin synthesis in Kc cell lines indicated that β3 tubulin gene expression is regulated[26] by 20E. The present study with cultured CNS of *B. mori* also demonstrated a stimulatory role for 20E on tubulin synthesis. The developmental expression of β-tubulin and also of its probable isoform β3, observed in the present study, correlates with the reported high levels of 20E in the haemolymph of *B. mori*[19]. These observations suggest that the cytoskeletal components, i.e. tubulin and actin, probably play a role in the reorganization of CNS during the metamorphosis of insects.


1. Tissot, M. and Stocker, R. F., Metamorphosis in *Drosophila* and other insects: the fate of neurons throughout the stages. *Prog. Neurobiol.*, 2000, **62**, 89–111.
2. Truman, J. W., Metamorphosis of the insect nervous system. In *Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells* (eds Gilbert L. I., Tata, J. R. and Atkinson, B. G.), Academic Press, San Diego, 1996, pp. 283–320.
3. Carlier, M. F. and Pantaloni, D., Control of actin dynamics in cell motility. *J. Mol. Biol.*, 1997, **269**, 459–467.
4. Caroni, P., Intrinsic neuronal determinants that promote axonal sprouting and elongation. *Bioessays*, 1997, **19**, 767–775.
5. Kawasaki, H., Sugaya, K., Quan, G. X., Nohata, J. and Mita, K., Analysis of alpha and beta tubulin genes of *Bombyx mori* using an EST database. *Insect Biochem. Mol. Biol.*, 2003, **33**, 131–137.
6. Buttgereit, D., Leiss, D., Michiels, F. and Renkawitz-Pohl, R., During *Drosophila* embryogenesis the β1 tubulin gene is specifically expressed in the nervous system and apodemes. *Mech. Dev.*, 1991, **33**, 107–118.
7. Mounier, N. and Prudhomme, J. C., Differential expression of muscle and cytoplasmic actin genes during development of *Bombyx mori*. *Insect Biochem.*, 1991, **2**, 523–533.
8. Truman, J. W. and Riddiford, L. M., Endocrine insights into the evolution of metamorphosis in insects. *Annu. Rev. Entomol.*, 2002, **47**, 467–500.
9. Truman, J. W., Hormonal approaches for studying nervous system development in insects. *Adv. Insect. Physiol.*, 1988, **21**, 1–35.
10. Pipa, R. L., Patterns of neural reorganization during the postembryonic development of insects. *Int. Rev. Cytol. Suppl.*, 1978, **7**, 403–438.
11. Amos, T. M., Gelman, D. B. and Mesce, K. A., Steroid hormone fluctuations regulate ganglionic fusion during metamorphosis of the moth *Manduca sexta*. *J. Insect Physiol.*, 1996, **42**, 579–591.
12. Shanavas, A., Dutta-Gupta, A. and Murthy, Ch. R. K., Identification, characterization, immunocytochemical localization and developmental changes in activity of $Ca^{2+}$/calmodulin dependent protein kinase II in the CNS of silkworm *Bombyx mori*. *J. Neurochem.*, 1998, **70**, 1644–1651.

13. Bradford, M., A rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. *Anal. Biochem.*, 1976, **72**, 248–254.
14. Laemmli, U. K., Cleavage of structural protein during the assembly of the head of bacteriophage $T_4$. *Nature*, 1970, **227**, 680–685.
15. Blum, H., Beier, H. and Gross, H. J., Improved silver staining of plant proteins, RNA, DNA in polyacrylamide gels. *Electrophoresis*, 1987, **8**, 93–99.
16. Towbin, H., Staehelin, T. and Gordon, J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets; procedure and some application. *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4350–4354.
17. Arif, A., Shanavas, A., Murthy, Ch. R. K. and Dutta-Gupta A., Juvenile hormone stimulated tyrosine kinase mediated protein phosphorylation in the CNS of the silkworm, *Bombyx mori*. *Arch. Insect Biochem. Physiol.*, 2002, **50**, 139–146.
18. Plantevin, G., Bosquet, G., Calvez, B. and Nardon, C., Relationships between juvenile hormone levels and the synthesis of major haemolymph proteins in *Bombyx mori* larvae. *Comp. Biochem. B, Physiol.*, 1987, **86**, 501–507.
19. Calvez, B., Him, M. and De Reggi, M., Ecdysone changes in the haemolymph of two silkworms (*Bombyx mori* and *Philosamia cynthia*) during larval and pupal development. *FEBS. Lett.*, 1976, **71**, 57–61.
20. Bonner, W. M. and Laskey, R. A., A film detection method for the tritium labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.*, 1974, **46**, 83–88.
21. Mogilner, A. and Oster, G., Cell motility driven by actin polymerization. *Biophys. J.*, 1996, **71**, 3030–3045.
22. Couderc, J. L., Hilad, L., Sobrier, M. L. and Datugue, B., 20-Hydroxyecdysone regulates cytoplasmic actin gene expression in *Drosophila* cultured cells. *Nucleic Acids Res.*, 1987, **15**, 2549–2561.
23. Marsh, L. and Letourneau, P. C., Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B., *J. Cell Biol.*, 1984, **99**, 2041–2047.
24. Hoffman, P. N., Distinct roles of neurofilament and tubulin gene expression in axonal growth. *Ciba Found. Symp.*, 1988, **138**, 192–204.
25. Kimble, M., Incardona, J. P. and Raff, E. C., A variant of β-tubulin isoform of *Drosophila melanogaster* (β3) is expressed primarily in tissues of mesodermal origin in embryos and pupae and is utilized in populations of transient microtubules. *Dev. Biol.*, 1989, **131**, 415–429.
26. Chapel, S., Sobrier, M. L., Montpied, P., Micard, D., Bruhat, A., Couderc, J. L. and Dastugue, B., In *Drosophila* Kc cells 20-OHE induction of 60 C β3 tubulin gene expression is a primary transcriptional event. *Insect Mol. Biol.*, 1993, **2**, 39–48.
27. Shanavas, A., Nayak, B. P. and Dutta-Gupta, A., Ecdysteroid mediated muscle actin synthesis during the larval development of rice moth, *Corcyra cephalonica*. *Biochem. Mol. Biol. Int.*, 1996, **40**, 955–963.
28. Vitek, M. P., Morganelli, C. M. and Berger, E. M., Stimulation of cytoplasmic actin gene transcription and translation in cultured *Drosophila* cells by ecdysterone. *J. Biol. Chem.*, 1984, **259**, 1738–1743.

ACKNOWLEDGEMENTS. Financial support from the Council of Scientific and Industrial Research, New Delhi is acknowledged. A.A. thanks University Grant Commission, New Delhi for financial support through a direct fellowship.

Received 12 November 2003; revised accepted 15 March 2004

# *Gegeneophis nadkarnii* – a caecilian (Amphibia: Gymnophiona: Caeciliidae) from Bondla Wildlife Sanctuary, Western Ghats

**Gopalakrishna Bhatta[1,*] and P. Prashanth[2]**

[1]Department of Zoology, Bhandarkars' College, Kundapura 576 201, India
[2]Sri J.C.B.M. College, Sringeri 577 139, India

***Gegeneophis nadkarnii* is described as a species of Caeciliidae (Amphibia: Gymnophiona) based on two specimens collected from Bondla Wildlife Sanctuary, Goa, India. This species is differentiated from all *Gegeneophis* except *G. danieli*, in having many secondary annuli that begin on the anterior of the body. *G. nadkarnii* differs from *G. danieli* in having substantially fewer teeth.**

UNTIL 1999, the endemic Indian caeciliids were represented by five species belonging to two genera, viz. *Gegeneophis* Peters and *Indotyphlus* Taylor. These five species are *G. carnosus*, *G. fulleri*, *G. krishni*, *G. ramaswamii* and *I. battersbyi*[1]. Recently two more species from India were added to the genus *Gegeneophis*. Ravichandran *et al.*[2] reported *Gegeneophis seshachari* from Ratnagiri District, central western Maharastra and Giri *et al.*[3] reported *Gegeneophis danieli* from Sindhudurg District, southern Maharastra. In September 2001 we had collected two specimens resembling each other from Goa, India which fit into the generic diagnosis given by Taylor[4] and Ravichandran *et al.*[2] for *Gegeneophis*, but differ from all known species of the genus according to the recently developed key to the species of *Gegeneophis* by Giri *et al.*[3]. Here we describe this form (*G. nadkarnii* sp. nov.) that differs from all other species of the genus *Gegeneophis* except *G. danieli*, as having many secondary annuli that begin on anterior of the body. The species differs from *G. danieli* in having substantially fewer teeth.

*G. nadkarnii* sp. nov. is described as follows:

Holotype: Zoological Survey of India, Calicut, India (ZSI, CLT. No. V/A-573). A mature male, collected in Bondla Wildlife Sanctuary, Goa in September 2001, while conducting field-work.

Paratype: Bombay Natural History Society, Mumbai, India (BNHS) 4234. A mature male. Other data same as holotype.

This is a *Gegeneophis* differing from all other species of the genus except *G. danieli*, in having many secondary annuli (around 80, of which nearly 70 are complete middorsally) that appear on anterior part of the body. The anterior-most primary annuli (around 40) appear incomplete middorsally.

*For correspondence. (e-mail: gkb@sancharnet.in)