Evolution of the Cephalopod Head Complex by Assembly of Multiple Molluscan Body Parts: Evidence From Nautilus Embryonic Development

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ABSTRACT Cephalopod head parts are among the most complex occurring in all invertebrates. Hypotheses for the evolutionary process require a drastic body-plan transition in relation to the life-style changes from bentho to active nekton. Determining these transitions, however, has been elusive because of scarcity of fossil records of soft tissues and lack of some of the early developmental stages of the basal species. Here we report the first embryological evidence in the nautiloid cephalopod Nautilus pompilius for the morphological development of the head complex by a unique assembly of multiple archetypical molluscan body parts. Using a specialized aquarium system, we successfully obtained a series of developmental stages that enabled us to test previous controversial scenarios. Our results demonstrate that the embryonic organs exhibit body plans that are primarily bilateral and antero-posteriorly elongated at stereotyped positions. The distinct cephalic compartment, foot, brain cords, mantle, and shell resemble the body plans of monoplacophorans and basal gastropods. The numerous digital tentacles of Nautilus develop from simple serial and spatially-patterned bud-like anlagen along the anterior–posterior axis, indicating that origins of digital tentacles or arms of all other cephalopods develop not from the head but from the foot. In middle and late embryos, the primary body plans largely change to those of juveniles or adults, and finally form a “head” complex assembled by anlagen of the foot, cephalic hood, collar, hyponome (funnel), and the foot-derived epidermal covers. We suggest that extensions of the collar-funnel compartment and free epidermal folds derived from multiple topological foot regions may play an important role in forming the head complex, which is thought to be an important feature during the body plan transition.

KEY WORDS: evolution; homology; squid; brain; transcription factor; engrailed

Cephalopods (nautiluses, squids, cuttlefishes, and octopods) are highly derived animals capable of fascinating behaviors, such as floating, crawling, or fast swimming with multistep hunting, defense, and cognitive performance (Wells, 1978; Hanlon and Messenger, 1996; Nixon and Young, 2003). These animals generally have an elaborate head composed of numerous long tentacles or arms, cup-shaped eyes, statocysts, rhinophore or olfactory organs, the centralized brain, attached collar, and flexible hyponome or funnel (Budelmann et al., 1997) (Fig. 1). Evolution of these structures is presumed to be a pivotal event for the successful adaptation of cephalopods in the ocean during the Late Cambrian, ~505 million years ago (Holland, 1987; Saunders and Landman, 1987; Young et al., 1998). In spite of interest in the origin and evolution of the “head” parts of other bilaterian groups, particularly in arthropods (e.g., Budd, 2002; Angelini and Kaufman, 2005; Carrol et al., 2005; Maxmen et al., 2005) and vertebrates (e.g., Holland, 2000, 2003; Puelles and Rubenstein, 2003; Kuratani, 2005), how the cephalopod head and associated structures evolved remains largely uncertain (Packard, 1966, 1972; O’Dor and Webber, 1986).

Evolutionary hypotheses for cephalopod body plans have been proposed on the basis of combined...
neotological and paleontological observations. From fossil records, cephalopods have been hypothesized to have originated from a cup-shaped monoplacophoran-type ancestor (Lemche and Wingstrand, 1959; Wingstrand, 1985; but see Girybet et al., 2006 for a controversy) through intermediate forms, with a dorsoventrally elongate, multisepate, and symmetrical shell (Yochelson et al., 1973; Holland, 1987). To date, there are no direct anatomical observations from fossils concerning the origin of soft-body structures except the muscle scar on the shell. The origin of cephalopod cephalic appendages (digital tentacles in nautiluses or arms and tentacles in coleoids) has been differently interpreted either as “head” or as “foot.” On the basis of innervation patterns and muscle scars on the shell, the cephalopod arms are hypothesized to have derived from the ancestral head with a part of the anterior foot, whereas the funnel has been derived from the foot of the primitive mollusces (the arms as head hypothesis (Yochelson et al., 1973; Salvini-Plawen, 1980; Holland, 1987; see also Clarkson, 1998; Lee et al., 2003)). In contrast, previous embryological research in coleoids has suggested that cephalopod arms appeared from the foot (the arms as foot hypothesis (Naef, 1928; Bandel and Boletzky, 1988; Boletzky, 2003)). The posterior foot became the funnel/hyponome (Bandel and Boletzky, 1988; Boletzky, 1989) or the funnel was derived as a dorsal extension of posterior arm rudiments (Boletzky, 2003).

The morphological analysis of Recent Nautilus embryos offers a rare opportunity to discuss the origin of cephalopod body plans (Arnold, 1987; Landman et al., 1989; Jacobs and Landman, 1993; Tanabe and Uchiyama, 1997). Five living embryos were reported by Arnold and Carlson (1986), two in the blastoderm stage, one in the middle organogenetic stage, and two had shells and visceral mass with largely developed circulatory systems. The analysis of these embryos indicated that the embryonic development of Nautilus is characterized by a large amount of yolk, meroblastic blastoderm, possible epibolic gastrulation, and direct development without typical molluscan larval stages, as had been reported in coleoid cephalopods (Naef, 1928; Boletzky, 2003). However, the earliest stages, necessary to infer cephalopod ancestors, are entirely unknown to date, and no details thus far have been examined in Nautilus for the organogenesis of soft parts based on successive developmental stages (Arnold and Carlson, 1986; Arnold, 1987).

Gene expression studies, such as Hox genes in a squid Euprymna scolopes (Lee et al., 2003) with comparison to those of a basal gastropod, such as Haliotis asinina (Hinman et al., 2003), have provided new insights into the evolution of molluscan heads, arms, and visceral parts. However, the primitive condition necessary to infer the link between a molluscan ancestor and primitive cephalopods remains largely uncertain, because of the difficulty of interpreting the multiple cooption of genes in many tissues in the sepiolid squid (Lee et al., 2003). Also, some Hox genes break the usual anterior–posterior colinearity of expression as reported in arthropod body plans (Angelini and Kaufman, 2005; Carrol et al., 2005).

In this study, we describe possible primitive features in embryonic stages of Nautilus pompilius to propose an alternative scheme for the evolution of cephalopod body plans. This article concentrates on the comparative morphology of the cephalopod head parts; their cleavage patterns, gastrulation, and histological details in each organ are not analyzed. The homologies for cephalopod arms and resulting implications for developmental variation are discussed. We also present the first whole-mount immunocytochemical documentation, using cross hybridized antibodies against transcription factors in Nautilus and Idiosepius embryos.

MATERIALS AND METHODS

Animals

The embryos of Nautilus pompilius Linné, 1758 were obtained from captive females that were purchased commercially from Philippine coastal waters near Bohol Island. For comparison, the embryos of the squid Idiosepius paradoxus

Fig. 1. Comparative scheme of the body plans in nautiluses and coleoids (e.g., squid), lateral view. The head complex is distinct from the visceral mass and mantle particularly in squid. These schemes were described by the physiological orientation defined by Hoyle (1886). Anterior is toward the left of panel.
(Ortmann, 1888) were collected as described in Yamamoto et al. (2003) near the Ushimado Marine Laboratory, which is located on the coast of the Seto Inland Sea of Japan. This squid was used as a representative of coleoids as it develops with a mode typical in other coleoids except for a marked retardation of tentacle formation (see Fig. 5e–g). The *Idiosepius* embryos examined were assigned to developmental stages according to Yamamoto (1988).

Aquarium System

Immature males and females of *Nautilus* were maintained in a closed aquarium system (3.8 m width/1.6 m length/1.3 m depth, at 21–22°C) at the Toba Aquarium, Mie prefecture, Japan. For long-term maintenance of specimens, a recycled seawater system with ultraviolet sterilizer (Hanovia) and automatic microceramic filter (Nihon Rosuiki Kogyo) for removal of particles larger than 0.4 μm was used. The health and growth of adults were carefully monitored. To avoid “black shell syndrome”, adhesive particles were carefully removed every week. This aquarium has succeeded in hatching cephalopods by incubation of fertilized eggs (Uchiyama and Tanabe, 1999) since May 1995, and in March 2000, a third-generation was produced under artificial conditions for the first time (Moritaki et al., personal communication). Deposited eggs were transferred to warmer recycled seawater at 24–25°C in a small special incubation tank to avoid interference from adults.

One female usually spawned from 1 to 6 eggs per month. During 5 years beginning in 2000, 1,035 eggs were produced in the aquarium (Moritaki et al., personal communication). However only 81 embryos succeeded in hatching during the 5 years, because many eggs were infertile or developed as abnormal embryos. The abnormal embryos have generally opaque tissues and they could easily be removed. Embryos were identified as "normal" based on transparency of tissues, in ovo movement, pulsation of the embryonic circulatory organs, and bilateral symmetry of bodies without any indication of degeneration. In this study, we opened over 50 normal egg cases beginning from 1-month-old and fixed specimens at different embryonic stages for comparison with hatchlings (8–12-month-old) and adults (Table 1).

### Table 1. Collected samples of *Nautilus pompilius* in this study

<table>
<thead>
<tr>
<th>Age</th>
<th>Sample number</th>
<th>Figures</th>
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<tr>
<td>2 month</td>
<td>1*</td>
<td>Blastoderm stage</td>
<td></td>
</tr>
<tr>
<td>3 month</td>
<td>020401</td>
<td>Fig. 3a–c; Fig. 5a; Fig. 6</td>
<td>Distributed organ anlagen</td>
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<tr>
<td></td>
<td>020412</td>
<td>Fig. 8a–d; Fig. 9a–c</td>
<td>Slightly centralized organ anlagen</td>
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<tr>
<td></td>
<td>020326</td>
<td>Fig. 5b; Fig. 6</td>
<td>Differentiation of visceral mass</td>
</tr>
<tr>
<td></td>
<td>020328</td>
<td>Fig. 4a–c; Fig. 5c; Fig. 6; Fig. 7a</td>
<td>Eye thickening</td>
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<tr>
<td></td>
<td>020304a</td>
<td>Fig. 4d–f; Fig. 5d; Fig. 6; Fig. 7b,d</td>
<td>Large shell, hearts, viscera</td>
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<td>020304b</td>
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<td>010930</td>
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<td>Fig. 7f</td>
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<td>000211 (hatch001115)</td>
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*Samples were observed in a live condition and were not fixed.*

Scanning Electron Microscopy

Specimens were fixed according to standard methods (Wanneringer and Haszprunar, 2001). A solution of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) with 0.1 M NaCl and 0.4 M sucrose was used as the primary fixative. After extensive washes with the 0.1 M sodium cacodylate buffer, the specimens were postfixed with 1% OsO4, 1% K-ferrocyanide, 0.1 M sodium cacodylate buffer (pH 7.4) with 0.4 M NaCl for 1 h, followed by the sodium cacodylate buffer washes. Finally, samples were dehydrated in an acetone series, critical-point dried, sputter coated with gold, and observed with SEM (JEOL, JSM-100).

Whole-mount Immunocytochemistry

Following anesthesia in a 3% ethanol-seawater solution, specimens of *Nautilus* (>4-month-old) and *Idiosepius* were fixed with 4% paraformaldehyde in phosphate buffered saline (4% PFA/PBS, pH 7.5) at 4°C for 6 h, washed in PBS, dehydrated in a graded ethanol series (30, 50, 75% in PBS), and stored in 80% ethanol at −30°C. Some specimens were fixed sufficiently in relaxant solution (1% NH4O2, 2.25% formalin, 50 μM MgSO4) for 2 h at 4°C and were similarly stored.

To analyze external morphology, we dissected the embryonic body from the large outer yolk sac, then briefly soaked the
embryo in a fluorescent nuclear marker, 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma, 5 μg ml⁻¹ in PBS) for 30 min, washed in PBS, and observed using fluorescent microscopy under UV light. Whole-mount immunohistochemistry was completed according to a standard protocol as described by Shigeno and Yamamoto (2002) with some modifications. Fixed embryos were treated with 0.02 mg ml⁻¹ proteinase K (GibcoBRL) in PBST (PBS, 0.3% Triton X-100) for 15 min at 37°C, and postfixed in 4% PFA in PBST. After blocking for 1 h in PBST containing 0.25% BSA (PBSTB), samples were incubated in PBSTB overnight at room temperature with the primary antibody: rabbit polyclonal antibody against distalless homeodomain (Panganiban et al., 1997) diluted 1:2,000 (kindly provided by Dr. N. Niwa) or a monoclonal antibody (Mab4D9) diluted 1:5. This Mab4D9 was raised against a portion of Drosophila engrailed as the primary antibody (Patel et al., 1989) (obtained from Developmental Studies Hybridoma Bank, University of Iowa, IA) and has been shown to bind selectively to the engrailed protein in the major classes of molluscs (Wanninger and Haszprunar, 2001). We used only one Nautilus specimen (no. 020401) for the same immunocytochemical study. No control experiment was conducted to test the reactivity of these poly- and monoclonal antibodies against Nautilus. However, nonspecific staining of tissues in the embryos and hatchlings was not observed in the control experiments without primary antibodies for Idiosepius paradoxus (n = 10 for each antibody experiment, not shown).

For the secondary antibody, Alexa Fluor 488-conjugated anti-rabbit IgG antibody (Molecular Probes) was diluted 1:500 for anti-distalless antibody or Alkaline Phosphatase (AP)-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch) for Mab4D9 (1:500) for 12 h at room temperature. To detect the chromogenic AP substrates 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indoxyl phosphate, a standard method was used according to the manufacturers’ directions (Boehringer Mannheim Biochemicals). For counter staining on the fluorescent centrum, Hoechst No.33342 (Sigma, 5μg ml⁻¹ PBS) for nuclei or rhodamine-conjugated Concanaavalin A (ConA, Sigma, 10 μg ml⁻¹ PBS) for cell membranes was used to detect morphology of tissues. Samples were cleared in 50% glycerol/PBS on a poly-L-lysine coated chamber-slide and observed using a dissecting fluorescent microscope, and were photographed using a Nikon Coolpix 995 digital camera.

Image Processing

Embryos were examined as whole-mounts by LSM-510 confocal laser scanning microscopy (Carl Zeiss). Laser power was settled with UV 351/364 nm, Argon 458/488 nm, HeNe1 543 nm, HeNe2 633 nm and the appropriate filter set was selected according to fluorescent markers. Using the multitracking mode according to the manufacturer’s protocol, the multicolor staining was protected. Additional processing of images for contrast, brightness, and color balance was made as needed with Adobe Photoshop CS2 (Adobe Systems Incorporated, USA) or Paintshop Pro 7 (Jasc Software, USA). Schematic diagrams were created with Paintshop Pro 7 or Adobe Illustrator CS2 (Adobe Systems Incorporated, USA).

RESULTS

Body Plans of Nautilus Hatchlings

The hatchlings of Nautilus pompilius have taken on the general form, in miniature, of the juveniles or adults (Figs. 1 and 2a); therefore, major components of the head complex are easily identifiable in the hatching stage. The head complex consists of: (1) digital, precocular, postocular, and buccal tentacles. Similar to those of adults, tentacles of the hatchlings are lined with alternating grooves and ridges that allow them to grip objects; (2) the head part covered by a large pigmented and the shelf-like bulge of hood; (3) sensory organs, such as pinhole eyes and rhinophores; (4) the buccal mass, which is functional and feeding begins during 2 or 3 days after hatching (Moritaki et al., personal communication); and (5) a distinct “neck” part, including collar and hyponome (or funnel). The collar and funnel are continuous with each other and exhibit synchronous pulse movement (not shown). The orientation of cephalopod bodies has typically been defined as seen in the swimming posture of Nautilus (Fig. 2a), i.e., the funnel ventral, and the opposite side dorsal, the head anterior, the apex of the mantle posterior (Hoyle, 1886) (Fig. 1). Rather than this physiological orientation, the embryological orientation (Fioroni, 1978) is used in this study; arms ventral, the apex of the mantle dorsal, the funnel posterior, and the opposite side anterior (Fig. 2b).

Unique Topographic Patterns in the Early Organ Primordia

The earliest body plans appear in a series of morphogenetic events before the embryonic stage
attained at 3 month of age (cf. Fig. 3a–c). The embryonic bodies are regionalized as simple distinct sets of organ primordia that display a roughly concentric-circle geometric pattern with anterior–posterior and medio-lateral body axes (Fig. 3a). The shell field that will form the cicatrix (shell primordium, Arnold et al., 1987), and mantle are situated medially. One pair of gill buds is detectable at the posterior end in the mantle cavity (Fig. 3c). In the medio-lateral position, bilaterally symmetrical and serial structures are visible as the first sign of the hood, collar, and funnel, referred to here as the collar-funnel compartment. Serially repeated bud-like single lobed anlagen of digital tentacles (Fig. 3b) are situated laterally. They are divided into five compartments (I–V) composed of nine buds in total on each side (see later for the numbers and details). At the anterior most embryonic region, one large morphologically identifiable domain includes anlagen of the eyes, part of the hood, and the buccal mass (mouth). Here, we refer to this as the cephalic compartment (green color in Fig. 3a,b). In this stage, the hood and eyes form a continuous mass and the cephalic domain is distinct from other anlagen, such as tentacle buds and collar-funnel domain.

Ontogeny of Centralized Body Plan

**Three month-old.** The simple flat body plan in the previous period is now rather centralized along the anterior–posterior axis (Fig. 4a). In this period, the relative size of the mantle enlarges to cover most of the embryonic structures. The eye vesicles are distinct within the cephalic compartment, which is divided into the eye vesicles and two distinct bulges (arrowheads in Fig. 4b). In the collar-funnel compartment, a pair of funnel anlagen shifts relatively to posterior. The collar surrounds the visceral mass with the funnel. The anlagen of tentacles begin to differentiate into approximately twelve digital tentacles on each side (Fig. 4c). Part of the yolk mass shifts into the embryo to form an inner yolk sac. The visceral mass, two pairs of gills, and systemic and branchial hearts are visible on the inner yolk sac.

**Four month-old.** Three major changes are evident from the 3-month-old embryos. The first is the pronounced enlargement of the funnel and mantle in comparison to the eyes and digital tentacles (Fig. 4d,e). The mantle expands laterally and dorsoventrally to cover the eyes, digital tentacles, and other head structures (Fig. 4f). The second is that the three bulges of the head and eye are divided into more distinct regions (Fig. 4e). The eyes are still flattened and not ovoid. The third is completion of the hatchling-like funnel. Differentiation of the visceral mass, gills, and digital tentacles continues.

**Six month-old.** The body plan is largely similar to that of a hatchling (i.e., from 8–12-month-old, Fig. 2b; Table 1). However, the hood and digital tentacles are not formed completely. The inner yolk sac has swollen and the visceral mass is now restricted to the anterior dorsal part of the embryo (Fig. 4g). The cephalic compartment is relatively small (Fig. 4h) and parts of digital tentacles have enlarged (Fig. 4i). The preocular tentacle and putative rhinophore are evident as described later.
The eye vesicles become ovoid with the pinhole situated at the center of the eye (Fig. 4h).

Details of Formation of the Head Complex

Digital tentacles. Whole-mount embryos stained by DAPI revealed an unexpected developmental pattern of the digital tentacles. The origin of numerous digital tentacles in adults can be traced in part to the five distinct compartments (I–V) in 3-month-old embryos (Fig. 3b). Each compartment is composed of two bud-like anlagen (bud numbers 1–6, and 8, 9), except for compartment IV, which consists of only one bud (bud number 7). Compartments IV and V are formed as a continuous structure (Fig. 5a). During the development of compartments I–III and V, a pair of buds in one compartment presumably fuse into one tentacle in the late 3-month-old embryo (Fig. 5b–d; for summary see Fig. 6). We could not detect the serial and detailed processes of bud fusion because of the morphological gaps in our specimens (no. 020412 and no. 020326; Fig. 6); however, the fusion is suggested by the similar size of each bud, relative position, and bud numbers (Fig. 5c,d). These transient fused buds may be called "bipartite structure or arms" as reported in Sepia officinalis (Naef, 1928). In the late-3- and 4-month-old embryos, many buds as anlagen of digital tentacles rapidly appear at the posterior most compartments IV and V (Fig. 5d). The earlier differentiated buds seem to be relatively small and are situated on the anterior and ventro-medial sides as seen in 4-month-old embryos (Fig. 5d; see also Fig. 6).

To compare the tentacle/arm formation in Nautilus and coleoids, we describe the arm development of the coleoid Idiosepius paradoxus by SEM (Fig. 5e–g). The development of arms and the arm base (arm-derived covers for the eyes and head) in coleoids has been investigated previously (e.g., Naef, 1928; Arnold, 1984; Boletzky, 1993). As seen in Nautilus, the origins of arms are composed of compartments I–V, and IV + V form a continuous structure (Fig. 5f,g). Compartments I–III, and V each develop by fusion of two buds. The enlargement of compartments IV and V (arm bases) covers the ventral region of the eyes (Fig. 5g). Finally, the
arm-head or foot–head complex is formed (Yamamoto, 1988).

**Multiple origins of the hood complex.** Based on the developmental patterns in 3–8-month-old embryos, the hood is a complex derived from several embryonic origins (dh [dorsal hood] and hood compartment 1–3). As described earlier in the 3-month-old embryo, the collar-funnel compartment (initial origins of a part of the hood, as well as the collar, and the funnel) are situated at the lateral position of the mantle and central shell field (Fig. 3a). During embryogenesis, the most anterior parts of the collar anlagen enlarge ventrally (Fig. 7a,b; see dh, dorsal hood) and laterally (Fig. 7c), and finally form a dorsal part of the hood (dh, Fig. 7e–g) (for summary, see Fig. 7j). The other parts of the hood are derived from three transient regions (h1-3) as seen in the 4- and 6-month-old embryos (Fig. 7d–g). We have already described that a part (h2) is derived from a fusion of bud-like compartments I and II whereas compartments III–V develop into digital tentacles (Fig. 6). The distinct hood parts (dh, h1, and h2) finally form one large continuous plate-like hood (Fig. 7h) with a medial groove (Fig. 7i). The origin of the hood region h3 is not clear, but it may be derived from a part of anterior tentacle bud-like anlagen or from a distinct region of the cephalic compartment, because of the typical bud-like shape of h3 (Fig. 7d, j). In hatchlings, the region h3 remains distinct from other hood parts, and the hood-like digital tentacles appear as described in adults (Griffin, 1900) (Fig. 7h).

**Preocular, postocular, and buccal tentacles.** The developmental processes of preocular, postocular, and buccal tentacles are quite different from those of digital tentacles. Based on topographical relationships with eyes, the hood, and digital tentacles, the postocular tentacles appear from a cephalic compartment situated at the anterior mar-

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**Fig. 5.** Development of digital tentacles in *Nautilus pompilius* or arms in *Idiosepius paradoxus* embryos. (a, b) Early arrangement of compartment III (yellow) and IV + V (red) in 3-month-old *Nautilus* embryos (a, no. 020401; b, no. 020412). The two buds in compartment V fuse to one as a bipartite arm, whereas bud IV is still single. The arm base-like projection of thick tissue is present (an arrowhead in a). Asterisks indicate newly differentiated tentacle buds. (c, d) Digital tentacles in the 3-month- (no. 020326) and 4-month-old (no. 020304b) embryos viewed from the ventral aspect without outer yolk sac. Derivatives of the cephalic compartment, lateral compartments I–III, and IV + V are indicated by green, yellow, and red, respectively. The buccal tentacles (buc) appear in a 4-month-old embryo (blue in d). A large projection is present toward the anterior (see apr) to form a base of the medial side of the digital tentacles. Asterisks indicate small buds considered to be newly formed tentacles. The digital tentacles arranged with similar topological manner are indicated by dotted lines. apr, anterior projection. The identity of compartment III is unclear. Hoechst nuclear staining (a–d). (e–g) The scanning electron micrographs show the early arrangement of arms and arm bases in *I. paradoxus* (e, stage 20, lateral view) compared with 3-month-old embryos of *N. pompilius* (Fig. 3b). In *Idiosepius* (f, stage 21; g, stage 24, posterior views), the two buds of a bipartite arm (I–III, yellow and V, red) fuse into one arm anlage except for IV (red) in later stages. The arm bases begin to cover the eye region to form a head–foot complex (arrowheads, red). The developed cephalic bulges (ceb) are shown by closed dots lines (in e, the head compartment is shown by green). fu, funnel. Scale bars, 200 μm.

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**Fig. 6.** Diagrammatic views for the development of tentacle compartments from 3 to 4-month-old in *Nautilus pompilius*. The initial five compartments (composed of nine buds) develop into a part of the hood and numerous digital tentacles as shown by arrows. In later stages, the digital tentacles are arranged in a similar topology (dotted lines, see also Fig. 5). The newest tentacles are indicated by arrowheads. The anterior projection forms a basis for late tentacle buds.
gin of the eyes in the 4–6-month-old embryos (Fig. 7d,f). The origin of the preocular tentacles is unclear, but topographically, the prospective origin may be traced from a cephalic compartment situated ventral to the eyes in the 6-month-old embryos (see Fig. 7j). The buccal tentacles also appear at a later stage than the digital tentacles in the 4-month-old embryo (Fig. 5d). The first buds are initially restricted to the posterior of the body (Fig. 6) and later develop to anterior (figures not shown), and finally they surround the whole-mouth in adults (Griffin, 1900).

Rhinophores. The rhinophore, an olfactory organ of Nautilus, seems to develop from a part of distinct region of bulges at the posterior of the cephalic compartment (Fig. 7d–f; see the position of rhi). In the 4–6-month-old embryos, two bulges are visible at each side of the posterior part of each eye as the anlagen of rhinophores (Fig. 7d,e). However, more detailed histological analysis is required, since there is no early external morphological feature to trace origins of rhinophores.

The early tripartite organization of brain cords. Brain structure is one of the major compo-
nents to consider in cephalopod evolution (Young, 1977, 1988; Nixon and Young, 2003). The Nautilus brain exhibits a primitive nature composed of cerebral, pedal, and visceral cords surrounding the esophagus (Young, 1965). Here, we describe the characteristic early organization of the brain anlagen in one 3-month-old Nautilus embryo (Fig. 8a–d) and in squid (Idiosepius) embryos (Fig. 8e–h) using cross-hybridized anti-distalless antibodies (Dil) as a marker to detect a population of neural progenitor cells (Panganiban et al., 1997). This Dil protein is usually localized to developing appendages, but it is also useful to identify the basic organization of neural cell populations in various bilaterians (Panganiban et al., 1997). In embryonic Nautilus and Idiosepius, we identified a similar cord-like topographic organization of the early brains; the cerebral, pedal, and visceral/palliovisceral cords or ganglia (red color in Fig. 8i for summary). The Dil-positive cells are situated in the cephalic compartment in Nautilus (Fig. 8a), whereas the cells exhibit a continuous band-like feature as

Fig. 8. Shared cord-like features in the tripartite brain anlagen of Nautilus pompilius (a–d, 3-month-old) and a squid Idiosepius paradoxus (e–h, stage 20 or 22). Confocal sections using anti-distalless antibody (green) and cell membrane background staining (concanavalin A, red) reveal the distribution of neural progenitor cells. a, e: Eye region. b, f: Similar expression patterns are detected at interface of the optic lobes (op) with the retina (re). Asterisks in b and f indicate similar expression domains in the optic lobes. c, g: Cerebral cord (ce) and cord-like anlage of supraesophageal mass (cerebral ganglion, ce) on the mouth (mo). d, h: Visceral (pv) and pedal (pe) cord compared with squid cord-like palliovisceral (pv) and pedal (pe) ganglia. The pedal cord (pe) of Nautilus elongates more anterior-ventrally and only some cells are shown. i: Comparative scheme in the early embryonic bodies between the Nautilus and squid. Similar characters are represented by the same colors (e.g., the cephalic compartment and brain cords are shown by orange and red, respectively). Bipartite arms are indicated by dotted lines. ceb, cephalic bulge. Scale bars, a, d, e–g, 200 μm; b, c, h, 50 μm.
seen from the position of the eye to the putative cerebral ganglion in *Idiosepius* (Fig. 8e, see also 8i). In both species, strongly expressing cells are located in the boundaries between the retina and optic cord/ganglia, suggesting a similar histological domain is present in this region (Fig. 8b,f). The cerebral cord and ganglia form a cellular bridge above the mouth anlage (Fig. 8c,g). The visceral and pedal cords elongate along the anterior–posterior axis in *Nautilus* (Fig. 8d). A similar condition is identifiable in the palliovisceral and pedal ganglia in squids (Fig. 8h) as has been described previously (Yamamoto et al., 2003).

The 4D9/Engrailed Protein Expression

To identify molecularly distinct embryonic compartments in the head complex and head-related structures, we used an immunocytochemical marker 4D9, which detects a transcription factor, *engrailed* protein, known for a role in segmentation and neural development in a variety of groups, such as arthropods, annelids, and vertebrates (Patel et al., 1989). In non-cephalopod molluscs, the shared expression patterns have been described in various embryos (Moshel et al., 1998; Jacobs et al., 2000; Wanninger and Haszprunar, 2001; Nederbragt et al., 2002). In the 3-month-old embryonic *Nautilus*, 4D9-positive cells are detected in the cephalic compartment as a narrow band between the hood and eyes (see asterisks in Fig. 9a,c), most of digital tentacles except a part of compartment IV (Fig. 9a), an edge of the mantle, the hyponome/funnel, anterior hood-collar compartment, putative visceral cord, arm bases, a part of the gills (Fig. 9b), and an edge of the shell field (Fig. 9c, marked by arrowheads). In squid embryos, similar expression patterns are also distinguished (Fig. 9d–g): the marginal edge of the mantle, anterior funnel folds, a part of the collar, the gills, a medial region of posterior funnel folds, which are probably the funnel gland, and the putative palliovisceral ganglion (Fig. 9f).

In both *Nautilus* and *Idiosepius*, no positive cells are found in the anterior part of the cephalic compartment (cf. cc in Fig. 9c and cg in Fig. 9e). In these cephalopods, the digital tentacle/arm bases are commonly positive (Fig. 9a,g). On the other hand, in contrast to the uniform expression pattern in *Nautilus* digital tentacles, the expression is detected more intensely in arm II of the squid than in arms I and V. These patterns probably are not transient, because the expression in squid arms is constantly maintained to the middle embryonic stages (cf. Fig. 9d,g).

Fig. 9. Localized immunocytochemical expression of 4D9, a marker for *engrailed* protein. *Nautilus pompilius* (a–c, 3-month-old embryo, no. 020401) and a squid *Idiosepius paradoxus* (d–f, embryonic stage 20 and g, stage 23). (a) Lateral view; arm bases (arrowheads), a narrow part between head and eye (asterisk). (b) Posterior view, a medial part of funnel (arrowhead). (c) Dorsal view, positive cells at the margin of shell field (arrowheads), a narrow band between head and eye in the cephalic compartment (asterisk). (d) Lateral view, a weak expression of eye (asterisk). (e) Frontal view. Asterisks indicate the same parts of d. (f) Posterior-dorsal view, putative funnel gland (arrowhead), weak expression of the margin of shell sac. (g) Lateral view of the middle embryonic stage. Asterisk indicates an external margin of eye vesicle. ab, arm base; cc, cerebral cord; I–V, digital tentacles/arms; mo, mouth; olf, olfactory organ; pv, palliovisceral ganglion; sh, shell sac; st, statocyst; vis, putative part of visceral cord.
DISCUSSION
Developmental Mode of Nautilus pompilius

Many aquarium facilities in the world have succeeded in maintaining juveniles and adults of Nautilus (e.g., Mikami et al., 1980; Carlson, 1991; Okubo et al., 1995; Westermann et al., 2004). However, many conditional limits still prevent procurement of numerous normal embryos for analysis. In this study, we obtained a series of embryos, including previously unknown earlier stages of N. pompilius. In our collected specimens (no. 020401, no. 020412, and no. 020326), there are still large gaps limiting our ability to trace precise morphogenetic events for early tentacle development (see Fig. 6). However, the collected embryos in the present study seem to provide enough information to discuss body-plan evolution in cephalopods. The embryogenesis of N. pompilius was very similar to that of N. belauensis as far as we compared: several layers of membranes that form a leathery protective egg capsule, large yolky embryos with formation of the inner and outer yolks sacs, embryonic body plans, the external shell, and direct development as miniature juveniles (Arnold and Carlson, 1986; Landman et al., 1989; Tanabe and Uchiyama, 1997). Hatching of N. pompilius took place 8 months from oviposition, whereas N. belauensis hatched after ca. 11 months (Arnold et al., 1990). It is not obvious whether these differences of the embryonic period are related to species-specific development or simply are dependent on water temperature, as has been reported in many cephalopod development studies (Boletzky, 1994); we incubated the N. pompilius embryos at warmer temperatures, 24–25°C versus 18°C in N. belauensis (Arnold et al., 1990).

The Embryonic Head Complex in Nautilus and Coleoid Cephalopods

The body plan of Nautilus has attracted considerable attention by evolutionary biologists and paleontologists as a “living fossil” since the classic anatomical studies by Owen (1832), Griffin (1900), and Willey (1902) (for reviews, see Saunders and Landman, 1987; Ward, 1987). Although they share many characteristics with other cephalopods, they differ in several major characters: an external shell, an incompletely formed tube-like funnel, numerous tentacles without suckers, primitive sensory organs, nonconcentrated brain cords, the lack of a branchial heart and ink sac. These characters have been viewed either as primitive characters inherited from a common cephalopod ancestor, or as secondary changes unique to Nautilus. In the following section, we mainly discuss evolutionary implications of cephalopod head development by comparing embryogenesis of Nautilus, coleoid cephalopods and other molluscs.

Eyes and associated structures. As found by Arnold and Carlson (1986), the embryonic eyes and hood are closely associated as an anteriorly located “cephalic component” in the early organogenetic period of Nautilus. We found that the cephalic component is a transient and distinct compartment that is composed of embryonic precursors for the dorsal part of the hood, rhinophores, ocular tentacles, eyes, optic lobes, and cerebral cord in the earliest body plans of N. pompilius. Similar embryonic patterns are identifiable in the squid Idiosepius, but differences are obvious in that these parts enlarge and shift posteriorly in I. paradoxus (Fig. 8i). The relative position of the eye vesicles is also different from that of Idiosepius (Fig. 8i). Surrounding the eye vesicles in coleoids are some distinct bulges or regions as described by Naef (1928) (Fig. 5e). While no such distinct cephalic bulges are identified around the eye vesicles in the 3-month-old Nautilus embryos (Fig. 3a), small bulges or compartments develop later (Fig. 4b,e). In both Nautilus and squid, these distinct regions possibly include ectodermal anlagen of the rhinophore/olfactory organs (Fig. 7j for Nautilus; Wildenburg and Fioroni, 1989 for squid). However, most of the region may be composed of the anlagen of juxtaganglionic or neurovenous tissues (anterior chamber organ, paravertical tissues, and subpapunculate tissues; Young, 1970). Therefore, the absence of such cephalic bulges around embryonic eyes in the early embryo may reflect a less developed condition of neurovenous tissues in Nautilus but more detailed analysis is required for further discussion of cephalopod neurovenous tissues (Shigeno et al., 2001b,c; Yamamoto et al., 2003).

Digital tentacles. Early topographic patterns of embryonic tentacles were previously reported based on one specimen of Nautilus belauensis (Arnold and Carlson, 1986; Arnold, 1987). Seven anlagen (six buds and one lobed cirral part) were described on each side of the embryo. The two anterior most buds on each side were expected to develop into the ocular tentacles, and the middle four buds with the posterior most lobed cirral part were inferred to be anlagen for digital tentacles, but their developmental process has not been investigated so far. In this study, five compartments (I–V) composed of four bipartite arm-like buds and one bud on each side were identified in 3-month-old embryos (Fig. 6). Unlike previous descriptions, the anterior most two compartments I and II were fused like the bipartite arms of coleoids (Naef, 1928) and formed a ventral part of the hood. The remaining compartments (III–V) differentiated into numerous digital tentacles. Compartments IV and V are continuous, and therefore these compartments correspond to the “one lobed cirral anlagen” of the previous description (Arnold and Carlson, 1986). In this study, we propose a different naming convention, IV and V, on the basis
of the unique manner of the fusion of the buds. It is not clear whether all of the adult digital tentacles are derived from anlagen III–V or compartments IV + V or only the single compartment V.

Homologies of the tentacles and arms remain uncertain among Nautilus and other cephalopods. A five-pair formula is well conserved in decapodiformes and in Vampyroteuthis infernalis, a basal species of octopodiforms (=vampyropods) (Naef, 1928; Boletzky, 1993; Young and Vecchione, 1999). The bipartite arms appear only in some species of coleoids (Naef, 1928) (Fig. 10). These facts suggest that the common coleoid ancestor may have had five pairs of arm compartments with the bipartite arm condition, totaling 10 distinct arm buds on each side (Naef, 1928). In this study, obvious topographical similarities were revealed in the early embryonic tentacles/arms of Nautilus and other cephalopods (Fig. 8i). Nautilus also has five compartments of bud-like anlagen with a bipartite arm-like condition (Fig. 10). Therefore, our results support a “five compartment hypothesis” for the archetype or phylotype of arm/digital tentacles in cephalopods.

The modification of the posterior compartments IV and V may suggest some flexibility in cephalopod arm/tentacle evolution. Only Nautilus has a specialized developmental program (Fig. 6), namely, compartments I and II develop into part of the hood and the posterior compartments III–V differentiate into numerous digital tentacles. This fact suggests that the mode of tentacle formation is secondarily modified in the lineage leading to Nautilus.

**Ocular and buccal tentacles.** Ocular and buccal (labial) tentacles are unique organs in Nautilus (Griffin, 1900; Willey, 1902). Our results in 3-month-old embryos indicated that the ocular tentacles differentiated from a part of the cephalic compartment and not from peripherally situated anlagen as suggested previously (Arnold and Carlson, 1986). Though the precise origin of the precellular tentacles is unclear, these tentacles appear in 6-month-old embryos (Fig. 7j). The anlagen of the buccal tentacles also appear later than 4 months old at the posterior ventral tissue at the buccal mass. Thus, the ocular and buccal tentacles have developmental origins that are obviously distinct from those of digital tentacles.

**Arm bases.** The arm bases are usually derived from the base of each arm anlage and cover the head in most coleoid embryos and these ecto- and mesodermal covers play an important role in the formation of part of the secondary cornea and the integration of the arms and head (Naef, 1928; Arnold, 1984; Boletzky, 2003; Fig. 5e–g). In Nautilus, we could not detect such a structure and no tissues cover the whole head part during embryogenesis; however, similar arm base-like structures are seen in 3-month-old Nautilus embryos. Elongated projections from compartment II (Fig. 3b) and from compartments IV and V are visible laterally (Fig. 5a). Thus, in coleoids, the elaboration of the arm bases appears to be accelerated and they cover the whole head part to form the eyelid and secondary head cover (Naef, 1928; Arnold, 1984; Bandel and Boletzky, 1988; Boletzky, 2003), although a common cephalopod ancestor might not have had such a covered head as is the case in Nautilus.

**Hood, collar, and funnel complex.** The development of the hood is an example of drastic mor-

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Fig. 10. Comparative scheme and arm homologies in Nautilus and coleoid cephalopods. The plesiomorphic arrangement of arms in coleoid cephalopods is considered to be 10 equal arms on each side with the five bipartite arm condition (Roman numerals) shown as a cephalopod phylotype composed of shared characters (Naef, 1928; Flower, 1955; Young et al., 1998; Boletzky, 2003). In Nautilus, tentacle anlagen IV and V have a unique differentiation manner. Other species have a conserved five arm formula (I–V) except lack or degeneration of arms [Vampyroteuthis (Pickford, 1946; Young and Vecchione, 1999); Octopoteuthis (Nixon and Young, 2003), asterisks indicate the adult stage] and delay of differentiation timing in each arm during the early ontogeny (arm IV in the embryos of Idiosepius paradoxus), arm III and V in the embryo and paralarvae of Todarodes pacificus (Watanabe et al., 1996; Shigeno et al., 2001a). In octopodiformes, there are some unsolved hypotheses for arm homologies (Naef, 1928; Boletzky, 1978–1979, 1993; Zell, 1988). In Octopus, the Arabic numerals indicate arm formula adopted by each author.
phogenic changes in *Nautilus* embryogenesis. The cephalic hood, a cap to close the shell aperture, was previously assumed to be formed by the fusion of sheaths of the digital tentacles seen in two modified hood-like digital tentacles of adults (Griffin, 1900) or to be a derivative from the anterior region of the eyes (Arnold and Carlson, 1986). Our observation partly supports both of those suggestions, because the hood is formed as a complex by assembly of three different embryonic precursors: the main frontal part of the collar-funnel compartment, the anterior region of the eyes, and the anterior compartments of the tentacle buds as described earlier. In coleoids, it is possible to identify homologous parts; however, the hood is not developed and the collar is considerably smaller (see Fig. 8i). The anlagen of the funnel are situated relatively anteriorly in *Nautilus*, whereas they are more posterior in coleoids.

**The brain cords.** The embryonic development of *Nautilus* brain (cerebral, pedal, and visceral cords) was entirely unknown before this study. Using *distalless* (*Dll*) antibody staining, we could successfully detect the position of the brain anlagen of *Nautilus* and *Idiosepius*. Currently there are many useful markers to detect neural cell types in various animals, but this antibody is the only useful immunohistochemical marker for neural progenitor populations in cephalopod brains, as far as we have determined (Shigeno et al., personal communication). The *Dll*-like proteins seem to be distributed in some neural or glial progenitor cells because of their characteristic morphology of lacking long neurites (e.g., Fig. 8h). Interestingly, similar expression patterns were reported in annelid and arthropod nervous systems (Panganiban et al., 1997), suggesting that this marker could bind to similar cell types across protostomes. In the 3-month-old-embryo of *Nautilus*, *Dll* positive cells were distributed in the cerebral, visceral, and pedal cords. They were situated in regions adjacent to the anlagen of the hood, collar, and digital tentacles, respectively (Fig. 5i). These expression patterns exhibit striking similarities to those of squid embryos, indicating the homology between the brain cords in *Nautilus* and massive brain lobes in coleoids as has been inferred previously (e.g., Young, 1965, 1977, 1988; Salvini-Plawen, 1980).

**Unique Engrailed-Like Protein Expression Patterns in Cephalopods**

Examination of the expression patterns of transcription factors can provide some insights into conserved developmental programs in different body plans (Lee et al., 2003). Localization of the 4D9*engrailed* expression had not been attempted so far in cephalopods. In both *Nautilus* and *Idiosepius*, many positive cells are located at a marginal zone of anlagen (Fig. 9g), indicating that a developmental role may exist as reported among other bilaterians, such as arthropods and annelids, in which similar expression and functional involvement in tissue specification are suggested (e.g., segment and boundary formation) (Prud’homme et al., 2003; Seaver and Kaneshige, 2006). In cephalopods, interestingly, *engrailed*-like proteins were commonly expressed in the arm base-like structure of compartment II of *Nautilus* (Fig. 9a) and the base of arm II of *Idiosepius* (Fig. 9g). There are positive tissues in the dorsal part of compartment V (see no. 020326 of Fig. 6 for details) and similar parts are also observed in *Idiosepius*. On the other hand, in contrast to the uniform expression pattern in *Nautilus* digital tentacles, the expression in arm II of the squid is stronger than those in arm I and IV. Thus, these expression patterns do not always provide unequivocal basis for morphological arm homology. More detailed analysis is apparently required. In particular, *Hox* gene expression patterns in *Nautilus* embryos may also provide important insights, because the homologous *Hox* genes are present in both *Nautilus* and coleoids (Iijima et al., 2006).

The *engrailed*-like protein is also localized at marginal cells of the shell gland (Fig. 5c, f, g), suggesting a conserved ancestral role for shell or shell sac formation among molluscs, as reported in studies using *engrailed* mRNA of a basal gastropod *Patella vulgata* (Nederbragt et al., 2002) and using 4D9 in a scaphopod *Antalis entalis* (Wanning and Haszprunar, 2001). In addition, many unique expression sites were identified in the embryonic tissues of *Nautilus* and *Idiosepius*. This may be explained by an added gene function of making new morphological parts in cephalopod body plans as is also seen in the unique *Hox* gene expression patterns in a sepioid squid *Euprymna scolopes* (Lee et al., 2003) compared with those of a gastropod (Hinman et al., 2003).

**Evolution of the Cephalopod Head Complex as an Assemblage of Coordinates**

One of the marked features in cephalopod evolution is development of the head complex, including the hood, arms, eyes, brains, and the neck parts (Fig. 1; Naef, 1921–1923; Teichert, 1988; Engeser, 1990; Nixon and Young, 2003 for reviews). As stated in the introduction, two major evolutionary hypotheses have been proposed to explain drastic changes of cephalopod head complex: (i) the *arms as head hypothesis* and (ii) the *arms as foot hypothesis*. Both of these hypotheses have remained controversial, because there are large gaps between the body plans of cephalopods and other conchiferan molluscs, and no fossil records have been found for soft parts of basal cephalopods. However, our results provided new insights into this subject in terms of ontogeny and phylogeny. (1) Topograph-
Fig. 11. A suggested alternative scenario for the evolution of the cephalopod head complex by assembly of multiple molluscan body parts. (a) Simplified schematic figures of conchiferan body-plans to show comparable and derived features. (Each color represents suggested homologous parts in a primitive form of the gastropod *Patella* (Sasaki, 1998), *Nautilus* (this study), and a representative derived form of the coleoid *Idiosepius* (Yamamoto et al., 2003 and this study)). The homologies between gastropod “head” part and cephalopod cephalic compartments are not certain (the orange color). The similar topographical arrangement of body plans is emphasized for comparison. (b) Cephalopod body plans are characterized by elaboration of the head complex. The arm bases cover the whole head part and funnel fused to them (Naef, 1928; Boletzky, 2003) to originate a more integrated head-arm part in coleoids (only the transient condition is represented in this figure). No such cover is identified in *Nautilus* embryo, although some arm-base-like structures develop during tentacle formation. Homology inferences are still controversial, but loss of arm II in octopodiformes (or vampyrops) composed of vampyroteuthids and octopods, might occur as seen in late embryos of a basal coleoid, vampire squid (Young and Vecchione, 1999, for digital tentacles/arm homologies, see Fig. 10). ab, arm base; olf, olfactory organ; rhi, rhinophore. (c) A conchiferan and cephalopod phylotype is composed of shared morphological characters to show a drastic transition from the benthic to nektonic forms. These figures indicate that the cephalopod tentacles and arms are derived from the ancestral foot. Morphological novelty is seen in the appearance and elaboration of collar-funnel compartments as a part of the head complex (black). The various organs including cephalic compartment (orange) and neural cords (red) are assembled to form an integrated head complex along the anterior–posterior body axis.
ical patterns of early Nautilus embryos never support the hypothesis that the digital tentacles of Nautilus have been derived from an ancestral molluscan head (Fig. 11a). Actually, the anlagen of the digital tentacles are obviously distinct from the embryonic head components (what we called the “cephalic compartment”), including the anlagen of the hood, eyes, and cerebral cords. (2) Our results are consistent with the arms as foot hypothesis, because the digital tentacles of Nautilus (and also coleoid arms: see Boletzky, 2003) are derived from a pedal region under the control of the pedal cords (Figs. 8i and 11a). (3) Our results also reject a pedal origin of the funnel, since the anlagen of the funnel are situated laterally along the mantle and distinct from supposedly pedal regions (the tentacle anlagen) in the 3-month-old Nautilus embryo (Fig. 8i). In previous studies, the innervation from the pedal ganglia was particularly emphasized in favor of the pedal origin of the funnel (Salvini-Plawen, 1980), but in fact, the funnel is innervated both from the pedal ganglia (pedal cords) and the pallio-visceral ganglia (visceral cords) according to descriptive studies in adult cephalopod nervous systems (Young, 1965, 1976; Shigeno and Yamamoto, 2002). Concerning homology criteria, we suspect that the innervation pattern is not necessarily conserved in the case of the highly concentrated brains of cephalopods. Instead, Nautilus embryogenesis indicates that the funnel has arisen from a posterior collar region as a part of the “neck” (Fig. 11a). This transition has possibly developed in relation to the constant buoyancy of individuals (e.g., Denton and Gilpin-Brown, 1973; Denton, 1974) as inferred in Cambrian Plectronoceras (Yochelson et al., 1973).

In summary, we suggest an alternative evolutionary scenario for cephalopod body plan and head complex formation as follows (Fig. 11b,c).

1. The tentacles/arms were derived from the foot region. Acquisition of a nektonic life from a benthic ancestor accelerated the loss of a creeping pedal sole and the development of tentacles from a freely mobile foot (Yochelson et al., 1973; Young et al., 1998).

2. In an ancestral cephalopod, the number of tentacles/arms was five pairs (or 10 pairs of bipartite arms). This means that the large number of tentacles in Nautilus results from secondary multiplication. Alternatively, the 10-arm condition of coleoids could be neotenous.

3. The mouth surrounded by foot-derived tentacles/arms is unique among molluscs. This body plan was created by enwrapping the head part by epidermal tissues of pedal origin. During embryogenesis the pedal region shifts forwards on the body surface, and eventually the “foot” is displaced anterior to the head (see also Boletzky, 2003).

4. The rhinophores of Nautilus and olfactory organs of coleoids are presumably homologous, since they develop at similar posterior parts of the cephalic compartment as discussed earlier. Therefore, ancestral olfactory organs might have been present at an early stage of cephalopod evolution (Fig. 11c).

5. An unfused hyponome as a primitive funnel might have arisen from the posterior part of the hood-collar compartment, which is possibly derived from an intermediate zone between the head–foot and visceral mass in the monoplacophoran ancestor (Fig. 11a). Alternatively, there is a possibility as suggested by Naef (1928) that a region of epipodial (dorsal) tentacles may differentiate into the hood-collar compartments. Then, with modifications for free-swimming behavior, the collar became distinct at the lateral region of the mantle and well-developed, including the funnel (Fig. 11a,c).

6. Cephalopod brain masses centralized from the primitive tripartite neural-cord condition (Young, 1965; Salvini-Plawen, 1980; Budelmann, 1995) as seen in the embryonic nervous system of coleoids (Marquis, 1989; Shigeno et al., 2001b,c) (Fig. 11a,b). Early cephalopods probably had a cord-like brain (not ganglia) as is found in the pedal cords of primitive gastropods (Fig. 11c).

7. The optic lobes innervating cerebral eyes were derived from the cerebral cord, since this connection is found in the early embryos of Nautilus (Fig. 8i).

8. The hood seems to be a secondarily-derived structure, convergent with the operculum of gastropods, which was coopted from two dorsal arm pairs together with ocular tissue and part of the collar/funnel complex.

9. The ancestral function of transcription factor engrailed was conserved during the shell formation process, given that similar expression patterns were seen in Nautilus, Idiosepius, and other molluscan embryos (Wanninger and Haszprunar, 2001; Nederbragt et al., 2002). Further analysis is required; however the expression patterns may suggest a role for arms (pedal components), funnel, collar, and eyes in the evolution and development of molluscs.

With regard to orientation, shape and axis formation of body plans, the cephalopod head is one of the most complicated examples in bilaterian evolution.

ACKNOWLEDGMENTS

We thank S. von Boletzky for critical reading of the whole manuscript and suggestions. We also thank K. Tanabe, M. K. Nishiguchi, K. Warnke, J. von Byern, R. Parnaik, C. W. Ragsdale, and an
anonymous reviewer for reading of early manuscripts, members of our laboratories for suggestions and technical assistance, N. Niwa for the gift of anti-distalless antibody and Kuratani for laboratory space.

LITERATURE CITED


