BRAIN EVOLUTION AND COGNITION

Edited by

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A JOHN WILEY & SONS, INC. and SPEKTRUM AKADEMISCHER VERLAG CO-PUBLICATION

WILEY New York · Chichester · Weinheim · Brisbane · Singapore · Toronto

Spektrum AKADEMISCHER VERLAG Heidelberg · Berlin

John Wiley & Sons, Inc.
605 Third Avenue
New York, NY 10158-0012
USA

Telephone: (212) 850-6000

Spektrum Akdemischer Verlag
Vangerowstrasse 20
D-69115 Heidelberg
Germany

Telephone: ++ 49 6221 91260

Address all inquiries to John Wiley & Sons, Inc.

Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data

Brain evolution and cognition / edited by Gerhard Roth and Mario F. Wullimann.
p. cm.
Includes index.
ISBN 0-471-33170-8 (cloth : alk. Paper)
1. Cognition. 2. Brain-Evolution.
I. Roth, Gerhard, 1942 Aug. 15- II. Wullimann, Mario F.
QP360.5. B734 2001
573.8'6-DC21 00-023095

Die Deutsche Bibliothek – CIP-Einheitsaufnahme

Brain evolution and cognition / ed. By Gerhard Roth and Mario F. Wullimann. – New York ; Chichester ; Weinheim ; Brisbane ; Singapore ; Toronto: Wiley ; Heidelberg ; Berlin : Spektrum, Akad. Verl., 2001
ISBN 0-471-33170-8

This book is printed on acid-free paper.

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

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Chapter 1

BRAIN PHENOTYPES AND EARLY REGULATORY GENES: THE *BAUPLAN* OF THE METAZOAN CENTRAL NERVOUS SYSTEM

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INTRODUCTION

In this chapter, I discuss certain aspects of the relationship between cladistically oriented comparative neuroanatomy and developmental neurogenetics. These two fields meet in the scientific search for the biological roots of the metazoan nervous system, one focusing on the phenotypic reconstruction of the basic *Bauplan* and its subsequent evolutionary alterations, the other focusing on the fundamental developmental mechanisms creating that *Bauplan* and its variations. At first sight, one might get the impression that the conclusions reached in these fields are contradictory regarding the early evolution of the central nervous system (CNS). A closer look, however, reveals that this need not be the case. Cladistic methodology is used with great success in comparative biology to reconstruct evolutionary patterns (e.g., ancestral phenotypes and their subsequent alterations). As is detailed later, recent conclusions regarding the ancestral phenotypic condition of the nervous system (e.g., with respect to its segmentation) are often based on patterns of gene expression alone. The message purported here is simple: It may be fatal to ignore the results of an otherwise successful methodology of comparative biology (i.e., cladistics) in the special case of the nervous system because the genes appear to tell us a different story.

Revolutionary studies in molecular genetics during the past decade showed that very many genes relevant for early neural development have orthologues in animals as remotely related as fruit fly and mouse. The *pax-6* gene is a prominent example (Callaerts et al., 1997). Orthologues of this gene are present in most metazoans where they are involved in eye morphogenesis at a hierarchically high level. By gene technology means, *pax-6* can experimentally be interchanged interspecifically and still function within its host developmental program (Halder et al., 1995). This could be taken as proof that all phenotypes produced by the *pax-6* gene are homologous. Thus, despite the fact that developmental programs with the *pax-6* gene at the top have been altered during metazoan evolution and led to similar eyes (*Octopus* eye

and vertebrate eye) and dissimilar eyes (insect compound eye and vertebrate camera eye), the resulting phenotypes all become homologous according to the logic outlined above. Thus, usage of the term *homology* apparently is very critical. Wiley (1981) offered a useful definition that excludes convergence and parallelism: *A character of two or more taxa is homologous if this character is found in the common ancestor of these taxa, or, two characters (or a linear sequence of characters) are homologues if one is directly (or sequentially) derived from the other(s).* As far as their detailed similarity is concerned, the *Octopus* eye and the vertebrate eye represent neither case, but are in fact a case of parallelism. The novelty value of the *pax-6* story is, however, that the underlying developmental program is partly identical in all metazoans and that we can, thus, assume that the *Octopus* eye and the vertebrate eye are homologous to the eye of their last common ancestor.

Even more relevant to the discussion raised here are those regulatory genes (e.g., the homeotic genes of the *Hox* complex) that are expressed in similar spatiotemporal patterns in various developing metazoan animals and control regionalization in the anteroposterior axis, especially during head and CNS formation. Some of these genes involved in head and brain formation can also be functionally replaced among metazoan species (see later discussion). One might conclude that the ancestral condition of the metazoan brain must have been rather complex already, for example, including a multisegmental structure (Reichert and Boyan, 1997). Such direct inference from patterns of gene expression in recent species to an ancestral phenotype is not, however, unchallenged by researchers who find alternative explanations for the same facts (Akam, 1989; Slack et al., 1993). In the following, I attempt to reconcile interpretations resulting from neurogenetics with those resulting from cladistic analysis of the phenotype of the metazoan CNS.

Cladistically oriented neuroanatomy using phenotypic characters reveals that there is indeed a pattern of ancestral (plesiomorphic) neural characters. Some of those may not change during evolution and may be similar and homologous for that reason (non-neural example: many characters in the tetrapod foreleg). Other characters may, however, reach similarity independently (i.e., not inherited from a common ancestor), and they therefore represent *homoplastic* features as far as their similarity is concerned (e.g., bat and bird wings). Again, another class of characters may change (i.e., become divergent in their phenotype) but remain homologous (e.g., reptilian foreleg/bird wing). Note that if, hypothetically, all recent and extinct amniotes had wings, we would, based on the very same data, conclude that bat and bird wings *are* homologous.

Another enlightening example of the relationship of similarity and evolutionary descent is the evolutionary loss of teeth in birds. Developmentally, the formation of teeth can be experimentally induced in chicken (Kollar and Fisher, 1980). Thus, the genetic basis for teeth is retained in birds, although no recent bird species displays teeth in the phenotype. Were a future bird to redevelop teeth phenotypically, these could not be considered homologous to the teeth of other vertebrates. The critical point for an evolutionary biologist here would be the phenotypic absence of teeth in the ancestor of the hypothetical future toothed bird. Thus, vertebrate teeth would not have a continuous history, and the hypothetical *new* bird teeth would be considered a case of parallelism, despite the fact that the genetic basis was largely identical.

It is generally accepted in evolutionary biology that these distinctions are valuable and of great heuristic value. In the foreleg (and teeth) case, we can safely assume that the cascade of interactions of genes and their products is very similar in all vertebrates, and yet nothing is gained for the understanding of tetrapod foreleg evolution by proclaiming that the underlying developmental program and all its subsequent alterations are homologous. This would extend the definition of homology to the point of becoming meaningless (e.g., the fruit fly *Drosophila melanogaster* is homologous to humans because the same ancestral developmental program with certain modifications is at work).

In the following, I first use cladistic analysis on prominent neural characters of extant metazoans in order to identify major events in the evolution of the nervous system. Alternative cladograms are used to exemplify how such hypotheses critically depend on the choice (and, ultimately, the adequacy) of the cladograms used. In light of this analysis, I then, discuss the conclusions regarding the early metazoan head and brain proposed in some of the neurogenetic literature.

COMPARATIVE PHENOTYPIC ANALYSIS OF METAZOAN CENTRAL NERVOUS CHARACTERS

The Cladistic Framework

A common procedure chosen for textbook contributions on the evolution of nervous systems consists of describing these systems along a phylogenetic tree and to assume that, by doing so, a more or less adequate picture of nervous system evolution emerges. Often, two assumptions are implicit to this approach: (1) Animals, and with them nervous systems, evolve as whole organisms in certain directions, and (2) the direction of evolution is always toward increasing complexity (i.e., nervous systems range from simple/primitive to more complex/advanced states in a linear fashion). For example, the urodele CNS retains early ontogenetic character states into adulthood, (i.e., the CNS is *paedomorphic*). The resulting simple morphological appearance of the urodele CNS was often interpreted as representing the ancestral tetrapod condition. The urodele CNS can, however, be demonstrated to represent a case of secondary simplification (Roth et al., 1993; Roth and Wake, this volume, chapter 8), a phenomenon that must remain principally unconsidered as an evolutionary possibility if one uses the two assumptions outlined above.

In contrast to this traditional *scala naturae* approach, the cladistic approach of analyzing CNS evolution has explicit epistemological foundations. Cladistic methodology (Hennig, 1950, 1966), once introduced into comparative neurobiology (Northcutt, 1984), has been widely used to determine the evolutionary polarity of nervous system characters by establishing whether a certain neural character represents an ancestral feature (plesiomorphy) or a derived feature (apomorphy) (Wullimann and Northcutt, 1988; Striedter, 1991; Roth et al., 1993; McCormick,

1992; Wicht and Northcutt, 1992; Northcutt, 1995; Roth and Wullimann, 1996; Wullimann, 1997).

Brain characters, like all characters, are traits that can evolve independently of each other (i.e., brains [or organisms] are not ancestral or derived as entities but represent a mosaic of plesiomorphic and apomorphic characters). The composition of this mosaic can be investigated by determining the evolutionary polarity of neural characters. Before determining the evolutionary polarity of certain characters, one has to accept a phylogenetic hypothesis, commonly proposed in the form of a cladogram. Cladograms are branching diagrams of biological taxa and are based on the hierarchical occurrence of evolutionary novelties (i.e., new characters or *apomorphies*) that characterize one (*autapomorphy*) or several (*synapomorphy*) taxa. A synapomorphy unites two or more taxa relative to other taxa (i.e., *outgroups*). The cladogram requiring the least amount of convergent character transitions is given preference by an argument of parsimony. Consequently, suspected synapomorphies supporting alternative cladograms are interpreted as cases of convergence.

Some of the best corroborated cladograms based largely on *non-neural* characters are used below. Thus, circular reasoning is avoided when the simple tool of outgroup comparison (Hennig, 1966) is applied to analyze the evolutionary polarity of metazoan neural characters with the help of these cladograms. In short, if two taxa show a different character state of a homologous character (e.g., presence vs. absence of lamination in the mesencephalic tectum in frogs compared with salamanders), then the one occurring in the outgroup(s) is considered the plesiomorphic condition (e.g., lamination present in bony fishes and cartilaginous fishes). I cannot list here, but simply cite, the sources for the hierarchy of non-CNS synapomorphies that support the chosen cladograms or the sources for the dendrograms resulting from molecular systematic studies. In many cases, alternative dendrograms exist, and the consequences for phenotypic CNS evolution are discussed.

The backbone of information on the diverse metazoan CNS is the classic monograph by Bullock and Horridge (1965), in addition to a bulk of more recent literature. The following analysis delivers a rough picture of the order, in which new CNS characters (apomorphies) appear to have arisen during metazoan evolution and, thus, highlights some longstanding and controversial topics of CNS evolution.

A Can of Worms: Plathelminths, Nemathelminths, and Nemertines

Although coelenterates display many ancestral eumetazoan characters, they—in contrast to sponges—have neurons forming a peripheral nervous system (nerve plexus), which is by no means simple and is beautifully adapted to guide coelenterate behaviors (Mackie, 1990). Nevertheless, the absence of a CNS may be considered a plesiomorphic condition for eumetazoans. Ring-shaped condensations of neurons at the oral as well as at the aboral animal pole in hydrozoans and scyphozoans as well as longitudinal neuronal aggregations in siphonophores and ctenophores (Grimmelikhuijzen et al., 1996, T.H. Bullock, personal communication) occur as

secondary specializations. If one considers ctenophores as a taxon not included in the ceolenterates, the former would already share longitudinal nerve cords as a synapomorphy with plathelminths (platyhelminths), which are conventionally viewed as the outgroup of the remaining bilaterians (see later discussion.).

What are the first steps in evolution toward a CNS in bilaterians? The hypothesis that a CNS evolved independently in each major bilaterian clade from a nerve plexus is extremly unparsimonious. Therefore, if we accept the cladogram of metazoans by Jefferies' (1986, Fig. 1.2), the ancestral condition for the bilaterian CNS (Fig. 1.1, lower panel) is characterized by a brain (supraesophageal or cerebral ganglion) and longitudinal medullary cords, which, by definition, contain nerve fibers as well as neuronal cell bodies. Respective medullary cords of each body side are interconnected by commissures. In many bilaterians, a more superficial nerve plexus (peripheral nervous system) exists in addition; this represents a symplesiomorphy shared with coelenterates. The CNS condition outlined above can be recognized as the plesiomorphic set of characters for bilaterians. In the plesiomorphic condition, the medullary cords may have been located dorsally, ventrally, and laterally as seen in at least some nemertean and plathelminth species, but many wormlike taxa need further comparative analysis. Molluscs retain the basic bilaterian *Bauplan* ancestrally (see later discussion), and so do tentaculates (including bryozoans, phoronids, and brachiopods), albeit in a simplified form. Within deuterostomes, especially chordates, changes in life history complicate the comparative interpretation, but the basic bilaterian *Bauplan* may be concluded to be retained, if somewhat simplified, in some stages of life history of at least some species (see below). In Jefferies, and other cladograms (Fig. 1.2; Fig. 1.7). annelids are the sister group of arthropods. It is, thus, parsimonious to assume that the cerebral and ventral cord ganglia forming a *strickleiter* nervous system originated once for the articulates (Figs. 1.1 and 1.2; see also Fig. 1.7). Within the articulates, only onychophorans must be interpreted as having partially regressed from the more complex *strickleiter* nervous system back to the described ancestral bilaterian condition, because these animals exhibit medullary cords.

An important alternative branching diagram based on a variety of molecular (18S rRNA sequences) and paleontological data has been proposed by Conway-Morris' (1993) (Fig.1.3). Similar to Jefferies' cladogram (1986), the clade designated as nemathelminths in Figure 1.3 appears monophyletic and includes nematomorph, nematode, gastrotrich, rotiferan, as well as acanthocephalan species. Also, tentaculates—although only data from brachiopods and phoronids, but not bryozoans, were included—are considered monophyletic (S. Conway-Morris, personal communication), as are arthropods. Protostomes, if plathelminths are included, are polyphyletic. Different from Jefferies' (1986), however, articulates (arthropods and annelids) would represent a polyphyletic group. An outgroup comparison of major CNS characters leads to a similar set of characters typical of the basic bilaterian CNS *Bauplan* outlined above (Fig. 1.3). As in Jefferies' cladogram (1986), deuterostomes (in certain life history stages; see later discussion) would plesiomorphically retain—if somewhat simplified—the basic bilaterian CNS *Bauplan*. Assuming that the *strickleiter* nervous system evolved only once (x in Fig. 1.3), not only onychophorans but additionally the nemertines, the pogonophorans, and the taxon including molluscs

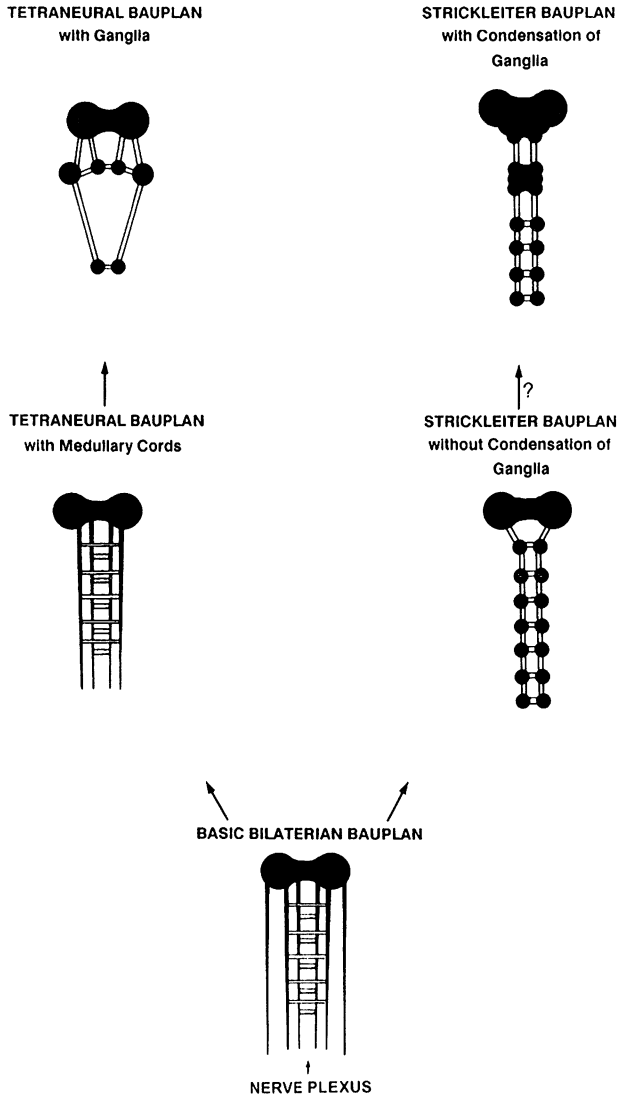


Figure 1.1. Schematic sketches of major invertebrate CNS *Bauplan* characters and an interpretation of the direction of evolutionary change in the molluscan and articulate lineages, respectively. The five drawings represent dorsal views (anterior is at the top) and show the set of likely ancestral CNS characters for certain metazoan taxa. The basic bilaterian *Bauplan* characterizes most nonsegmented wormlike taxa (e.g., plathelminthomorphs, nemathelminths, and nemertines) as well as onychophorans, and it may be ancestral for bilaterians. The tetraneural *Bauplan* with medullary cords is found in aplacophorans, polyplacophorans, and monoplacophorans and is ancestral for molluscs. The tetraneural *Bauplan* with discrete ganglia characterizes gastropods, bivalvians, scaphopods, and cephalopods. The *strickleiter Bauplan* without condensation of ganglia is seen in annelids, kinorhynchs, and tardigrades, and the *strickleiter Bauplan* with condensation of ganglia characterizes all remaining arthropods. Black: CNS contains neuronal somata. White: cords, commissures, or connectives without neuronal somata.

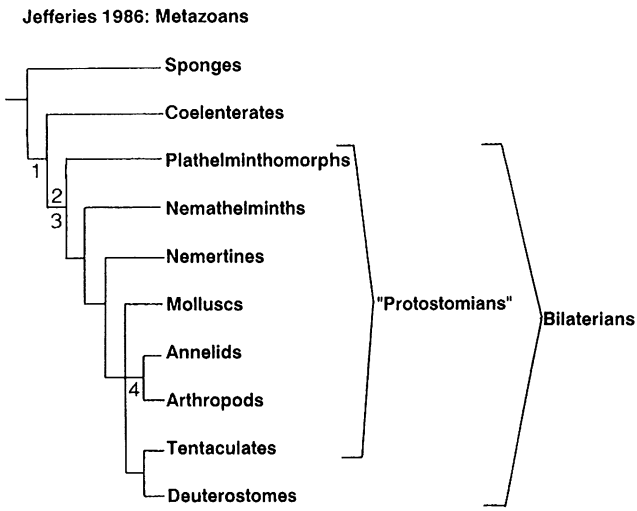


Figure 1.2. Legend on p.26.

(the latter only in their plesiomorphic condition), sipunculids, tentaculates, and the latter's sister taxon (including kinorhynchs and priapulids) would secondarily regress to the plesiomorphic bilaterian CNS state. The segmental organization of ventrally located ganglia in kinorhynchs would, however, be the retention of an ancestral feature. This scenario would, thus, involve at least four cases of secondary simplification. In contrast, the assumption that the *strickleiter* nervous system evolved independently in annelids and arthropods (4 in Fig. 1.3) clearly is more parsimonious in the dendrogram of Conway-Morris' (1993). Accordingly, nemertines, pogonophorans, as well as molluscs, sipunculids, tentaculates, priapulids, and kinorhynchs would retain the plesiomorphic CNS condition; kinorhynchs, however, would independently form segmental ventral cord ganglia. Only onychophorans would be a case of secondary simplification of the *strickleiter* nervous system, as was the case in the first scenario. Importantly, after considering the distribution of neural characters in two dendrograms as different as those of Jefferies' (1986) and of Conway-Morris' (1993), the major conclusion regarding the plesiomorphic condition for the bilaterian CNS remains the same.

Recently, another very different branching diagram for metazoans (Fig. 1.4) has been suggested based on phylogenetic analysis of 18S ribosomal DNS sequences (Halanych et al., 1995; Aguinaldo et al., 1997). In contrast to both Conway-Morris' and Jefferies' dendrograms, tentaculates are now considered to be polyphyletic, but protostomes (if plathelminths are included) are the sister group of deuterostomes and represent a monophyletic taxon here (Fig. 1.4), consisting of two sister taxa, the lophotrochozoans (including molluscs, annelids, inarticulate and articulate brachiopods, phoronids, bryozoans, rotiferans, and plathelminths) and the ecdysozoans (moulting animals; i.e., all arthropod groups plus nematodes, nematomorphs,

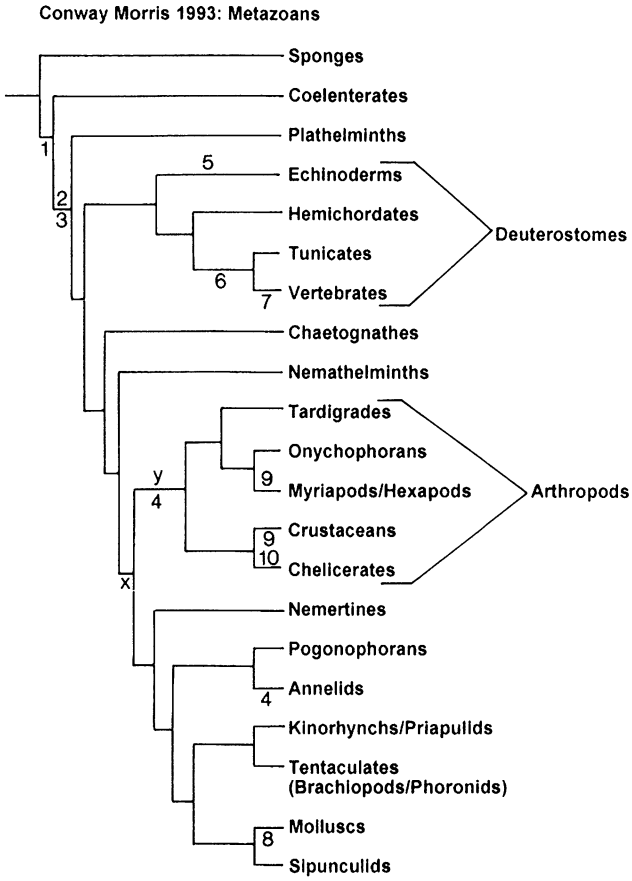


Figure 1.3. Legend on p.26.

kinorhynchs, and priapulids). Thus, nemathelminths are polyphyletic because rotiferans belong to the lophotrochozoa, while nematomorphs and nematodes are ecdysozoans. Furthermore, arthropods do not form a monophyletic taxon (see Fig. 1.4). Morever, the most drastic departure from other branching diagrams is that the plathelminths are not the outgroup of all other bilaterians, but are part of the lophotrochozoa (see Fig. 1.4).

What are the consequences for CNS evolution if neural characters are interpreted in light of this branching diagram? In applying the outgroup comparison, a rather simple set of neural characters resembling much the plesiomorphic metazoan CNS condition outlined above (i.e., an anteriorly located brain and at least some medullary cords) results at the basis of both the lophotrochozoa and the ecdysozoans. Thus, similar to the conclusion reached above, this *Bauplan* likely was present in the last common ancestor of all protostomes as defined in this dendrogram. No clear picture emerges, however, for the ancestral condition of the deuterostome CNS using this

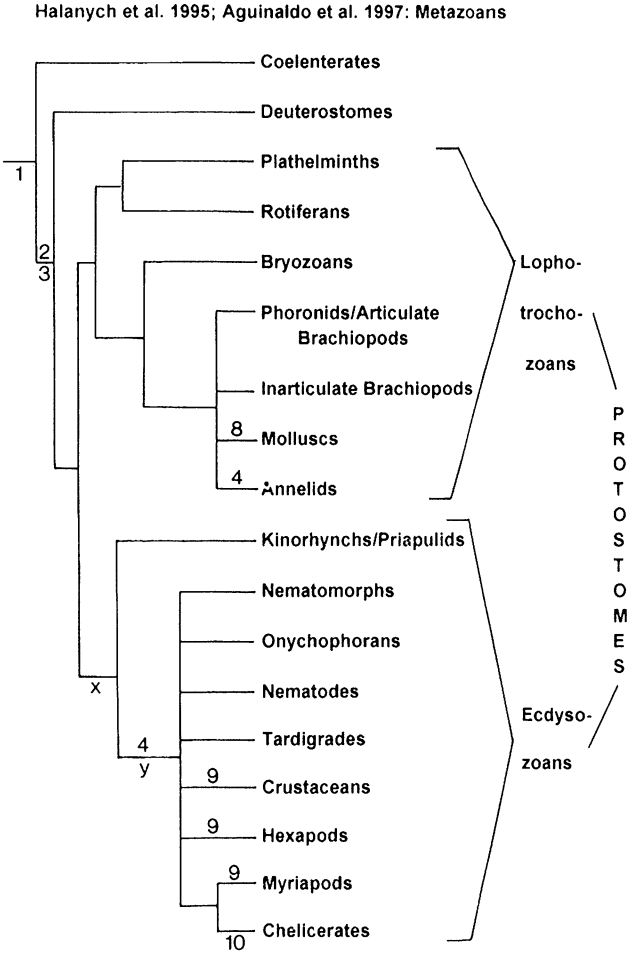


Figure 1.4. Legend on p.26.

dendrogram (see later discussion). A *strickleiter* nervous system—as in Conway-Morris' branching diagram—would have evolved twice, once in annelids and a second time at the base of the node, which includes all arthropods (4 in Fig. 1.4). Following this dendrogram, it is even more unlikely than in that of Conway-Morris' that annelids and arthropods share a segmented ancestor with a *strickleiter* nervous system. Furthermore, many of the ecdysozoan taxa remain cladistically unresolved in this dendrogram, and a *strickleiter* nervous system may well constitute a synapomorphy for tardigrades and arthropods only (Fig. 1.4). Interestingly, onychophorans would then simply retain the plesiomorphic CNS condition, but kinorhynchs would independently form a segmented ventral cord.

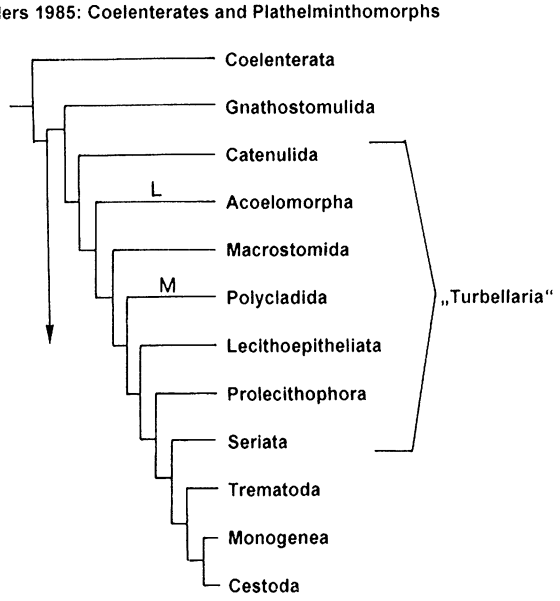


Figure 1.5. Legend on p.26.

Using the presently best corroborated cladogram for plathelminthomorphs (Ehlers, 1985) (Fig. 1.5), the basic bilaterian *Bauplan* must be interpreted to have undergone secondary simplification as well as increasing complexity. Some, but not all, species within the acoelomorphs (Fig. 1.5) exhibit the simplest nervous systems among plathelminthomorphs in that they only have a nerve plexus instead of medullary cords or lack a CNS (including a brain) entirely. These simple nervous systems resemble to a varying degree those of coelenterates. However, the acoelomorphs have two outgroups (the catenulids and the gnathostomulids) with a well-developed CNS, including a brain and longitudinal medullary cords. This strongly suggests that the simple nervous system of some acoelomorphs results from secondary simplification and loss of the bilaterian *Bauplan* (L in Fig. 1.5). Likewise, apomorphic within plathelminthomorphs is the complex brain of some polycladids (*Notoplana*, *Stylochoplana*), which is differentiated into five lobes (M in Fig. 1.5).

The Molluscan Controversy

A survey of CNS characters among the different molluscan taxa (an often used dendrogram is given in Fig. 1.6) reveals that the primitive condition for the molluscan CNS is characterized by a paired supraoesophageal ganglion (cerebral ganglion, brain) and two pairs of longitudinal medullary cords, the more dorsal pair being the pleurovisceral cords and the more ventral pair being the pedal cords (Fig. 1.1, left

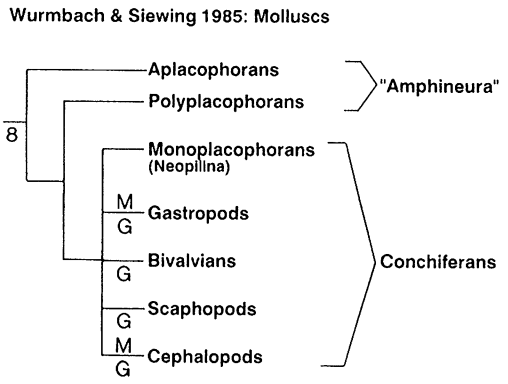


Figure 1.6. Legend on p.26.

middle panel). This is very close to the basic bilaterian *Bauplan* outlined above. A synapomorphy for molluscs, however, is the consolidation to four medullary cords (tetraneural CNS). This tetraneury is evident even in the highly derived CNS of gastropods and cephalopods.

Both aplacophorans and polyplacophorans (sometimes together called amphineurans) exhibit this simple molluscan condition, which appears to be plesiomorphic to the more complex CNS of gastropods, bivalvians, scaphopods, and cephalopods. It has alternatively been suggested, however, that molluscs and articulates share a common ancestor, which was already segmented. This would imply that a *strickleiter* nervous system is plesiomorphic for molluscs as well. An early definition of segmentation by Bateson (1894; reviewed in Jeffs and Keynes, 1990) involves that animals show a repetition of more or less identical body segments (containing most organ systems, including the coelom) along the anteroposterior axis as is seen in annelids or arthropods. Although the term *strickleiter nervous system* is used for the CNS of such overall segmented animals, the term *segmentation* today is universally used for any repetitive structures in metazoans. Do molluscs show signs of segmentation in the CNS or elsewhere?

The discovery of the monoplacophoran *Neopilina galathea* (Lemche, 1957; Lemche and Wingstrand, 1959) first fueled the theory that molluscs were ancestrally segmented. *Neopilina* shows evidence for segmental organization in a limited number of organ systems (eight pairs of muscles, six pairs of nephridia, and five pairs of gills). These organs could as well, however, have been secondarily multiplied—as is commonly assumed for the gills of polyplacophorans—and therefore would not represent remnants of a segmental organization. More importantly, in *Neopilina* there is no evidence for a segmental organization of the gonads, the coelom, and especially the nervous system. The latter conforms to the plesiomorphic molluscan *Bauplan* described above (Fig. 1.1, left middle panel): A cerebral ganglion gives off two pairs of medullary cords (i.e., neurons are distributed continuously inside the cords and are not organized into segmental ganglia as in annelids). The commissures seen in

Neopilina, which appear to have been suggestive of segmentation, also occur in plathelminths and provide no evidence of a *strickleiter* nervous system.

The distribution of central neural characters within molluscs (Fig. 1.6) strongly suggests that a cerebral ganglion with tetraneural medullary cords and commissures is the primitive condition for molluscs. This *Bauplan* is retained in monoplacophorans, and the presence of discrete ganglia instead of medullary cords in other conchiferans clearly is a derived state (Fig. 1.1, left upper panel) and may represent a synapomorphy uniting gastropods, bivalvians, scaphopods, and cephalopods, as already suggested by Hennig (1980). Furthermore, in contrast to annelids, each pair of ganglia in all derived conchiferan taxa is functionally related to a different organ system (e.g., mantle, foot, intestine; G in Fig. 1.6). Thus, there is no single mollusc exhibiting segmental ganglia associated with a segmentally organized body (i.e., a *strickleiter* nervous system). Gastropods and cephalopods in addition develop a multilobed cerebral ganglion (M in Fig. 1.6).

In contrast to Jefferies' cladogram (Fig. 1.2), the dendrograms of Conway-Morris' (1993) (Fig. 1.3) and of Halanych et al. (1995) and Aguinaldo et al. (1997) (Fig. 1.4) suggest that annelids are more closely related to molluscs than to arthropods. As demonstrated above, these dendrograms render it more parsimonious that segmentation and a *strickleiter* nervous system evolved independently in annelids and arthropods and thus offer no reason to assume that molluscs were ancestrally segmented. Again, even considering dendrograms as different as those three, the interpretation of the mollusc CNS system being very close to the basic bilaterian *Bauplan* in its plesiomorphic state and becoming more complex in derived conchiferan taxa remains the most parsimonious scenario.

The Arthropod CNS, Rather Than Being Ancestral to the Vertebrate CNS, is Equally Remote from the Basic Bilaterian *Bauplan* as the Craniate Brain

Often, annelids are viewed as the sister group of arthropods, and the two taxa would form the articulates (Ax, 1984) (Fig. 1.7). Alternatively, as has been suggested based

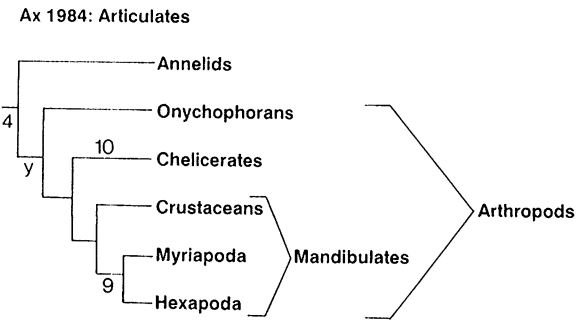


Figure 1.7. Legend on p.26.

on molecular data, annelids are only distantly related to arthropods (Conway-Morris 1993; Halanych et al., 1995; Aguinaldo et al., 1997) (Figs. 1.3 and 1.4). Contrary to the case of the origin of the plesiomorphic bilaterian and mollusc CNS condition as discussed earlier, accepting one dendrogram over the others has profound consequences for the interpretation of CNS evolution in this case.

If annelids are the sister group of arthropods (Fig. 1.7), then a *strickleiter* nervous system undoubtedly evolved only once from the basic bilaterian *Bauplan* in the last common ancestor of articulates and is ancestrally composed of a paired cerebral ganglion located in the first body part, the prostomium (Fig. 1.1, right middle panel). This cerebral ganglion is homologous to the supraoesophageal ganglion present in the basic bilaterian CNS *Bauplan* discussed earlier. The annelid supraoesophageal ganglion is connected via suboesophageal connectives to ventrally located paired cords (e.g., Telkes et al., 1996). These ventral cords (Bauchmark) are not medullary cords, however, but consist of a series of paired ganglia that are interconnected across the midline via commissures and anteroposteriorly via connectives. Ventral cord ganglia are integral parts of body segments, each of which contains a set of almost all organs including a coelomic cavity. In contrast to this plesiomorphic *Bauplan* of the *strickleiter* nervous system, the development of a trilobed cerebral ganglion through elaboration of the single prostomium ganglion (and not through fusion of several segmental ganglia; see later) in some predatory polychaetes (*Nereis, Eunice*) clearly is apomorphic within annelids. Likewise, apomorphic is the simplification of the CNS in hirudineans.

The outlined plesiomorphic condition for the *strickleiter* nervous system is altered in arthropods in many ways from the beginning. An apomorphy of arthropods is that the brain consists of fused ganglia (Fig. 1.1, right upper panel) and thus includes additional parts to the one that would appear to be homologous to the supraoesophageal ganglion located in the prostomium of annelids (which corresponds to the acron of insects). The plesiomorphic number of segmental ganglia contributing to the arthropod brain is controversial, however, because in chelicerates the minimal number is two segments (i.e., a protocerebrum containing at least the prostomium ganglion and a tritocerebrum consisting of the cheliceran ganglion), while in mandibulates (crustaceans, myriapods, and hexapods) the minimal number of brain segments is three (a protocerebrum containing at least the prostomium ganglion, a deutocerebrum consisting of the ganglion of the first antennal segment, and a tritcerebrum consisting of the ganglion belonging to the second antennal segment). The cheliceran segment is often considered to be homologous to the second antennal segment of mandibulates. Thus, the deutocerebrum with its associated segment is sometimes viewed as having been secondarily lost in chelicerates. Recently, based on homeotic gene expression patterns, it has been proposed that the deutocerebrum is present in chelicerates (Telford and Thomas, 1998). Accordingly, the cheliceran segment and the pedipalp segments would be homologous to the first antennal segment of insects/crustaceans and intercalary/second antennal segment of insects/crustaceans (i.e., to the deutocerebrum and tritocerebrum), respectively. If so, chelicerates, crustaceans, hexapods, and myriapods might share ancestrally a trisegmented brain. A recently described Cambrian arthropod with a three-segmented

head supports this assumption (Chen et al., 1995). The next outgroup of chelicerates and mandibulates, the onychophorans, are ambiguous in that respect. Although the onychophoran brain is said to arise embryonically from three neuromeres, this gives no final evidence for the fusion of segmental ganglia, because the adult multiple-lobed brain of onychophorans is located in the prostomium (Schürmann, 1987). The brain of annelids as the next outgroup definitely consists of no more than the cerebral ganglion located in the prostomium.

The cladistic position of onychophorans as the outgroup of all other arthropods (except myriapods) has recently been confirmed with molecular data (Ballard et al., 1992). Surprisingly, in this molecular study, myriapods, otherwise considered to be mandibulates (Fig. 1.7) were suggested to represent the outgroup to all other arthropods, including onychophorans. This would support the assumption that the arthropod brain consists of at least three ganglia (that of the prostomium and two additional segmental ganglia) in its plesiomorphic state (y in Fig. 1.7). Also, the brain and medullary cords of onychophorans would, then, clearly be considered secondarily simplified.

In summary, while the whole brain of annelids is homologous to that of their bilaterian outgroups, in the arthropod brain two segmental ganglia—which appear to be homologous to the most anterior ventral cord ganglia of annelids—likely were added to the plesiomorphic bilaterian brain.

Increasing fusion of additional ventral cord ganglia took place independently within chelicerates, crustaceans, and hexapods (Roth and Wullimann, 1996); the most rostral of these condensations is often called *suboesophageal ganglion*. Although within chelicerates the xiphosurans and scorpionids retain many unfusioned ventral cord ganglia, the more derived arachnids have a single, fused suboesophageal ganglionic mass. Immediately posterior to the pedipalp segment (which might be homologous to the tritocerebral brain segment, see earlier), however, the suboesophageal cell mass always involves the ganglia of leg and of abdominal segments. A similar phylogenetic trend toward increasing fusion of ventral cord ganglia has occurred independently in crustaceans (Sandeman, 1982) and hexapods. In the latter, the suboesophageal ganglion ancestrally consists of the three ganglia belonging to the mandibular, maxillary and labial segments carrying the mouth appendages. Therefore, while at least part of the supraoesophageal ganglion (brain) has a continuous evolutionary history and may be homologous even within all bilaterians, the various manifestations of a suboesophageal ganglion definitely are homoplastic. The composition of the latter is heterogeneous in different bilaterian taxa (e.g., consisting of all ventral cord ganglia in derived arachnids and of only the most anterior three ventral cord ganglia in insects) and clearly evolved independently in annelids, chelicerates, crustaceans, and hexapods/myriapods.

If, alternatively, annelids are only distantly related to arthropods (Figs. 1.3, and 1.4), segmentation and a *strickleiter* nervous system more likely have developed twice independently from the basic bilaterian *Bauplan* (i.e., once in annelids and once in arthropods (see discussion of the molluscan controversy). Furthermore, in Conway-Morris' dendrogram (Fig. 1.3), mandibulates do not form a monophyletic group within the arthropods, and in the dendrograms by Halanych et al. (1995) and

Aguinaldo et al. (1997) not even arthropods are monophyletic. In comparison to Ax's cladogram (1984), neither dendrogram offers a more parsimonious explanation for the emergence of a multisegmented brain in arthropods or ecdysozoans, respectively. The tardigrades however, remain part of the arthropods and ecdysozoans (Figs. 1.3 and 1.4, respectively); they have a relatively simple *strickleiter* nervous system, including a brain consisting of the prostomium ganglion and very few body segments with ventral cord ganglia. Thus, there might be an independently evolved similarity of ancestral features of a *strickleiter* nervous system in the arthropod and in the annelid lineages. Strausfeld (1998), using exclusively neural characters, recently proposed a dendrogram for metazoans naturally explaining neural evolution most parsimoniously (e.g., the *strickleiter* nervous system).

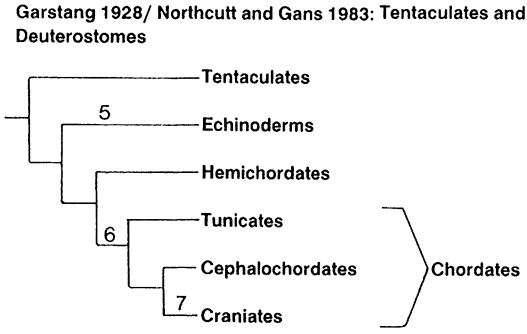
Irrespective of the cladistic position of annelids, there is no good reason for assuming that segmentation and a *strickleiter* nervous system evolved before the divergence of deuterostomes and arthropods, because this would require independent losses of typical overall segmental and *strickleiter* nervous system characters in many taxa. Such an assumption is extremly unparsimonious. Furthermore, the *strickleiter* nervous system (and especially the insect brain) can in no way be considered plesiomorphic to the vertebrate CNS. Both types of CNS are equally apomorphic, and they may have arisen from the same plesiomophic condition (i.e., the bilaterian *Bauplan* as described earlier).

Deuterostome Nervous Systems

Craniates include the myxinoid fishes plus all vertebrates, and the search for the evolutionary roots of the craniate brain is of immense interest to neurobiologists. It is, however, obscured by three problems. The first problem lies in the unsatisfactorily resolved systematic position of deuterostomes, and another two problems relate to their biology.

Although deuterostomes are regarded monophyletic in all three metazoan dendrograms discussed here (Figs. 1.2–1.4), their position could not be more different in each single case. In the first case (Fig. 1.2), they are the sister group of tentaculates and range among the most derived taxa. In the second one (Fig. 1.3), they represent the sister group of all other bilaterians, with the notable exception of the plathelminths, which again form the outgroup to *all* other bilaterians. In the third dendrogram, however, deuterostomes are the outgroup of all other bilaterians, including the plathelminths. This has profound consequences for the interpretation of the phenotypic evolution of the deuterostome CNS.

At first sight, the last mentioned dendrogram allows for a provocative hypothesis. Because echinoderms are generally viewed as the outgroup to all other deuterostomes (an often used dendrogram is shown in Fig 1.8), echinoderms may have retained and altered the radial nervous system seen in their outgroup, the coelenterates. The life history and development of echinoderms, however, clearly show that they are bilaterians and acquire radial symmetry secondarily. Also, using the first and second metazoan dendrograms (Figs. 1.2 and 1.3), a hypothetical adult deuterostome



Figures 1.2–1.8. Dendrograms of diverse metazoan taxa as indicated. Numbers refer to neural characters (listed below) and, except for Figure 1.6, are plotted according to the most parsimonious explanation for their evolutionary emergence. 1, neurons; 2, cerebral ganglion; 3, longitudinal medullary cords (maximally: ventral, lateral, dorsal); 4, *strickleiter* nervous system; 5, secondary radial nervous system; 6, dorsal hollow neural tube; 7, craniate brain; 8, consolidation to four medullary cords (tetraneury); 9, ganglia of 1 and 2 antennal segment fused with cerebral ganglion; 10, ganglion of cheliceran segment fused with cerebral ganglion; L, loss of basic bilaterian *Bauplan* in some species; M, multilobed cerebral ganglion in some species; G, discrete ganglia replace medullary cords; x, alternative position for *strickleiter* nervous system (see text); y, alternative to 9 and 10, the brain consists of minimally 3 fused ganglia here.

ancestor would be concluded to exhibit the basic bilaterian CNS *Bauplan* discussed earlier. Thus, echinoderms would simply represent a special case of secondary radial symmetry and loss of the basic bilaterian CNS *Bauplan*. Two other deuterostome issues of importance here are life style (sessility vs. mobility) and life history (larval and adult nervous systems). Many deuterostomes (and tentaculates, for that matter) are characterized by sessility, and this lifestyle appears to be correlated with a certain simplification of the basic bilaterian CNS *Bauplan* as established earlier. This *Bauplan* might alternatively, however, have been simpler initially in bilaterian evolution given that deuterostomes might be the outgroup of all other bilaterians (Fig. 1.4). Clearly, more comparative studies on various critical taxa are needed to decide this question.

The most profound and related problem is that of life history of deuterostomes. Which life history stages and related nervous structures may be homologized at all? Some deuterostomes (echinoderms and hemichordates) have planktonic larvae like many other bilaterians (essentially all taxa belonging to the lophotrochozoa; see Fig. 1.4), which subsequently metamorphose into adults (Schwartz, 1973). If this biphasic sequence is ancestral for bilaterians (which would be strongly supported by the dendrogram shown in Fig. 1.4, but also by that in Fig. 1.3), it is reasonable to look in this two-step life history for how neural tissue is transformed into an adult nervous organ and to homologize nervous tissues or organs during this developmental process among bilaterians. The fact that many bilaterian taxa (the ecdysozoans, Fig. 1.4) have no planktonic larvae may be explained as a loss of that life stage (e.g., in terrestrial arthropods) and thus poses no problem for an evolutionary analysis of adult nervous structures: Although insects skipped that early stage of larval development, we may still compare their adult CNS with that of annelids because these life stages are equivalent.

Garstang (1894) has proposed such a scenario for deuterostomes: A deuterostome ancestor resembling the echinoderm auricularia-larva gave rise to the various adult recent deuterostome forms (the Auricularia hypothesis). Not all recent deuterostome taxa (Fig. 1.8), however, retain a planktonic larva. In tunicates, planktonic larvae are lost, and a more actively mobile larval type comes into existence, displaying a set of characters (i.e., a similar early embryology of the neuroectoderm leading to a hollow dorsal neural tube [neurulation] associated with a notochord and axial musculature) that is commonly recognized as the diagnostic complex of synapomorphies uniting (larval) tunicates, cephalochordates, and craniates (chordates). Garstang's additional hypothesis (1928) of secondary mobility of cephalochordates and craniates through neoteny of a tunicate ancestor is widely accepted and highly plausible (Fig. 1.8). Accordingly, the life history stage that becomes the adult mode of life in cephalochordates and craniates initially is intercalated between the planktonic and adult stages of a chordate ancestor. Thus, although it is reasonable to compare the *adult* CNS of tunicates with that of any other bilaterians, the neural tube of chordates would represent an evolutionary novelty that has no homologue in any other taxon. In fact, the whole tissues giving rise to the adult tunicate body originate from the head portion of the chordate larva; the tail containing the neural tube, chorda, and musculature is resorbed (Jeffery and Swalla, 1997). The primordial neural cells forming the adult nervous system (cerebral ganglion) of tunicates reside as an undifferentiated cell mass in the chordate stage larval head (Koyama and Kusunoki, 1993). The larval neural tube is thus not transformed into the adult tunicate CNS.

What about the collar ganglion (*Kragenmark*) of hemichordates, which, based on a neurulation like development (Schwartz, 1973) during metamorphosis of the planktonic (dipleurula) larva and adult location, has been homologized with the neural tube of chordates? For the latter to be true, one would have to accept that the adult stages of hemichordates (enteropneusts, pterobranchs) correspond to the larval (chordate) and not to the adult stage of tunicates. Although many missing developmental and adult characters (e.g., absence of a chorda and axial musculature) speak against this scenario, more detailed developmental studies are needed in hemichordates. Unfortunately, hemichordates are not treated in a recent excellent comparative embryology text of Gilbert and Raunio (1997). Alternatively, the collar ganglion of hemichordates could be interpreted as the retention of the basic bilaterian cerebral ganglion rather than being homologous to the neural tube of chordates. The existence of extensive ventral and dorsal medullary cords in enteropneusts (Knight-Jones, 1952) supports this interpretation.

Nevertheless, it is possible that a hypothetical deuterostome ancestor had a triphasic life history (i.e., a planktonic larva, a freely mobile chordate larva, and adult stage). In such a scenario, one could compare and eventually homologize the adult nervous system of echinoderms to the chordate CNS. Accordingly, we would have to assume that echinoderms alter their development during the very early chordate larval stage and become radially symmetrical, including their nervous systems. In both hemichordates and echinoderms, one would have to look during metamorphosis of the dipleurula larvae for indications of synapomorphies typical of the chordate larval stage of tunicates, such as neurulation (for echinoderms, compare Heinzeller and

Welsch, this volume, chapter 2). Neurulation clearly does not occur in nervous system development during the metamorphosis of a (trochophora-type) planktonic larva in any other bilaterian. Lacalli (1994) provides clear evidence that the development of the adult plathelminth CNS (i.e., the basic bilaterian *Bauplan*) develops differently and independently from the nervous system (i.e., the ciliary bands) of the planktonic Müller larva. This might well be the ancestral developmental pattern that is altered in deuterostomes where the ciliary bands are assumed to transform into a neural tube as part of the altered ontogeny leading to the chordate larval stage (the Auricularia hypothesis; see earlier). If this ontogenetic change occurred only at the base of the chordates (and not of the deuterostomes), however, the echinoderm and hemichordate nervous systems could be reasonably compared and homologized only with the altered ancestral basic bilaterian *Bauplan*. Because we will probably never know for sure at what stage of deuterostome evolution the triphasic life history originated, this problem may never be resolved satisfactorily. If deuterostomes turned out to be the outgroup of all other bilaterian taxa (Fig. 1.4), they might have had a different life history and consequent development from the beginning.

A rather different scenario of deuterostome evolution is given by Jefferies' (1986), who assumes that hemichordates are the outgroup of both echinoderms and chordates and that a hypothetical sessile hemichordate ancestor gave rise to both echinoderms and (secondarily) motile chordates (calcichordates, mitrates). This hypothetical ancestor is assumed to already have had a craniate-type nervous system (including major craniate brain parts and cranial nerves; compare Fig. 1.9, below). In this scenario, cephalochordates form the outgroup to a tunicata/craniate sister taxon and have a reduced nervous system, and tunicates would have become secondarily sessile again. Because this scenario is based entirely on highly controversial paleontological data, I will not further discuss it regarding CNS evolution.

Conclusion

A phylogenetic analysis at this rather general morphological level suggests that a brain (i.e., cerebral or supraoesophageal ganglion) and medullary cords originated at the base of bilaterian evolution. A cerebral ganglion and medullary cords were retained in an evolutionary continuous history in almost all evolutionary bilaterian lineages, possibly including the deuterostome lineage, and are thus homologous among them. Many alterations of the medullary cords (segmentation, fusion of ganglia) and especially of the cerebral ganglion (expansions, inclusion of other parts of the CNS) can be recognized in various bilaterian lineages independently. Besides many independent increases in complexity of the brain (insects, cephalopods, craniates), simplification must also have occurred (aceolomorph plathelminths, various times within the major arthropod groups, maybe tentaculates and early deuterostomes, salamanders among craniates). Regarding chordate CNS evolution, one must conclude that if Garstang's theory of neoteny is correct, then the craniate brain and spinal cord, as well as the cephalochordate and tunicate neural tube (but not the adult tunicate, hemichordate, and echinoderm nervous systems) are

homoplastic to all other bilaterian CNS manifestations. If Garstang's Auricularia hypothesis is correct, at least part of the adult echinoderm and hemichordate CNS might be homologous to the craniate CNS.

EARLY GENES IN NEURAL DEVELOPMENT—DO THEY TELL A DIFFERENT STORY?

Development and *Bauplan* of the Vertebrate CNS

The morphogenetic events and molecular genetic mechanisms during early brain development are fundamental for the understanding of the craniate (vertebrate) brain *Bauplan*. In the last decade, two findings marked a considerable progress in that understanding. First, there was a rediscovery of the fact that the conventionally described five parts of the adult vertebrate brain are preceded in early development by a more fundamental segmentation (neuromery) of the brain (Puelles und Rubenstein, 1993) (Fig. 1.9A). Although segmental elements (neuromeres) in the vertebrate brain had already been described morphologically decades ago (e.g., Rendahl, 1924; Bergquist, 1932; Vaage, 1969), the reality of neuromeres was accepted only after modern methods confirmed their existence. For example, there is a spatiotemporally ordered gene expression in the early embryonic vertebrate brain, and certain neuromeres may be characterized by a selective gene expression pattern. A second important realization was that many early developmental genes also occur in invertebrates, for example, in *Drosophila*, where they are expressed in a similar fashion. In the following, I discuss these two major results of developmental biology and point out some consequences for brain evolution.

Classic embryology states that the vertebrate brain traverses a three-vesicle stage by exhibiting a most caudal rhombencephalic vesicle (rhombencephalon, including the metencephalon and myelencephalon), a middle mesencephalic vesicle (mesencephalon), and an anterior prosencephalic vesicle (prosencephalon, including the diencephalon and telencephalon). Subsequently, the brain enters the five-vesicle stage, representing the *Anlage* of the five major adult brain parts. Proponents of the neuromeric theory emphasize that slightly earlier in vertebrate development, a more fundamental compartmentalization along the longitudinal brain axis exists. According to this view, at least the rhombencephalon (hindbrain)—if not the whole brain—is subdivided into a larger number of transitory elements (neuromeres). Originally, the description of neuromeres was largely based on repetitive alternating swelling and narrowing of the rhombencephalic neural tube. These descriptions were, however, viewed as artifactual for most of the twentieth century. Nowadays, the existence of neuromeres in the rhombencephalon (i.e., rhombomeres) is widely accepted because a wealth of modern studies document specifically the segmental organization of the rhombencephalon. For example, cellular clonal restriction within a neuromere, segmental patterning of first neurons and of axonal sprouting, distribution of glia, and certain gene expression patterns respect rhombomere boundaries

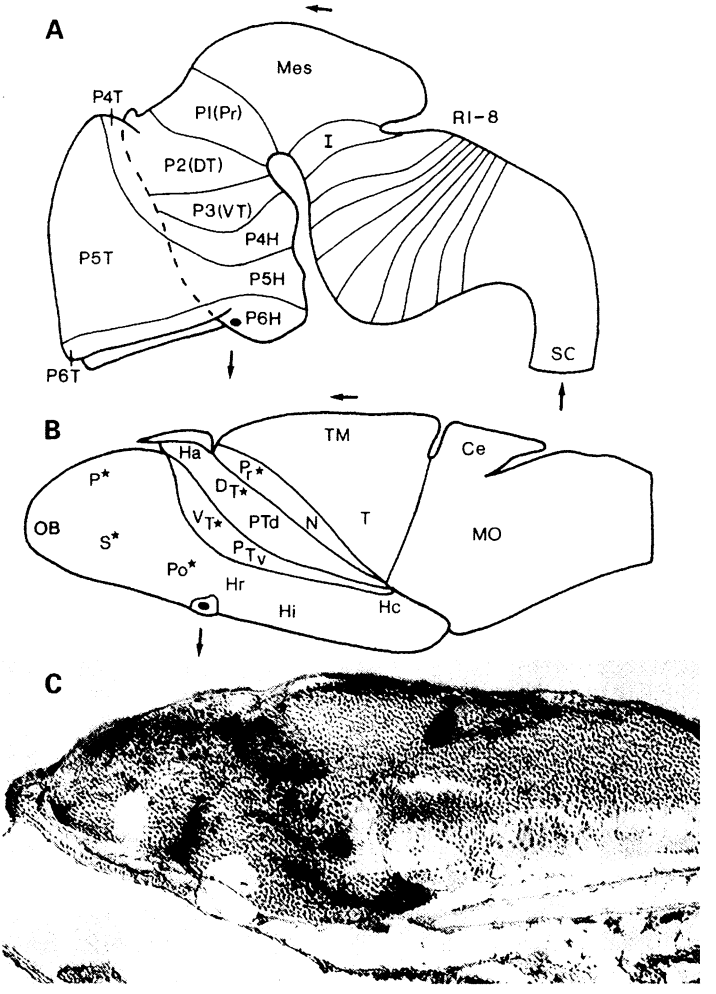


Figure 1.9. (*A*) Sagittal view of neuromeric model for the amniote brain (Puelles and Rubenstein, 1993). (*B*) Its application to the zebrafish forebrain, based on the distribution of proliferation zones (Wullimann and Puelles, 1999). (*C*) A sagittal section of the zebrafish brain immunoreacted for the proliferation marker proliferating cell nuclear antigen. In A and B, arrows designate axis of neural tube and black dot indicates the optic chiasma. The distribution of distinct proliferation centers in the mesencephalon and forebrain is consistent with the prediction of the neuromeric model that three prosomeres exist rostral to the mesencephalon, that is, the pretectal one (P1), the dorsal thalamic one (P2), and the ventral thalamic one (P3), because each of those prosomeres has separable alar plate (Pr*, DT*, VT*) and basal plate (N, PTd, PTv) proliferation zones. More rostrally (telencephalon and hypothalamus), the distribution of proliferation zones is not apparently related to the existence of three additional prosomeres in the zebrafish. Ce, corpus cerebelli; DT, dorsal thalamus; Ha, habenula; Hc, Hi, Hr, caudal, intermediate, rostral hypothalamus; I, isthmic segment; Mes, mesencephalon, MO, medulla oblongata; N, proliferation in the area of the nucleus of the medial longitudinal fascicle; OB, olfactory bulb; P*, pallial proliferation zone; Po*, preoptic proliferation zone; Pr, pretectum; PT, posterior tuberculum area; PTd, PTv, dorsal, ventral proliferation zone of PT; R1–8 rhombomeres 1–8, P4H, P5H, P6H, prosomeres 4–6 (hypothalamic portions); P4T, P5T, P6T, prosomeres 4–6 (telencephalic portions); S*, subpallial proliferation zone; SC, spinal cord; T, tegmentum, TM, tectum mesencephali; VT, ventral thalamus.

(Holland und Hogan, 1988; Lumsden, 1990; Wilkinson and Krumlauf, 1990). There is also evidence for neuromeres in the prosencephalon (forebrain, including the diencephalon and telencephalon), for example, clonal restriction of cell lines (Figdor and Stern, 1993). Also, the spatial pattern of early proliferation activity in the forebrain supports a prosomeric organization (Wullimann and Puelles, 1999), at least in the posterior forebrain (P1–P3 in Fig. 1.9B,C). The most comprehensive neuromeric model of Puelles und Rubenstein (1993) currently available integrates data from classic morphology with those mentioned from modern studies, including gene expression data.

According to this model, the vertebrate brain consists of an isthmic (0), plus seven to eight more caudally located rhombencephalic neuromeres (rhombomeres), a mesencephalic neuromere (mesomere), and six more neuromeres in the prosencephalon (prosomeres; Fig. 1.9A; note that the direction of numbering is opposite for rhombomeres and prosomeres). The essentials of the model are as follows. Early emerging neural tube flexures tilt the originally straight longitudinal axis of the brain, for example, the axis of the prosencephalon deviates almost 180° in comparison to that of the rhombencephalon. Thus, the rostral tip of the brain lies in the region of the optic chiasma (black dot located at the lower—not the left—boundary of the prosencephalon in Fig. 1.9A,B). According to this longitudinal axis, the ventral prosencephalon is directly adjacent to the ventral rhombencephalon. Furthermore, the diencephalon is not simply the caudal part of the forebrain as was assumed in some traditional models. In the neuromeric model, the diencephalon consists of three complete prosomeres plus the basal parts of three additional prosomeres. The first, most caudal prosomere (P1) includes the pretectum; the rostrally adjacent second prosomere (P2) includes the epithalamus and dorsal thalamus; and the third prosomere (P3) represents the ventral thalamus of the traditional diencephalon. The ventral portions of the final three more rostral prosomeres represent the hypothalamus (P4H–P6H in Fig. 1.9A) and complete the diencephalon. Accordingly, the hypothalamus is—with respect to the above-mentioned longitudinal axis—not considered to be the ventral part of the classic diencephalon, but is in fact the ventral part of those prosomeres giving rise to the telencephalon with their dorsal portions (P4T–P6T). The telencephalon (as well as the hypothalamus), in contrast to the remaining four classic brain parts, is not composed of complete neural tube segments, because it lacks their respective basal parts.

These differences between the neuromeric and traditional models in the allocation of brain regions based on a newly defined axis are of great importance because the ventral and dorsal aspects of the neural tube differ in many respects (e.g., origin of motor and sensory neurons from ventral basal and dorsal alar plates, respectively). In the neuromeric model, the classically recognized four longitudinal zones of the neural tube (i.e., from ventral to dorsal, the floor, basal, alar, and roof plates) do all continue up to the anterior end of the brain in the area of the optic chiasma. This newly defined longitudinal and assumed overall segmental organization of the brain of the neuromeric model is of great predictive value and is open to be tested on all levels of investigation.

Early Regulatory Genes and Neuromeres in the Vertebrate Bain

The activity of many early regulatory genes has been visualized meanwhile by in situ hybridization in various metazoans. The homeotic genes of the *Hox-B* complex are expressed (e.g., in portions of the CNS and other segmental organs; Graham et al., 1989; Hunt and Krumlauf, 1992) in an anteroposterior order that parallels their spatial order in the vertebrate genome. More specifically, the anterior ends of different *Hox-B* gene expression domains proceed successively more rostrally in a graduated manner and respect various rhombomere boundaries (Fig. 1.10). The expression domains of certain additional early regulatory genes, such as *Krox-20*, outline particular rhombomeres and thus respect anterior as well as posterior rhombomere boundaries. Such evidence is generally taken as the most convincing proof for the existence of rhombomeres. Like many other early regulatory genes, the *Hox* genes are transcription factors, (i.e., their proteins interact with the DNA and regulate the expression of various other genes). Thus, many of those regulatory genes not only act early in embryogenesis, but, by activating the transcription of other genes, they also stand at a rather high hierarchical level during development. Evidently, such genes have a much greater influence on the phenotype than structural genes, for example.

The anteroposteriorly graduated rostral expression boundaries of various *Hox-B* genes (as well as the expression of other genes) lead to a particular combination of gene activity in each rhombomere during early brain development that is thought to specify the interrhombomeric differences and, consequently, the adult hindbrain phenotype. Indeed, experimental extension of the expression domains of particular *Hox-B* genes to more anterior rhombomeres results in an altered phenotype of those rhombomeres (Krumlauf, 1993). While the homeotic genes of the *Hox* complex have no expression domains in the prosencephalon (forebrain) and mesencephalon, various other regulatory genes containing a homeobox (e.g., *Otx, Emx, Dlx, Gbx*; Simeone et al., 1992, Boncinelli et al., 1993; Bulfone et al., 1993; Millet et al., 1996) are expressed there during early development (Fig. 1.10). The caudal expression boundary of *Otx2* coincides with the midbrain–hindbrain boundary, and the rostral one extends almost to the tip of the brain in the region of the optic chiasma (see earlier discussion). Because *Otx1* as well as various *Emx* and *Dlx* genes have more restricted expression domains, a graduated expression pattern similar to that formed by the *Hox-B* genes in the rhombencephalon is observed in the midbrain and forebrain. Studies on null mutants and their phenotypes (*Otx2*: Bally-Cuif and Boncinelli, 1997; *Emx1/2*: Yoshida et al., 1997) also suggest similar functions to that of the *Hox* complex (i.e., anteroposterior patterning of the more anterior brain parts).

Early Regulatory Genes and the Insect CNS

Orthologues of many regulatoy genes now known in vertebrates were discovered much earlier in the fruit fly *D. melanogaster*. The homeotic (HOM) genes of the

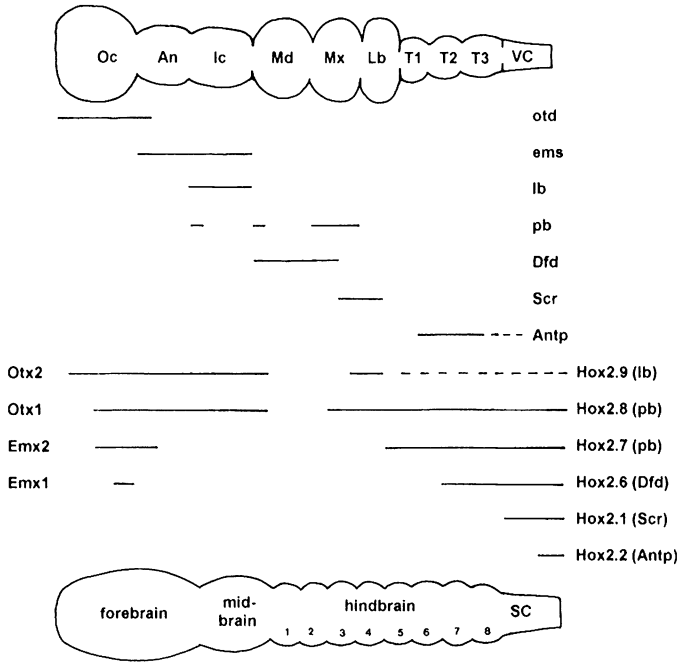


Figure 1.10. Early regulatory gene expression in insect CNS (*upper panel*) and vertebrate CNS (*lower panel*). Numbers indicate vertebrate rhombomeres. *Drosophila* genes: *Antp, antennapedia; Scr, sex combs reduced; Dfd, deformed; pb, proboscipedia*. Abbreviations for other genes are mentioned in the text. *Drosophila* head segments and corresponding neuromeres: Oc, ocular (protocerebrum); An, antennal (deutocerebrum); Ic, intercalary (tritocerebrum); Md, mandibular; Mx, maxillary; Lb, labium segment (last three containing together the subesophageal ganglion); T1–3, thoracal (leg) segments (and respective ganglia); VC, ventral cord ganglia. Note that whereas in vertebrates the anterior ends of expression domains of various *Hox* genes are exactly *at* the respective interrhombomeric boundaries (as summarized by Holland et al., 1992), some corresponding expression boundaries in the insect brain mark *hemisegments* (posterior boundary of *Dfd*, both boundaries of *Scr* and *Antp;* after Kaufman et al., 1990; *pb* after Telford and Thomas, 1998). The expression domains of vertebrate forebrain and midbrain genes (*Otx, Emx*) are not definitively assigned to neuromeric boundaries here. However, both *Otx1* and *Otx2* expressions appear to respect posteriorly the midbrain/hindbrain boundary. Although a small, most rostral telencephalic area is spared, the *Otx2* expression extends considerably more rostral than that of *Otx1*. Rostrally, the *Emx2* expression is coextensive with that of *Otx1*, and, posteriorly, it respects the P3/P2 boundary. *Emx1* has a more restricted, exclusively telencephalic expression domain (after Boncinelli et al. 1993). In insects, brain segment boundaries definitively are transgressed by the *otd*, but not the *ems*, expression pattern; in contrast to vertebrates, the two genes additionally have a more posterior CNS expression domain, that is, in the ventral cord ganglia (Reichert and Boyan, 1997).

antennapedia–ultrabithorax complex, which determine segment identity (Gehring, 1987), correspond to the *Hox-B* cluster of vertebrates. As in the latter, the corresponding homeotic genes of insects are aligned in the genome in the same order as they are phenotypically expressed in the anteroposterior axis. In particular, these orthologues in *Drosophila* of the vertebrate homeotic *Hox-B* genes have a similar

spatial expression pattern in parts of the CNS when compared with vertebrates (Graham et al., 1989; Carroll, 1995). Equally striking, there are orthologues of vertebrate *Otx* and *Emx* genes in *Drosophila* (i.e., *orthodenticle* [*otd*], and *empty spiracles* [*ems*], respectively), and their expression domains are restricted to anterior head neuromeres (supraoesophageal—but not suboesophageal—ganglion), and ventral cord ganglia (Finkelstein and Perrimon, 1990; Finkelstein and Boncinelli, 1994; Reichert and Boyan, 1997; compare Fig. 1.10). For example, the *otd* gene is expressed in the protocerebrum and in the most anterior part of the deutocerebrum, and the *ems* gene is expressed in deuto and tritocerebrum (Reichert and Boyan, 1997). Accordingly, *Drosophila* that are mutant in *otd* or *ems* lack a protocerebrum or deuto- and tritocerebrum, respectively (Hirth et al., 1995). Furthermore, the vertebrate *Otx1* and insect *otd* genes can functionally replace each other in development; murine *Otx1* null mutants with an introduced *Drosophila otd* gene show a rescued brain phenotype (Acampora et al., 1998; Leuzinger et al., 1998).

Apparently, many such early regulatory genes were present in the last common ancestor of vertebrates and insects. What does this fact reveal about the brain and CNS phenotype in that ancestor? An often heard argument is that those similarities in sequence, genomic and phenotypic alignment, and developmental function of early regulatory genes support the early existence of a complex brain and its concomitant developmental plan, which were both established only once close to the origin of bilaterians (Reichert and Boyan, 1997). The similarities sometimes are further taken as evidence that segmentation (including that of the CNS) is plesiomorphic for bilaterians or even that a *strickleiter* nervous system may be plesiomorphic for the vertebrate brain and spinal cord (De Robertis, 1997). Major differences in the *Bauplan* of the vertebrate and insect CNS exist, however, and are in need of explanation. Nobody would ever mistake an insect brain for a vertebrate brain. Furthermore, comparative analyses of phenotypic CNS evolution (even on a rather general level; see earlier) are in contradiction to easily digestable generalizations such as the often heard "There is but one animal".

Phylogenetic Interpretation of Molecular Genetic and Phenotypic Data

How can these apparently contradictory conclusions drawn from neurogenetic or morphological data be reconciled? Let us consider brain subdivisions of various invertebrates. The three divisions of the insect brain (i.e., proto-, deuto-, and tritocerebrum) are phylogenetically best interpreted as having arisen through fusion of originally similar, segmentally organized ventral cord ganglia. In contrast, the trilobed brain of some polychaete annelids (*Eunice*; see earlier) most likely did *not* arise through fusion of segments, but originated through elaboration of the prostomium ganglion. Its anteroposterior specification may, however, turn out to be controlled by orthologous homeobox genes active in the insect brain. Indeed, in one leech species (which does not have a trilobed brain), the *otd*- orthologue *Lox22–Otx* is reported to be expressed mainly in the prostomium ganglion (Bruce and Shankland, 1998). The developmental

program that creates a trilobed brain in polychaetes would nevertheless have a different evolutionary history in insects and annelids (i.e., the feature of having a trilobed brain could not be considered homologous in the phenotype).

Let us return to the *Hox-B* example. Craniates and, consequently, vertebrates had a rhombencephalon from the very beginning, and the said genes were undoubtedly always involved in the development of this most caudal brain part. The most rostral expression domains of the orthologous genes of the antennapedia–ultrabithorax complex of insects are in the suboesophageal ganglion, which subserves the various segmental mouth appendages, as well as in the tritocerebrum and most posterior part of the deutocerebrum, but *not* in the protocerebrum (Fig. 1.10). More specifically, only *labial* and *proboscipedia* are expressed in the brain proper (trito- and deutocerebrum); the other genes of the complex have more posterior expression domains in the suboesophageal ganglion. The latter is multisegmental and includes the ganglia of the mandibular, maxillary, and labium segments. Rogers and Kaufman (1996) recently confirmed the assumption of a six-segmented insect head with the *engrailed* protein pattern, although a total of seven segments (with an additional segment anterior to the ocular or protocerebral one) has also been suggested (Schmidt-Ott et al., 1994). If one accepts that the suboesophageal ganglion is the posterior part of the insect brain, then an outgroup comparison renders evidence that such a ganglion did not exist in the outgroups of insects in the primitive condition, but corresponds with several ganglia of the ventral chain that are not included in the brain. In the phenotype that represents a functional morphological unit (i.e., the brain has functions different from those of ventral cord ganglia), the expression domains of the said homeotic genes would not be included in the brain proper in the ancestral condition. Of course, the said homeotic genes can be expected to have expression domains in the corresponding ventral chain ganglia of these arthropods as well. Consistent with this, Kourakis et al. (1997) showed the anterior *Hox* gene expression boundary between ventral cord ganglia and prostomium ganglion in an annelid (i.e., leech). Although these corresponding ventral chain ganglia are homologous to the respective segmental ganglia forming the insect suboesophageal ganglion, they are not homologous when their phenotypic aspect of being a brain part is considered. These anterior ventral chain ganglia that correspond with the insect suboesophageal ganglion are not part of the brain in the plesiomorphic condition in arthropods, let alone in articulates, coleomates, or bilaterians.

Thus, the *Hox-B*/HOM cluster likely was always expressed in the posterior brain of vertebrates, but not in that of arthropods, because the latter included the suboesophageal ganglion only later into the cephalic portion of the CNS. With respect to the brain of the hypothetical ancestor of vertebrates and insects, this means that the corresponding (orthologous) genes were expressed in the CNS, but that a complex rhombencephalon or suboesophageal ganglion did not yet exist. *This shows that one cannot directly derive a particular phenotype from the mere existence and orderly expression of early regulatory genes. Molecular genetic and phenotypic data need to be jointly interpreted in a phylogenetic context.* A prediction to be made here is that plathelminths and nemertines should express orthologues of the *Hox-B* cluster in the anterior part of the medullary cords, but not in the cerebral ganglion.

The expression domains of the *Otx/otd* and *Emx/ems* genes are certainly located within the brain proper of both vertebrates and insects. A brain containing three to four neuromeres is unlikely, however, to be ancestral for all arthropods, and it certainly is not plesiomorphic for articulates or even protostomes (see earlier). Genes specific for the anterior brain would be expected to be expressed in these phenotypes within the most rostral ventral cord ganglia as well. Actually, in the leech, *labial*, which is the *Hox* gene with an expression domain extending into the trito- and deutocerebrum in *Drosophila*, is expressed up to the most rostral ventral cord ganglia, but not in the cerebral ganglion (Kourakis et al., 1997). In contrast, vertebrates had a constant anteroposterior sequence of brain parts from the very beginning, including a fore-, mid-, and hindbrain. It is therefore highly unlikely that the last common ancestor of arthropods and vertebrates had a *brain* showing a graduated gene expression pattern characterized anteriorly by *Otx/Emx* orthologues and posteriorly by *Hox* orthologues.

Current evidence suggests that the above discussed genes of the HOM/*Hox* complex and the *Otx/otd* and *Emx/ems* genes form part of the *zootype* and are plesiomorphic for metazoans (Slack et al., 1993), including those that are not segmentally organized. This suggests that homeotic genes are not strictly correlated with the phenotypic context in which they were first discovered (i.e., segment specification and *strickleiter* nervous system). Ten years ago, Akam (1989) proposed that the reason why genes of the HOM/*Hox* complex are highly conserved in bilaterian phylogeny is that they are plesiomorphically responsible for positional information, that is, for the anteroposterior specification of the bilaterian body, and that these already existing genes were used and further elaborated on by arthropods in the context of an overall segmentation (including that of the CNS) and by vertebrates in relation to segmental (metameric) organ systems independent of each other. This scenario certainly is more parsimonious than one assuming that segmentation as seen in articulates (including a *strickleiter* nervous system) was plesiomorphic for bilaterians because many phenotypic alterations leading away from an initially segmented body would then have to be invoked in all bilaterians outside the articulates.

CONCLUSION

The number of known early active genes common to invertebrates and vertebrates that share similar developmental functions is growing almost daily, and this fact is amazing in itself. There is clear evidence that genes of the HOM/*Hox* complex and eventually the *Otx/Emx* genes and their orthologues (and many other early regulatory genes) existed and were active in the developmental context of anteroposterior specification at the outset of bilaterian history. Head segmentation (including that of the CNS) of insects and neuromeres in vertebrates is a special case of this specification. The fact that these genes originated very early in bilaterian evolution does not mean that any recent phenotype (e.g., a complex insect or vertebrate brain or the *strickleiter* nervous system) may be deduced to be plesiomorphic for bilaterians or for the last common ancestor of vertebrates/insects.

Conversely, it was a misinterpretation of comparative morphology that seemingly convergent phenotypes (i.e., our example of a complex hindbrain or suboesophageal ganglion) *must* have a different genetic basis. Obviously, data from all levels of investigation have to be jointly interpreted. The stunning universality in developmental function and systematic distribution of many early regulatory genes is only half of the lesson to be learned here. The second half of the lesson is that modifications of the spatiotemporal interactions of these genes are *the* major force in creating new phenotypes on which natural selection might act in evolution. Comparative neurobiologists by definition are interested in evolutionary history and, thus, must still care about the study of phenotypic CNS evolution that complements—but cannot be replaced by—the fascinating molecular genetic work.

ACKNOWLEDGEMENTS

I am greatly indebted to the grandseigneur of comparative neurobiology, Theodore H. Bullock, for taking the time to make many illuminating remarks after reading a more immature version of the manuscript and Gerhard Roth for many clarifying discussions on metazoan brain evolution. I also thank Catherina G. Becker, Thomas Becker, Thomas Müller, Gerhard Roth, and Gerhard Schlosser for making valuable comments on the manuscript. The author's research is supported by the DFG (Wu 211/1–4).

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