ELSEVIER

Review

Evolution of striated muscle: Jellyfish and the origin of triploblasty

Katja Seipel, Volker Schmid*

Institute of Zoology, Biocenter/Pharmacenter, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Abstract

The larval and polyp stages of extant Cnidaria are bi-layered with an absence of mesoderm and its differentiation products. This anatomy originally prompted the diploblast classification of the cnidarian phylum. The medusa stage, or jellyfish, however, has a more complex anatomy characterized by a swimming bell with a well-developed striated muscle layer. Based on developmental histology of the hydrozoan medusa this muscle derives from the entocodon, a mesoderm-like third cell layer established at the onset of medusa formation. According to recent molecular studies cnidarian homologs to bilaterian mesoderm and myogenic regulators are expressed in the larval and polyp stages as well as in the entocodon and derived striated muscle. Moreover striated and smooth muscle cells may have evolved directly and independently from non-muscle cells as indicated by phylogenetic analysis of myosin heavy chain genes (MHC class II). To accommodate all evidences we propose that striated muscle-based locomotion coevolved with the nervous and digestive systems in a basic metazoan Bauplan from which the ancestors of the Ctenophora (comb jellyfish), Cnidaria (jellyfish and polyps), as well as the Bilateria are derived. We argue for a motile tri-layered cnidarian ancestor and a monophyletic descent of striated muscle in Cnidaria and Bilateria. As a consequence, diploblasty evolved secondarily in cnidarian larvae and polyps.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Basic Bauplan; bHLH genes; Cnidaria; Entocodon; Hydrozoan jellyfish; Medusa; Mesoderm; Metazoan evolution; Monophyletic descent; Origin of triploblasty; Prebilaterian ancestor; Striated muscle; Zootype

Introduction

In recent years molecular studies have provided much insight into developmental gene networks and how they may have cooperated in the evolution of the bilaterian body plans (Caroll, 2000; Erwin and Davidson, 2002; Koonin et al., 2000; Peterson and Davidson, 2000; Revilla-i-Domingo and Davidson, 2003; Wray, 2003). Gene networks interact in metazoans to regulate axis formation, gastrulation, as well as organ and limb formation. Rapidly accumulating molecular phylogenetic data are now beginning to provide a coherent picture of the relations between major animal groups (Collins, 1998; Collins and Valentine, 2001; Conway-Morris, 2003; Halanych, 2004). However, as recently exemplified by the cnidarian Wnt gene family (Kusserow et

al., 2005), there is no simple relationship between genetic and morphological complexity. Molecular information based on rRNA sequences or mitochondrial DNA structure may be helpful in classifying the basal metazoans in relation to the bilaterians, but it is not sufficient for reconstructing the anatomy and developmental patterns of the prebilaterian ancestor. The original bilaterian Bauplan may be reconstructed based on anatomical and genomic information in the bilaterian model organisms as well as on the anatomy of bilaterian fossils. In order to speculate on the ancestral prebilaterian Bauplan, however, additional information is required. To this end the analysis of developmental regulators of the basal metazoan outgroups is very promising, especially since poriferan and cnidarian anatomies may have changed little since Precambrian times (Chen et al., 2000, 2002; Li et al., 1998).

The goal of this review is to discuss recent cellular and molecular data providing information on the evolution of a hypothetical prebilaterian Bauplan (Fig. 1A) composed of a

* Corresponding author.
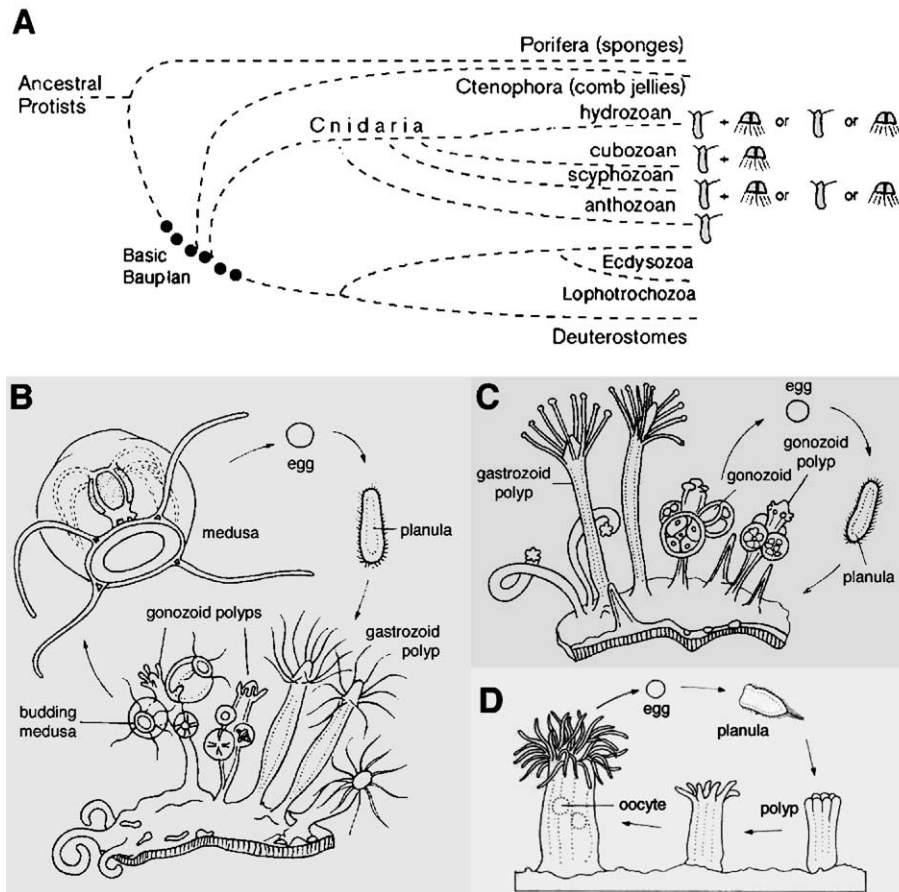*E-mail address:* V.Schmid@unibas.ch (V. Schmid).

Fig. 1. Cnidarian evolution and life cycles. (A) Overview of the evolutionary relations in the metazoan kingdom (modified after Galliot and Schmid, 2002). The evolutionary position of the Basic Bauplan is indicated by black dots. The adult stages of the four cnidarian classes are indicated by schematic drawings. While the Cubozoa always have polyp and medusa stages, either one can be reduced in the Hydrozoa and Scyphozoa. The Anthozoa have only the polyp stage. The life cycles of the hydrozoans *P. carnea* (B) and *Hydractinia echinata* (C) and a representative anthozoan (D) were adapted from Tardent, 1978.

digestive, reproductive, nervous, and locomotive system based on striated muscle. We discuss the evolution of striated and smooth muscle, the formation of the germ layers and axial symmetry and present a model. We propose that metazoan evolution did not necessarily include a diploblast stage, as the evolution of striated muscle-based locomotion most likely was based on an integrated anatomy assembled from three germ layers.

The outgroups to Bilateria and the early evolution of a common Bauplan

Muscle differentiation in the basal non-bilaterian phyla

Metazoan striated muscle may be monophyletic or polyphyletic. The former possibility implies the existence of a common metazoan ancestor with striated muscle, the latter the repeated emergence of striated muscle in several ancestral life forms lacking striated muscle. In the same manner there are two possibilities for the evolution of a metazoan lacking striated muscle. Either it derives from an ancestor lacking muscle or it has lost the muscle tissue of a common metazoan ancestor by adaptive processes. The outgroup phyla are the most promising animal groups for evaluating these possibilities (Fig. 1A). Porifera have neither muscle nor nerve cells. They have featured sessile life styles ever since Precambrian times (Li et al., 1998) and their anatomy, development, and consequently gene structure and gene arrangement may show few traces of a hypothetical presessile ancestor (Manuel et al., 2003). They likely diverged from the prebilaterian line before the basic Bauplan was established (Fig. 1A). Placozoa have a low level of tissue organization and apparently lack muscle and nerve cells (Grell et al., 1980) but some cells react with antibodies against the neuropeptide RFamide (Schuchert, 1993a). Furthermore they have genes thought to be specific for mesoderm in bilaterian animals (Martinelli and Spring, 2003). Hence the simple anatomy appears to hide a more complex genetic background and may either represent a reduced derivative of a metazoan, possibly medusozoan, Bauplan (Cavalier-Smith and Chao, 2003), or their own evolutionary line (Ender and Schierwater, 2003). Ctenophora have well-developed muscle and nerve systems

(reviewed in Hernandez-Nicaise and Franc, 1993). In the extant Ctenophores the striated muscle is absent from the main body but can occur as non-epithelial muscle in the tentacles of some cydippids, the group with the oldest Devonian fossils (Stanley and Stuermer, 1983). Unfortunately very little is known about their regulatory genes. Molecular phylogenetic data suggest that both the Ctenophora and Cnidaria arose independently within the prebilaterian line, possibly with the Ctenophora preceding the Cnidaria (Ball et al., 2004; Halanych, 2004; Medina et al., 2001; Podar et al., 2001). The phylogenetic position of the Ctenophora is still unclear, some characteristics place them close to the deuterostomes (Nielsen, 1995), others are shared exclusively with the Cnidaria, as the egg polarity, unilateral cleavage, the site of gastrulation in relation to body axis formation and the division of the embryo in four quadrants (Freeman, 1977, 1981; Goldstein and Freeman, 1996; Scholtz, 2004). The Cnidaria are the best-studied basal animals. The cnidarian medusa, or jellyfish, exhibits a well-differentiated and complex anatomy with striated and smooth muscles, nerve systems, and various types of sense organs, including lens eyes (reviewed in Bouillon, 1993; Hyman, 1940; Tardent, 1978). In recent years a number of patterning genes, as well as mesoderm and myogenic regulatory genes, have been analyzed in Cnidaria (Bode, 2001; Finnerty et al., 2004; Galliot, 2000; Galliot and Schmid, 2002; Hayward et al., 2002; Hobmayer et al., 2000; Holstein et al., 2003; Kusserow et al., 2005; Martindale et al., 2004; Miller et al., 2000; Müller et al., 2003; Seipel et al., 2004a,b,c; Spring et al., 2000, 2002). In the subsequent paragraphs this information is reviewed with special attention to jellyfish and the evolution of muscle tissue as well as the germ layers.

The complex life cycles of cnidarians and the mesoderm question

The cnidarian life cycle features the pelagic larva, the sessile polyp, and the free-swimming medusa (Fig. 1B). The full life cycle is present in the majority of cnidarian species pertaining to the Hydrozoa, Cubozoa, and Scyphozoa, also grouped together as Medusozoa. In Hydrozoa and Scyphozoa the polyp or the medusa stage can be reduced (Figs. 1A and C) or completely absent (Bouillon, 1993; Piraino et al., 2004). The Anthozoa represent the only cnidarian class that has no medusa stage (Figs. 1A and D). Most cnidarian species use nematocytes to prey on ecdysozoans, few live in part or entirely off symbiotic algae, and one juvenile medusa (*Obelia*) is known to feed on bacteria. In general, cnidarian larvae and polyps are composed of two epithelial cell layers. This anatomical trait accounts for the diploblast classification of the cnidarian phylum. Both epithelial layers are interspersed with other cell types (Bouillon, 1993; Hyman, 1940; Tardent, 1978). The majority of larval cells including all gastrodermal and most epidermal cells contain smooth muscle myofibers (Bouillon, 1993; Doumenc and Van Praet,

1987). Epithelial smooth muscles are generally regarded as primitive features and typical for Cnidaria. There are however reports for mesoderm-derived smooth muscle epithelia lining coelomic tissues in various bilaterian phyla including the Acrania (Storch and Welsch, 1974). In contrast to the bi-layered larva and polyp, the bell of all Medusozoa is basically composed of four cell layers, two of which are particular to the jellyfish, including a well-developed layer of striated muscle (Fig. 2A; Bölsterli, 1977; Gröger et al., 1999; Schuchert et al., 1993; Weber et al., 1987). Contrary to most schematized presentations the layer of mononucleated, non-fused striated muscle cells of the medusozoan jellyfish is covered entirely or partially by an epidermal layer (Fig. 2; Bouillon, 1993; Chapman, 1968, 1999; Franc, 1993; Hyman, 1940).

The evolutionary position of the Medusozoa is controversial and has been discussed since the 19th century (Ball et al., 2004; Boero et al., 1992; Bouillon, 1993; Brien, 1969; Brooks, 1886; Collins, 2002; Hyman, 1940; Schuchert, 1993b). In the classical phylogeny the Hydrozoa (Medusozoa) are positioned at the base of the cnidarian phylum (Ball et al., 2004; Hyman, 1940). Some phylogenetic studies based on sequences of partial large subunits (Odorico and Miller, 1997), or complete small and large subunits rRNA (Bridge et al., 1995; Collins, 2002; Medina et al., 2001; Podar et al., 2001) and mitochondrial DNA structure, circular in Anthozoa and linear in Medusozoa (Bridge et al., 1992), place the Anthozoa in a basal position within the Cnidaria. Since Anthozoa have no medusa stage this classification led to the hypothesis that the anthozoan ancestor and likely the common ancestor of all Cnidaria and Bilateria was a bi-layered polyp-like, sessile animal (archicoelomate hypothesis; Jägersten, 1955; Sedgwick, 1884) which evolved according to the gastrea hypothesis (reviewed in Grell et al., 1980). Recent data on mesodermal/ myogenic regulators in jellyfish and on the evolution of motor proteins challenge this scenario. The forthcoming discussion attempts to reconcile these divergent views.

The entocodon of the hydrozoan medusa, a mesoderm-like layer?

Direct development of medusae from fertilized eggs occurs in few medusozoan species, but very little is known about this mode of development (Bouillon, 1993). In general the medusa arises from the polyp either by budding (Fig. 1B, Hydrozoa) or by transformation of all or part of the polyp (Scyphozoa and Cubozoa, not shown). Budding is the best-studied mode of medusa development (Bouillon, 1993; Frey, 1968; Hyman, 1940; Kühn, 1910; Tardent, 1978; Weiler-Stolt, 1960). In *Podocoryne carnea* (syn. *Hydractinia carnea*, Anthomedusa, Hydrozoa) the young medusa buds are composed of rapidly dividing undifferentiated cells (Spring et al., 2000) with some resemblance to I-cells of the fresh water polyp *Hydra* (Bölsterli, 1977). These undifferentiated cells represent the
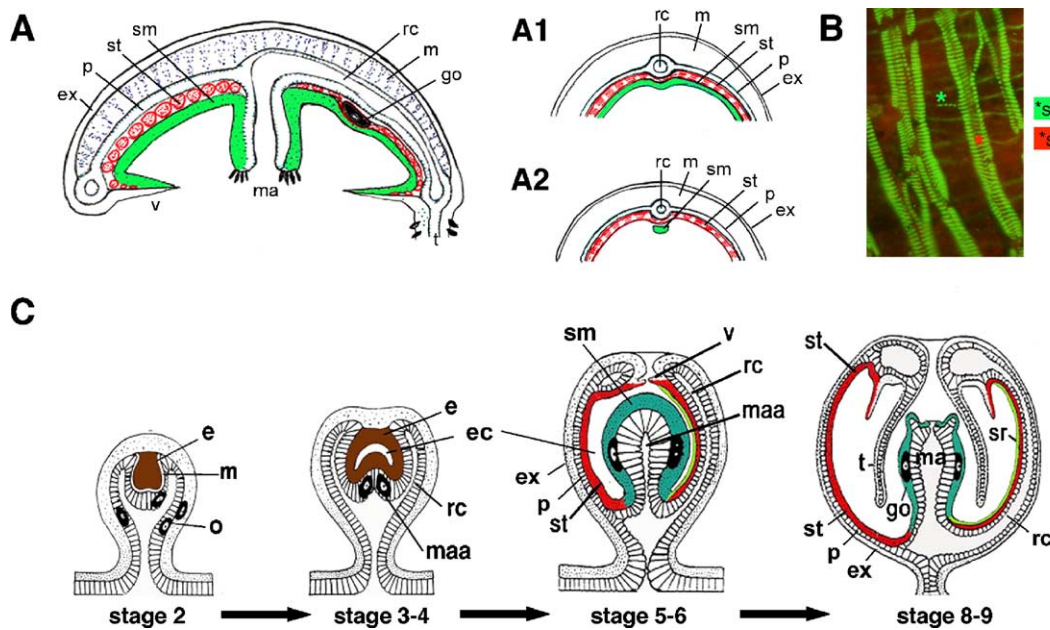
Fig. 2. Anatomy and development of hydrozoan medusae. (A) Distribution of entocodon-derived striated (red) and smooth muscle (green) in the bell of medusae. Sagittal section of a Leptomedusa (A). Cross section through part of the medusa bell of the Leptomedusa *Phiallidium hemisphaericum* (A1), and the Anthomedusa *P. carnea* (A2). In the Leptomedusa (Chapman, 1968) and few Anthomedusa (Hyman, 1940) the striated muscle (red) is completely covered (A, A1) by flagellated smooth muscle epithelia (green), while in the Scyphomedusa and Cubomedusae the striated muscle is covered by a non-muscled epithelium (Chapman, 1999; Franc, 1993). In many Anthomedusa (Bouillon, 1993) the striated muscle is only partially covered (A2) by flagellated smooth muscle over the radial canals. (B) Phalloidin stained preparation of the muscle systems in the bell of the Leptomedusa *P. hemisphaericum*. The radial smooth muscle runs perpendicular to the striated muscle. The tissue has been spread by squeezing for better visibility of the arrangement of muscle fibers. (C) The main stages of medusa development in *P. carnea*, with medusa bud stages 2, 3–4, 5–6, and 8–9, modified after Frey, 1968. Abbreviations: e, entocodon (brown); ec, entocodon cavity; ex, exumbrella; go, gonads; m, mesoglea (ECM); ma, manubrium (feeding organ); maa, manubrium anlage; o, migrating oocytes; p, plate endoderm; rc, radial canal; sm, smooth muscle of the manubrium (dark green); sr, smooth muscle over the radial canal (light green); st, striated muscle (red); t, tentacles; v, velum.

proliferative and migratory phase of myoepithelial cells (Bravermann, 1974; Bouillon, 1993). A candidate mesoderm layer is first observed in the early hydrozoan medusa at bud stage 2 (Fig. 2D), where undifferentiated cells separate exclusively from the distal ectoderm (Bölsterli, 1977; Frey, 1968; Weiler-Stolt, 1960). This additional layer has been called "Mesotheca" (Hamann, 1882), "Glockenkern" (Kühn, 1910), and entocodon (Hyman, 1940). Because the entocodon cells are clearly separated from the ectoderm and endoderm by the formation of an extracellular matrix (Bölsterli, 1977) the entocodon qualifies per definition as a third germ layer (Boero et al., 1998; Nielsen, 1995). At bud stage 3–4, the entocodon enlarges and forms a cavity, the future subumbrellar space (Fig. 2D). At this stage the entocodon cavity has no connection to the outside and is organized as a coelom-like structure. All of the striated and smooth muscles and the RFamide-positive nerve cells of the subumbrella derive from the outer layer of the entocodon (Seipel et al., 2004a; Tardent, 1978). The inner layer of the entocodon forms the smooth muscled epidermis of the manubrium into which the oocytes immigrate (Fig. 2D; Bölsterli, 1977). In some Eumedusozoa the medusa is partially reduced and remains attached to the polyp (Bouillon, 1993). In this case the entocodon-derived muscles are used to eject the gametes

liberated into the coelom-like entocodal cavity through the reduced (gonopore) velar opening. In summary, the developmental histology and the anatomy of the bell of the Medusozoa demonstrates that much of cnidarian anatomy and development cannot be sufficiently explained by diploblasty.

Origins of mesoderm and muscle in the Bilateria

While most bilaterian mesoderm may originate from the mesendoderm (Martindale et al., 2004; Technau and Scholz, 2003), the spiralian and vertebrate mesoderm and muscle cells appear to be of dual origin. In Spiralia mesoderm and muscle derive from the ectoderm (Nielsen, 1995, 2004), and also from the endodermal 4D blastomere (Boyer et al., 1996). The vertebrate striated muscles are predominantly derived from the mesendoderm, whereas the mesectoderm gives rise to the neural crest-derived mesenchymal tissues including smooth muscles, cartilage, and bone (Le Douarin et al., 2004). Additionally, there are reports of an ectodermal striated muscle in the entoproct tentacle (Nielsen and Rostgaard, 1976) and of mesectoderm-derived striated muscle cells in vertebrate ocular structures (Creuzet et al., 2005). It thus appears that muscle tissue can originate from the mesectoderm also in Bilateria.

The presence of striated muscle tissue in a "diploblast" phylum has raised questions about its evolutionary origin. Two observations corroborate the existence of a triploblast jellyfish ancestor: (1) jellyfish striated muscle develops from a mesoderm-like layer (entocodon), and (2) jellyfish myogenesis is controlled by regulators similar to bilaterian mesoderm and myogenic factors.

The molecular analysis of muscle and nerve cell formation in medusa development

The value of anatomical characteristics and of cellular and molecular data for evolutionary studies strongly depends to what extent they can be related to a Precambrian ancestor. Except for the Scleractiniidae (Anthozoa) and Milleporina (Hydrozoa) Cnidaria are soft-bodied animals with poor fossilization capabilities. The moribund jellyfish is prone to rapid decay. This is especially true for the extremely thin-layered, transparent medusa bell, a key structure for identification of fossils. In small-sized medusa the mesoglea of the bell dissolves rapidly followed by tissue fragmentation and dissociation in the benthos (Schmid, 1969); in larger species the buoyancy of the large mesoglea keeps the decaying body in the pelagos. Therefore benthic sediments hardly ever contain intact medusa bodies. Jellyfish fossils are found where animals were washed ashore and immediately covered by sediments. The same parameters are also valid for Ctenophores. Fossils interpreted as cnidarian-like larvae and hydrozoan-like polyps have been described in Precambrian phosphorite deposits of the Doushantuo Formation (Chen et al., 2002). The characteristic stinging cells of the Cnidaria, the cnidocysts, however, have not been described in these fossils. Since cnidocysts of the extant species are sufficiently large and extremely hard walled, they should be well suited for fossilization. Larger soft-bodied structures like jellyfish or ctenophores have not been observed in these formations. The oldest mid- to large-sized jellyfish fossils are reported from the Vendian (reviewed in Wade, 1993) and large scyphozoan-like medusae and ctenophores are reported from mid-Cambrian strata (Chen and Zhou, 1997; Hagadorn et al., 2002).

Although Cnidaria appear to be "primitive" animals they do not represent a "genetic museum" (Conway-Morris, 2003). Nevertheless it is remarkable that the vast majority of cnidarian genes show more similarity to the corresponding deuterostome than to protostome homologs (Ball et al., 2004; Kortschak et al., 2003; Spring et al., 2000, 2002). Furthermore, cnidarians and vertebrates share at least eleven of twelve known Wnt gene subfamilies whereas five subfamilies have been lost in the protostome lineage (Kusserow et al., 2005). Since cnidarian anatomy appears to have little changed since Precambrian times it can be assumed that the ancestral genomes are suitably well conserved in the extant cnidarian species to investigate developmental regulators and their differentiation products for evolutionary studies.

Myogenic and neurogenic regulatory genes

The close functional link of nerve and muscle cells in neuromuscular units has led to the hypothesis of a common evolutionary origin of both cell types (reviewed in Mackie, 1970). Key regulators of mesoderm, myogenic, and neurogenic differentiation are present in all eumetazoans (Table 1). Transcription factors of the basic helix−loop−helix (bHLH) family are classified according to function. The myogenic bHLH factors include the Twist, Id, and MRF families while the neurogenic branch comprises the Atonal and Achaete-scute super families. In Cnidaria genes of the Achaete-scute family are expressed in nematocytes and sensory neurons (Grens et al., 1995; Hayakawa et al., 2004; Holstein and Hausmann, 1988; Müller et al., 2003) and in endodermal cells (Seipel et al., 2004a). The cnidarian Atonal-like (*Atl1*) gene is expressed in the developing striated muscle as well as in mechanosensory and nerve cell precursors in the medusa tentacles (Seipel et al., 2004a). Moreover *Atl1* expression is upregulated in proliferating nerve cell precursors arising from adult striated muscle cells by transdifferentiation in vitro. Likewise the neuronal marker gene *NP* coding for the RFamide neuropeptide is expressed not only in mature nerve cells but also transiently in the developing muscle. The molecular evidence supports the hypothesis that muscle and nerve cells are closely linked in evolution and derive from a common myoepithelial precursor. Furthermore, recent investigations in the lens-eyed jellyfish *Cladonema radiatum* (Weber, 1981) demonstrate that genes of the Six family are involved in muscle and eye development and eye regeneration (Stierwald et al., 2004). Members of this gene family are also involved in muscle and nerve development in the Bilateria (Heanue et al., 1999).

Mesoderm and myogenic regulatory genes

In Bilateria the mesoderm contributes to the formation of a large number of organs, tissues, and cell types. In comparison, jellyfish have a simple anatomy and therefore the potential number of mesoderm-derived differentiation products is small. The hydromedusa structural genes coding for striated muscle-specific myosin heavy chain and tropomyosin (Gröger et al., 1999; Müller et al., 1999; Schuchert et al., 1993; Yanze et al., 1999), as well as the hydromedusa myogenic regulatory genes, resemble their bilaterian counterparts. The expression patterns of several key regulators for mesodermal, myogenic, and neurogenic differentiation are summarized for Bilateria, the hydrozoans *P. carnea* and *Hydra vulgaris*, and the anthozoan *Nematostella vectensis* in Table 1 and Fig. 3. The expression of the hydromedusa *Twist* gene is consistent with an inhibitory

Table 1
Eumetazoan regulatory genes in mesoderm formation, myogenesis, and neurogenesis

Gene family	Gene names			Expression/function		References
	Bilateria	Hydrozoa	Anthozoa	Embryo/larva	Developing/adult	
Basic helix–loop–helix						
Ash A	*Ash1,2*			Neurogenic lineages		Verma-Kurvari et al., 1996
		PcAsh1		Endoderm nematoblasts		Müller et al., 2003
		CnAsh		Nematocytes + sensory neurons		Hayakawa et al., 2004
Ash B	*Ash3*			Salivary gland duct cells		Yoshida et al., 2001
		PcAsh2		Endoderm secretory cells		Seipel et al., 2004a
Atonal	*Ath1*			CNS, GI	GI neurons	Akazawa et al., 1995
		PcAtl1		Endoderm	Entocodon + proneural	Seipel et al., 2004a
Id	*Id1-4*			Inhibitor of myogenesis + neurogenesis		Jen et al., 1997
		PcId		No	Endoderm + muscles	Müller et al., 2003
MRF	*MyoD et al.*			Myogenic lineages		Molkentin and Olson, 1996
		PcJellyD		Endoderm	Entocodon/muscles	Müller et al., 2003
Twist	*Twist*			Inhibitor of myogenesis		Spicer et al., 1996
		PcTwist		Ubiquitous	Plate endoderm	Spring et al., 2000
			NvTwist	Endoderm	Endoderm	Martindale et al., 2004
Homeo domain						
Otx	*Otx1,2*			Neuroectoderm	Brain	Simeone, 1998
		PcOtx		No	Entocodon/muscles	Müller et al., 1999
		HvOtx		?	Ectoderm branching	Smith et al., 1999
Msx	*Msx1,2*			Inhibitor of myogenesis + neurogenesis		Hu et al., 2001
		PcMsx		?	Entocodon/muscles	Galle and Seipel, unpublished
		HvMsh		?	Neuronal	Miljkovic-Licina et al., 2004
Nk-2	*Nkx2.1-2.9*			Lung, salivary, pancreas, heart, brain		Pabst et al., 1998
		HvNK-2		?	Endoderm	Grens et al., 1996
MADS-box						
Mef2	*Mef2A-D*			Myogenic + neurogenic lineages		Naya et al., 1999
		PcMef2		Endo + ecto	Entocodon + ectoderm	Spring et al., 2002
			NvMef2	Ectoderm	Ectoderm	Martindale et al., 2004
T-Box						
Bra	*Brachyury*			EMT, mesoderm		Showell et al., 2004
		PcBra		Blastopore + other	Entocodon + other	Spring et al., 2002
		HvBra		Blastopore + endoderm		Technau and Bode, 1999
			NvBra	Blastopore + endoderm		Technau and Scholz, 2003
Zn-finger domain						
C2H2	*Snail, Slug*			EMT, mesoderm		Hemavathy et al., 2000
		PcSnail		Endoderm	Entocodon/muscles	Spring et al., 2002
			NvSnailA,B	Endoderm	Endoderm	Martindale et al., 2004
C4	*Gata1-6*			Mesoderm	Mesoderm	Patient and McGhee, 2002
			NvGata	Endoderm	Endoderm	Martindale et al., 2004

Abbreviations: CNS, central nervous system; EMT, epithelial–mesenchymal transition; GI, gastrointestinal; Hv, *H. vulgaris*; Nv, *N. vectensis*; Pc, *P. carnea*.

function (Spring et al., 2000), similar to the function of the bilaterian homolog (Anant et al., 1998; Hebrok et al., 1994; Spicer et al., 1996). *Id*, an inhibitor of muscle differentiation in vertebrates, is expressed only in medusa development (Müller et al., 2003) both in non-muscle tissues (p in Fig. 3) and in the developing striated muscle (st in Fig. 3). *Msx*, however, another inhibitor of muscle differentiation in Bilateria, is strongly expressed in the entocodon and the differentiating muscle in medusa development (Galle and Seipel, unpublished). Together these data indicate that cognates of certain bilaterian myogenic genes are recruited to differentiate muscle and non-muscle tissues in medusa development. In the development of the bi-layered planula larva, expression of mesodermal, myogenic, and neurogenic

genes is observed in the ectodermal and endodermal myoepithelia (Table 1; Fig. 3). The difference in expression patterns between the hydrozoan and anthozoan larvae may be due to differences in their embryologies (Tardent, 1978). The expression of mesodermal/myogenic genes in the larval endoderm of *Podocoryne* (Hydrozoa) and *Nematostella* (Anthozoa) can be interpreted as evidence for an endodermal origin of the triploblast mesoderm derived from a diploblast planuloid ancestor (Martindale et al., 2004; scenario 2). Similar conclusions were drawn in a recent study of the Wnt gene expression patterns during larval development in *Nematostella* (Kusserow et al., 2005). It has to be pointed out, however, that neither the anthozoan *N. vectensis* nor the hydrozoan *H. vulgaris* have a medusa
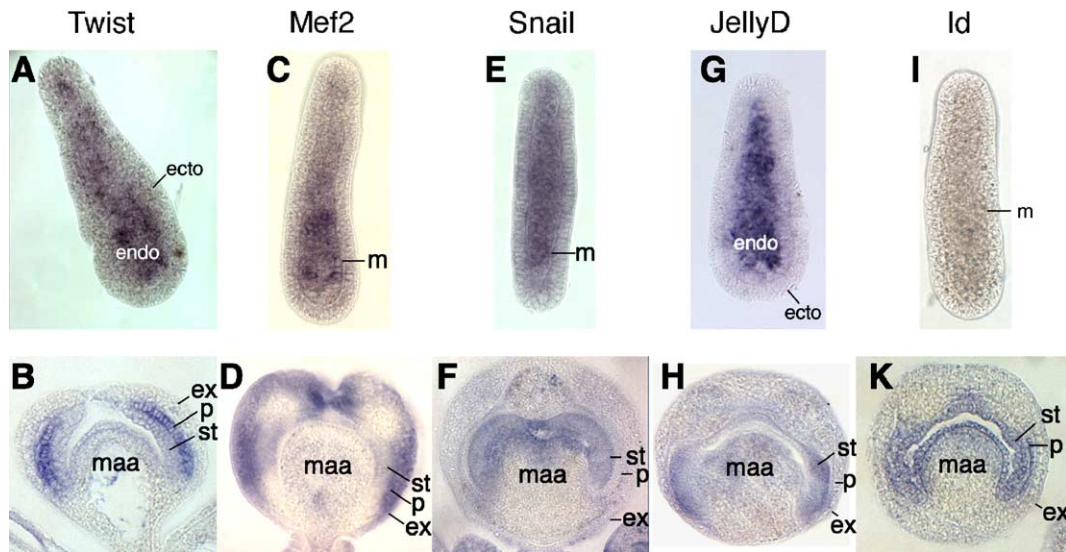
Fig. 3. Expression of mesoderm/myogenic genes in the development of the planula larva and medusa of *P. carnea*. Whole mount in situ hybridization with probes for *Twist* (A, B), *Mef2* (C, D), *Snail* (E, F), *JellyD* (G, H), and *Id* (I, K). Upper row: Larvae were fixed at the age of 1–3 days. Anterior poles are positioned to the bottom. Lower row: Sagittal sections are shown for medusa bud stages 4–6. For more details see publications by Müller et al., 2003; Spring et al., 2000, 2002. Abbreviations: ecto, ectoderm; endo, endoderm; ex, exumbrella; m, mesoglea (ECM); maa, manubrium anlage; p, plate endoderm (i.e., layer without myofilaments); st, presumptive striated muscle, entocodon-derived.

stage with striated muscle and there is no evidence for striated endomesodermal cells in extant cnidarian larvae and polyps, including anthozoans. The only cnidarian life stage that differentiates bilaterian-like striated muscle is the medusa.

In principal the abovementioned findings lead to two possible interpretations:

a. The medusa and bilaterian striated muscles evolved independently from the entocodon and the mesoderm, respectively, but in both cases the same genetic machinery was co-opted towards similar developmental and morphological ends. Entire pathways and/or single genes may have been co-opted and assembled (Erwin and Davidson, 2002). This principle of convergent evolution by co-option is not restricted to muscle but applicable to other tissues and organs in all animal phyla. In this case the common ancestor between Cnidaria and Bilateria may have been a planuloid type diploblast.

b. Both the jellyfish and bilaterian striated muscles are derived from mesoderm-like primordia in a common ancestor established before the Zootype with clustered Hox genes (Slack et al., 1993) evolved. In this case, the ancestor was not a diploblast planula type organism, but an organism with advanced anatomy including striated muscle.

While the planuloid diploblast hypothesis dominates the literature (Baguna and Riutort, 2004; Holland, 2000; Kusserow et al., 2005; Martindale et al., 2004; Salvini-Plawen, 1978; Valentine et al., 1996), the data and arguments in favor of the second hypothesis are presented in the following chapter.

The assembly of the basic anatomy in the common Ctenophora/Cnidaria/Bilateria ancestor

Multicellular animals first appear in the fossil record around 580 million years ago; however, authentic ancient DNA has not been obtained from fossils over 50,000 years of age (Poinar and Stankiewicz, 1999). Thus we do not have access to fossils or molecular information underlying the basic Bauplan and we are left with best guesses about the evolution of the mesodermal and myogenic lineages. Under the assumption that the principles of evolution have not changed since the first animals appeared, the replacement of ciliary motility by muscle-driven locomotion must have represented an important selective advantage in preying and escape. Furthermore the extent of conservation of the molecular control mechanisms in mesoderm and myogenic patterning throughout the triploblast phyla indicates that early muscle evolution passed a selective bottleneck.

The evolution of striated and smooth muscle

Muscle cells evolved by assembling new variants of motor proteins for fast and slow contraction and by forming adhesive substrates able to withstand and counteract the generated contraction forces (Rieger, 1994). Before the existence of exo- and endoskeletal structures the primordial muscle likely adhered to gelatinous material as present in a rudimentary form in the extant Porifera (Grell et al., 1980), and well developed in Ctenophora (reviewed in Hernandez-Nicaise and Franc, 1993) and Cnidaria (reviewed in Bouillon, 1993; Schmid et al., 1999). Muscle cells are subdivided into smooth and striated muscle characterized by

specific motor protein variants derived from ancestral eukaryote motor proteins (Cheney et al., 1993). The large family of myosin heavy chain (MHC) class II genes is divided into three subfamilies specifying non-muscle, smooth, and striated muscle myosins (Sellers, 2000; Weiss and Leinwand, 1996). Based on phylogenetic analysis the head, neck and tail domains of the MHC class II molecules co-evolved (Korn, 2000). Moreover, in a phylogenetic analysis of the myosin class II genes, head domains of smooth muscle myosin appear to be more related to non-muscle than to striated muscle myosin, implying that smooth and striated muscle myosin were independently derived from an ancestral myosin, with the possibility that striated may be older than smooth muscle myosin (Goodson and Spudich, 1993). Both vertebrate and medusa striated muscles are very similar in ultra structure including A and H bands as well as Z discs (Bölsterli, 1977; Schuchert et al., 1993). Sequence analysis of a *Podocoryne* striated muscle-specific MHC showed a higher similarity to bilaterian striated muscle than to smooth muscle or non-muscle MHCs from either invertebrates or vertebrates (Schuchert et al., 1993). Together these data suggest a very early origin of the striated muscle. Moreover, it appears unlikely that striated muscle derived from smooth muscle myoepithelia of a potential planula or polyp ancestors, but rather evolved directly from non-muscle cells.

Diploblasty

The transition from an early ciliated metazoan to a mesodermate-like animal has often been viewed as a stepwise evolution starting with one, then two, then three germ layers. For this reason a diploblastic planuloid ancestor is well represented in the literature discussing the early metazoan evolution. Within the anatomies of the extant phyla, however, diploblasty is properly documented only for the cnidarian larva and the sessile polyp stage. Moreover, Ctenophora are potentially triploblastic according to cell lineage analysis (Martindale and Henry, 1999) and anatomy (Hernandez-Nicaise and Franc, 1993). Furthermore it is remarkable that there is no evidence for the preservation of a diploblastic phase during the blastula–gastrula transition in any bilaterian embryo. This should be, at least occasionally, observed in the development of extant organisms if a major diploblastic period had occurred during metazoan evolution. Indeed, the term diploblast becomes questionable with respect to an animal phylum (Ball et al., 2004; Hyman, 1940; Willmer, 1990), as it is appropriate only for the anatomy of the cnidarian larva and polyp. The diploblast anatomy may be a reduced anatomy (scenario 3 in Martindale et al., 2004) or alternatively evolved by delayed completion of gastrulation after endoderm formation (Spring et al., 2000, 2002). The presence of functional nematocytes in early development, and thus the possibility of feeding, may have facilitated a delayed development.

The placement of muscle tissue and the symmetry axis

In this chapter we present a hypothetical model of early metazoan evolution (Fig. 4). The existence of a relatively small sized motile multicellular ancestor at the beginning of metazoan evolution is widely accepted (reviewed in Grell et al., 1980; Rieger and Weyer, 1998). Its cellular organization is highly speculative but according to the presented data we have based the model on a parenchymula or phagocytella-like ancestor (Grell et al., 1980). The diet likely included fellow metazoans trapped by adhesive structures (Fig. 4) similar to those found in Protozoa (Petroni et al., 2000), Cnidaria (nematocytes), Ctenophora (colloblasts), or Turbellaria (rhabdites). This ancestral organism featured internal gamete production and protist-like locomotion (Figs. 4A and B). Primordial striated muscle cells may have been placed between the digestive and epidermal layers to facilitate spawning (Rieger and Weyer, 1998). To generate striated muscle-based locomotion an ECM had to be developed, the contractile cells had to be aligned in parallel units and wired with nerve/pacemakers. Basically these units could be placed in two ways, either perpendicular or parallel to the digestive epithelium and body axis (Figs. 4F and G). To avoid negative effects of muscle contractions on digestion, the anatomy had to be adapted accordingly. The digestive region was either dislodged from the area of contraction and located centrally (Fig. 4F) or sealed off against loss of content by sphincter muscles at "mouth" and "anus" position (Fig. 4G). In the first case (Fig. 4F) the striated muscle tissue extended towards the periphery, thus inducing a radial medusa-like anatomy. Because all further differentiations were added later, the radial orientation of the muscles was largely conserved throughout medusozoan evolution. The second case (Fig. 4G) allowed the gradual evolution of a benthic, motile life style with dorsoventral differentiations possibly including acoelomorph life forms (Baguna and Riutort, 2004) followed by segmental growth regulated by clustered Hox genes as postulated for the Zootype (Slack et al., 1993). The third possibility (Fig. 4D) refers to a ctenophoran ancestor. The extant Ctenophora are characterized by a massive, ball-shaped ECM providing floatation support and a locomotion system with specialized flagella, the comb plates. Therefore the continued evolution or maintenance of the ancestral striated muscle tissue in the main body was abandoned in most ctenophores but survived in some cyddipids with fast contractile tentacles (Hernandez-Nicaise and Franc, 1993). The increase in metazoan size required the development of systems for transportation of nutrients to peripheral body parts. This was achieved in the Ctenophora by gastric pouches, in the Cnidaria and Turbellaria by gastrovascular systems, and in the more evolved Bilateria by vascular systems. The formation of the jellyfish gastrovascular system appears to be regulated by VEGF (Seipel et al., 2004c). Vascular endothelial growth factors also play important roles in formation of vertebrate vascular systems
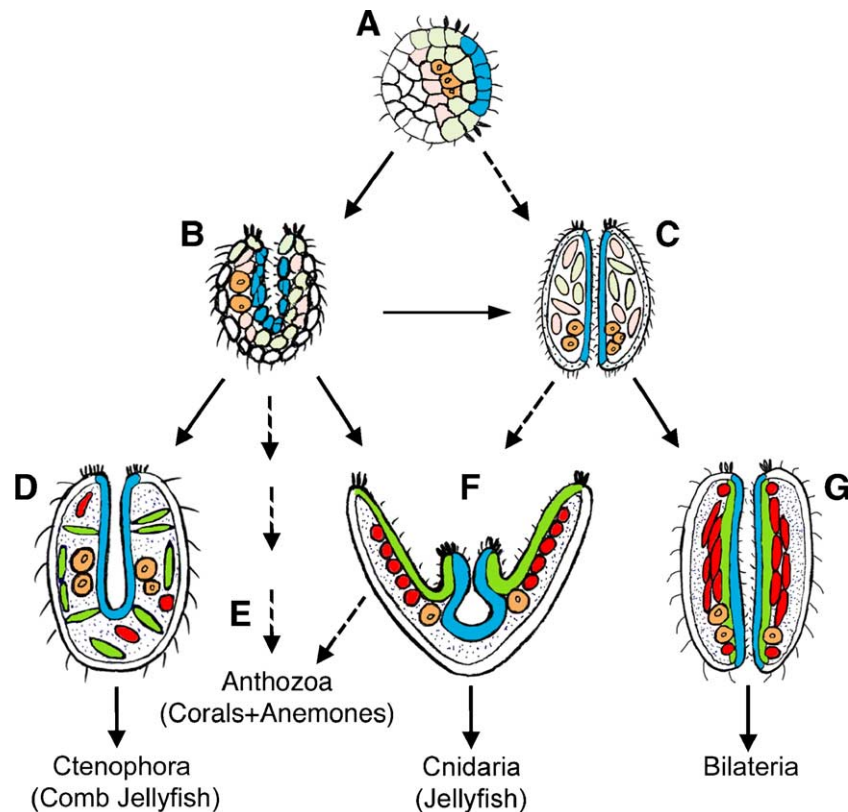
Fig. 4. Hypothetical model of the early metazoan evolution. Schematic drawings of sagittal sections with upward orientation of the oral opening illustrate the discussed hypothetical anatomies of metazoan ancestors. (A) Ancestral metazoan with flagella (thin black lines), adhesive structures (thick black spikes), digestive area (blue), gametogonia (orange), and primordial myocytes (light green and light red). (B and C) Intermediate stages of the basic metazoan Bauplan. (B) Intermediate stage formed from panel A. The digestive area has formed a pocket and primordial myocytes are found between the digestive and the flagellated epithelia. (C) Intermediate stage formed from panel B (or from panel A). It has a through gut and anterior–posterior polarity, primordial myocytes start aligning along the digestive tube. (D) Ctenophore ancestor, derived from B, a massive ECM has formed, myocytes mostly differentiate into the smooth muscle type (green) with a subepidermal location across the ECM. Locomotion is generated by specialized flagella, the comb plates. (E) Anthozoan polyp ancestor might have formed directly from panel B (or from panel F). (F) Cnidarian jellyfish-like ancestor, derived from B, or by closing the through gut from C (dashed arrow). Premordial myocytes differentiate into radial smooth muscle (green) and striated muscle (red) with a circumferential orientation. The digestive region is centralized in the radial animal. The inner layer is formed by a flagellated and smooth muscled epithelium. (G) Zootype ancestor, derived from panel C. Myocytes differentiate to smooth (green) and striated (red) muscle aligned in parallel around the digestive tube, at the ends sphincter muscles (red) control the aperture of the digestive tube. This stage develops into the bilaterian line.

indicating a common origin of vascular system formation in metazoan evolution.

It is difficult to speculate on the anatomy of a cnidarian ancestor at the point of divergence (Figs. 4B and C). Did it already have radial or bilateral symmetric properties (dorsal-ventral), or elements of both? The hypothetical common ancestor probably exhibited some bilateral characteristics before the definite placement of the muscle systems occurred. This theory is corroborated by the fact that certain bilateral traits are present in the development of both the extant Anthozoa and Siphonophora (Hydrozoa) as well as in the Ctenophora (Bouillon, 1993; Hernandez-Nicaise and Franc, 1993; Martindale and Henry, 1998; Martindale et al., 2002). Cnidarian axis development is regulated by Hox-like genes (Bode, 2001; Finnerty and Martindale, 1999; Galliot, 2000; Yanze et al., 2001). Moreover the anthozoan Decapentaplegic (Dpp) and Hox-like genes are expressed in a "dorsal–ventral" type of pattern (Finnerty et al., 2004; Hayward et al., 2002)

typical for bilateral systems. In fact the radial symmetry may be a secondary trait superimposed on a basically bilateral body plan (Ball et al., 2004), a situation similar to the pentamery of the adult echinoderms.

Cnidarian evolution

The molecular developmental analysis of the hydrozoan jellyfish and the evolutionary relationship of motor proteins like myosin support a metazoan phylogeny with a non-sessile, tri-layered, possibly medusa-like ancestor at the cnidarian base. In this scenario the anthozoan ancestor may have been the first to diverge from the main cnidarian line (Fig. 4E) and to engage in sessility. The transition from a motile to a sessile life style has led to the evolution of colonial life stages both in the Bilateria (Davidson et al., 2004) and in the Cnidaria as exemplified by the colonial polymorphism in the Anthozoa and Hydrozoa. The transition may have been achieved by pedomorphosis and/or

reduction of the anatomy of a motile, triploblast ancestral stage (Figs. 4B and F). As suggested previously this ancestor may have featured a medusa-like anatomy (Boero et al., 1998; Brien, 1969; Brooks, 1886; Hyman, 1940; Rees, 1966; Scholtz, 2004; Schuchert, 1993b). This scenario does not contradict the basal position of the Anthozoa within the Cnidaria based on rRNA phylogeny nor exclude the expression of homologs to bilaterian myogenic genes in the ectoderm and endoderm of cnidarian larvae (see previous chapters). In contrast to the three-layered bilaterian larvae the two layers of the cnidarian planula are smooth muscle epithelia with additional secretory functions. They are the first cell types to differentiate (Gröger and Schmid, 2001) and in many species also generate I-cells, nerve cells, and nematocytes (Van de Vyver, 1993). These cell types are also present in the medusa. Hence it is plausible that the same genes are used in the differentiation of both larval and medusa cell types.

Another characteristic often employed in evolutionary discussions is the linearity of the medusozoan mtDNA. Compared to the circular mtDNA of the anthozoans and bilaterians, linear mtDNA appears to be more derived. There is, however, no strict correlation of mtDNA structure with phylogeny. Linear mtDNA is known to occur in plants, fungi, slime molds, yeast, or ciliates, often randomly scattered in the phylogenies. This is carried to an extreme where two strains of the same species have circular or linear mtDNA, respectively (reviewed in Nosek et al., 1998). Thus the linearization of the mtDNA in the Medusozoa was likely of little selective value and occurred early, after the separation from the Anthozoa and/or Bilateria.

There are further arguments in favor of a motile cnidarian ancestor. If there was a sessile cnidarian ancestor it is difficult to understand why this highly successful life style was abandoned only in the ancestor of the Medusozoa while sharing the same habitats with that of the Anthozoa. Furthermore, according to Hyman (1940) the polyp first hypothesis would present "the curious spectacle of a lower type (polyp) evolving into a higher type (medusa) and continuing to exist simultaneously as part of its life cycle". The polyp stage as well as the medusa stage can be reduced or completely absent in Hydrozoa and Scyphozoa (Boero and Bouillon, 1987; Bouillon and Boero, 2000; Piraino et al., 2004; Tardent, 1978). A complete loss of the medusa has frequently occurred in several hydrozoan families (Boero et al., 1992) and a temperature shift may suffice to change from medusa budding to polyp budding (Werner, 1963). Furthermore the transition from a motile to a sessile life style is not exclusive to cnidarians but evidently has occurred repeatedly in various aquatic invertebrates, sometimes accompanied by reduction in neuromuscular complexity, as observed in the filter feeders. Additional support for a common motile "mesodermate" ancestor comes from the Ctenophora. There are no permanent sessile stages in this phylum, they have a mesodermate-like development and

histology and according to rRNA phylogeny this phylum is positioned basal to the Cnidaria (Fig. 1B; Ball et al., 2004; Cavalier-Smith and Chao, 2003; Collins and Valentine, 2001; Halanych, 2004; Medina et al., 2001; Podar et al., 2001). In summary it appears that the most parsimonious hypothesis taking into account the recent molecular, cellular, and developmental data is based on a motile life form with mesodermate-like development as a common ancestor of Ctenophora, Cnidaria, and Bilateria.

Conclusions

In summary it appears that Cnidaria derive from a motile pre-zootype metazoan featuring mesodermate and possibly bilaterian elements of anatomy. In this scenario the evolution of the basic Bauplan did not include a diploblast stage. The evolution of anatomical elements able to generate rapid locomotion required the simultaneous emergence of a digestive support system and a concurrent connection of musculature and nervous system. Additionally there were placement constraints for the basic anatomical elements within the body and with respect to each other. All the basic anatomical elements probably co-evolved as integrated functional units in the basic Bauplan. This scenario reflects a simplified evolutionary process leading to the major animal phyla.

Acknowledgments

References

Akazawa, C., Ishibashi, M., Shimizu, C., Nakanishi, S., Kageyama, R., 1995. A mammalian helix–loop–helix factor structurally related to the product of *Drosophila* proneural gene atonal is a positive transcriptional regulator expressed in the developing nervous system. J. Biol. Chem. 270, 8730–8738.

Anant, S., Roy, S., VijayRaghavan, K., 1998. Twist and Notch negatively regulate adult muscle differentiation in *Drosophila*. Development 125, 1361–1369.

Baguna, J., Riutort, M., 2004. The dawn of bilaterian animals: the case of acoelomorph flatworms. BioEssays 26, 1046–1057.

Ball, E.E., Hayward, D.C., Saint, R., Miller, D.J., 2004. A simple plan-Cnidarians and the origins of developmental mechanisms. Nat. Rev., Genet. 5, 567–577.

Bode, H.R., 2001. The role of hox genes in axial patterning in *Hydra*. Am. Zool. 41, 621–628.

Bölsterli, U., 1977. An electron microscopic study of early developmental stages, myogenesis, oogenesis and cnidogenesis in the Anthomedusa, *Podocoryne carnea* M. Sars. J. Morphol. 154, 259–289.

Boero, F., Bouillon, J., 1987. Inconsistent evolution and paedomorphosis among hydroids and medusae of athecatae/anthomedusae and thecatae/leptomedusae (Cnidaria, Hydrozoa). In: Bouillon, J., Boero, F., Cicogna, F., Cornelius, P. (Eds.), Modern Trends in Systematics, Ecology and Evolution of Hydroids and Hydromedusae. Oxford Univ. Press, pp. 229–250.

Boero, F., Bouillon, J., Piraino, S., 1992. On the origins of evolution of hydromedusan life cycles (Cnidaria, Hydrozoa). In: Dallai, R. (Ed.), Sex Origin and Evolution, U.Z.I., Sel. Symp. Monogr., vol. 6. Muchi, Modena, pp. 59–68.

Boero, F., Gravili, C., Pagliari, P., Piraino, S., Bouillon, F., Schmid, V., 1998. The cnidarian premises of metazoan evolution: from triploblasty, to coelom formation, to metamery. Ital. J. Zool. 65, 5–9.

Bouillon, J., 1993. Classe des hydrozoaires. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Cnidaires, Cténaires, Vol. III, Fascicule 2. Masson, Paris, pp. 29–416.

Bouillon, J., Boero, F., 2000. The Hydrozoa: a new classification in the light of old knowledge. Thalass. Salentina 24, 3–45.

Boyer, B.C., Henry, J.Q., Martindale, M.Q., 1996. Dual origins of mesoderm in a basal spiralian: cell lineage analyses in the polyclad turbellarian *Hoploplana inquilina*. Dev. Biol. 179, 329–338.

Bravermann, M., 1974. The cellular basis for colony form in *Podocoryne carnea*. Am. Zool. 14, 673–698.

Bridge, D., Cunningham, C.W., Schierwater, B., Desalle, R., Buss, L.W., 1992. Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. Proc. Natl. Acad. Sci. U. S. A. 89, 8750–8753.

Bridge, D., Cunningham, C.W., DeSalle, R., Buss, L.W., 1995. Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. Mol. Biol. Evol. 12, 679–689.

Brien, P., 1969. Principes de Phylogenèse animal appliqués à l'interprétation des Métazoaires diploblastiques et à leur evolution en Métazoaires triploblastique. Mém. Acad. R. Belg. 38, 9–110.

Brooks, W.K., 1886. Origin of medusae and significance of metagenesis. Boston Soc. Nat. Hist., 3.

Caroll, S.B., 2000. Endless Forms: the evolution of gene regulation and morphological diversity. Cell 101, 577–580.

Cavalier-Smith, T., Chao, E.E., 2003. Phylogeny of choanozoa, apusozoa, and other protozoa and early eukaryote megaevolution. J. Mol. Evol. 56, 540–563.

Chapman, D.M., 1968. A new type of muscle cell from the subumbrella of *Obelia*. J. Mar. Biol. Assoc. U. K. 48, 667–688.

Chapman, D.M., 1999. Microanatomy of the bell rim of *Aurelia aurita* (Cnidaria: Scyphozoa). Can. J. Zool. 77, 34–46.

Chen, J.Y., Zhou, G.Q., 1997. Biology of the Chengjang Fauna. Bull. Natl. Mus. Nat. Sci. (Taipei) 10, 11–105.

Chen, J.-Y., Oliveri, P., Li, C.-W., Zhou, G.-Q., Gao, F., Hagadorn, J.W., Peterson, K.J., Davidson, E.H., 2000. Precambrian animal diversity: putative phosphatized embryos from Doushantuo Formation of China. Proc. Natl. Acad. Sci. U. S. A. 97, 4457–4462.

Chen, J.Y., Oliveri, P., Gao, F., Dornbos, S.Q., Li, C.W., Bottjer, D., Davidson, E.H., 2002. Precambrian animal life: probable developmental and adult cnidarian forms from southwest China. Dev. Biol. 248, 182–196.

Cheney, R.E., Riley, M., Mooseker, M., 1993. Phylogenetic analysis of the myosin super-family. Cell Motil. Cytoskeleton 24, 215–223.

Collins, A., 1998. Evaluating multiple alternative hypothesis for the origins of Bilateria: an analysis of 18S molecular evidence. Proc. Natl. Acad. Sci. U. S. A. 95, 15458–15463.

Collins, A.G., Valentine, J.W., 2001. Defining phyla: evolutionary pathways to metazoan body plans. Evol. Dev. 3:6, 432–442.

Collins, A.G., 2002. Phylogeny of Medusozoa and the evolution of cnidarian life cycles. J. Evol. Biol. 15, 1–15.

Conway-Morris, S., 2003. The Cambrian "explosion" of metazoans and molecular biology: would Darwin be satisfied? Int. J. Dev. Biol. 47, 505–516.

Creuzet, S., Vincent C., Couly, G., 2005. Neural crest derivatives in ocular and periocular structures. Int. J. Dev. Biol. 49 (in press).

Davidson, B., Jacobs, M., Swalla, B., 2004. The individual as a module: solitarity to colonial transitions in metazoan evolution and development. In: Schlosser, G., Wagner, G. (Eds.), Modularity in Development and Evolution. University of Chicago Press, pp. 443–465.

Doumenc, A.D., Van Praet, M., 1987. Ordre des actinaires, des ptychodactiniares, des coralliomorphaires. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Cnidaires, Anthozoaires, Vol. III, Fascicule 3. Masson, Paris, pp. 257–401.

Ender, A., Schierwater, B., 2003. Placozoa are not derived Cnidarians: evidence from the molecular morphology. Mol. Biol. Evol. 20, 130–134.

Erwin, D.H., Davidson, E.H., 2002. The last common bilaterian ancestor. Development 129, 3021–3032.

Finnerty, J.R., Martindale, M.Q., 1999. Ancient origins of axial patterning genes: Hox genes and ParaHox genes in the Cnidaria. Evol Dev. 1, 16–23.

Finnerty, J.R., Pang, K., Burton, P., Paulson, D., Martindale, M.Q., 2004. Origins of bilateral symmetry: Hox and Dpp expression in a sea anemone. Science 304, 1335–1337.

Franc, A., 1993. Classe des cubozoaires. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Cnidaires, Cténaires, Vol. III, Fascicule 2. Masson, Paris, pp. 885–922.

Freeman, G., 1977. The establishment of the oral-aboral axis in the ctenophore embryo. J. Embryol. Exp. Morph. 42, 237–260.

Freeman, G., 1981. The cleavage initiation site establishes the posterior pole of the hydrozoan embryo. Roux Arch. Dev. Biol. 190, 123–125.

Frey, J., 1968. Die Entwicklungsleistungen der Medusenknospen and Medusen von *Podocoryne carnea* M. Sars nach Isolation und Dissoziation. Wilhelm Roux's Arch. 160, 428–464.

Galliot, B., 2000. Conserved and divergent genes in apex and axis development of cnidarians. Curr. Opin. Genet. Dev. 10, 629–637.

Galliot, B., Schmid, V., 2002. Cnidarians as a model system for understanding evolution and regeneration. Int. J. Dev. Biol. 46, 39–48.

Goodson, H., Spudich, J.A., 1993. Molecular evolution of the myosin family: relationship derived from comparison of amino acid sequences. Proc. Natl. Acad. Sci. U. S. A. 90, 659–663.

Goldstein, B., Freeman, G., 1996. Axis specification in animal development. BioEssays 19, 105–116.

Grell, K.G., Gruner, H.E., Kilian, E.F., 1980. Einführung. In: Graner, H.E. (Ed.), Lehrbuch der speziellen Zoologie, Vol. 1: Wirbellose Tiere: I. Einführung Protozoa, Placozoa, Porifera. Fischer, Stuttgart.

Grens, A., Mason, E., Marsh, J.L., Bode, H.R., 1995. Evolutionary conservation of a cell fate specification gene: the *Hydra* achaete-scute homolog has proneural activity in *Drosophila*. Development 121, 4027–4035.

Grens, A., Gee, L., Fisher, D.A., Bode, H.R., 1996. CnNK-2, an NK-2 homeobox gene, has a role in patterning the basal end of the axis in hydra. Dev. Biol. 180, 473–488.

Gröger, H., Schmid, V., 2001. Larval development in Cnidaria: a connection to Bilateria. Genesis 29, 110–114.

Gröger, H., Callaerts, P., Gehring, W.J., Schmid, V., 1999. Gene duplication and recruitment of a specific tropomyosin to striated muscle cells in the jellyfish *Podocoryne carnea*. J. Exp. Zool. 285, 378–386.

Hagadorn, J.W., Dott, R.H., Damrow, D., 2002. Stranded on a late Cambrian shoreline: medusae from central Wisconsin. Geology 30, 147–150.

Halanych, K.M., 2004. The new view of animal phylogeny. Annu. Rev. Ecol. Evol. Syst. 35, 229–256.

Hamann, O., 1882. Der Organismus der Hydroidpolypen. Jena. Z. Naturwiss. Bd. XV, N.F. VIII. pp. 1–72.

Hayakawa, E., Fujisawa, C., Fujisawa, T., 2004. Involvement of *Hydra* achaete-scute gene CnASH in the differentiation pathway of sensory neurons in the tentacles. Dev. Genes Evol. 214, 486–492.

Hayward, D.C., Samuel, G., Pontynen, P.C., Catmull, J., Saint, R., Miller, D.J., Ball, E.E., 2002. Localized expression of a dpp/BMP2/4 ortholog in a coral embryo. Proc. Natl. Acad. Sci. U. S. A. 99, 8106–8111.

Heanue, T.A., Reshef, R., Davis, J., Mardon, G., Oliver, G., Tomarev, S., Lassar, A.B., Tabin, C.J., 1999. Synergistic regulation of vertebrate muscle development by Dach2, Eyea2, and Six1, homologs of genes required for *Drosophila* eye formation. Genes Dev. 13, 3231–3243.

Hebrok, M., Wertz, K., Fuchtbauer, E.M., 1994. M-Twist is an inhibitor of muscle differentiation. Dev. Biol. 165, 537–544.

Hemavathy, K., Ashraf, S.I., Ip, Y.T., 2000. Snail/slug family of repressors: slowly going into the fast lane of development and cancer. Gene 257, 1–12.

Hernandez-Nicaise, M.-L., Franc, J.M., 1993. Embranchment des Cténaires: Morphologie, Biologie, Ecologie. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Cnidaires, Cténaires, Vol. III, Fascicule 2. Masson, Paris, pp. 943–1055.

Hobmayer, B., Rentzsch, F., Kuhn, K., Happel, C.M., von Laue, C.C., Snyder, P., Rothbächler, U., Holstein, T.W., 2000. WNT signaling molecules act in axis formation in the diploblastic metazoan *Hydra*. Nature 407, 186–189.

Holland, L.Z., 2000. Body-plan evolution in the Bilateria: early antero-posterior patterning and the deuterostome–protostome dichotomy. Curr. Opin. Genet. Dev. 10, 434–442.

Holstein, T., Hausmann, K., 1988. The cnidocil apparatus of hydrozoans: a progenitor of metazoan mechanoreceptors? In: Hessinger, D.A., Lenhoff, H.M. (Eds.), The Biology of Nematocysts. Academic Press, San Diego, pp. 53–73.

Holstein, T.W., Hobmayer, E., Technau, U., 2003. Cnidarians: an evolutionary conserved model system for regeneration. Dev. Dyn. 226, 257–267.

Hu, G., Lee, H., Price, S.M., Shen, M.M., Abate-Shen, C., 2001. Msx homeobox genes inhibit differentiation through upregulation of cyclin D1. Development 128, 2373–2384.

Hyman, L.H., 1940. The Invertebrates: Protozoa through Ctenophora. McGraw-Hill, New York.

Jägersten, G., 1955. On the early phylogeny of the Metazoa: the bilatero-gastrea theory. Zool. Bidr. Uppsala 30, 321–354.

Jen, Y., Manova, K., Benezra, R., 1997. Each member of the Id gene family exhibits a unique expression pattern in mouse gastrulation and neuro-genesis. Dev. Dyn. 208, 92–106.

Koonin, E.V., Aravind, l., Kondrashov, A.S., 2000. The impact of comparative genomics on our understanding of evolution. Cell 101, 573–576.

Korn, E.D., 2000. Coevolution of head, neck, and tail domains of myosin heavy chains. Proc. Natl. Acad. Sci. U. S. A. 97, 12559–12564.

Kortschak, R.D., Samuel, G., Saint, R., Miller, D.J., 2003. EST analysis of the cnidarian *Acropora millepora* reveals extensive gene loss and rapid sequence divergence in the model invertebrates. Curr. Biol. 13, 2190–2195.

Kühn, A., 1910. Die Entwicklunsgeschichte der Geschlechtsindividuen der Hydromedusen. Zool. Jahrb. Abt. Syst. Okol. Geogr. Tiere 30, 145–164.

Kusserow, A., Pang, K., Sturm, C., Hrouda, M., Lentfer, J., Schmidt, H.A., Technau, U., von Haeseler, A., Hobmayer, B., Martindale, M.Q., Holstein, T.W., 2005. Unexpected complexity of the Wnt gene family in a sea anemone. Nature 433, 156–160.

Le Douarin, N.M., Creuzet, S., Couly, G., Dupin, E., 2004. Neural crest cell plasticity and its limits. Development 131, 4637–4650.

Li, C.-W., Chen, J.-Y., Hua, T.-E., 1998. Precambrian sponges with cellular structures. Science 279, 879–882.

Mackie, G.O., 1970. Neuroid conduction and the evolution of conducting tissues. Q. Rev. Biol. 45, 319–332.

Manuel, M., Borchiellini, C., Alivon, E., Le Parco, Y., Vacelet, J., Boury-Esnault, N., 2003. Phylogeny and evolution of calcareous sponges:

monophyly of calcinea and calcaronea, high level of morphological homoplasy, and the primitive nature of axial symmetry. Syst. Biol. 52, 311–333.

Martindale, M.Q., Henry, J.Q., 1998. The development of radial and biradial symmetry: the evolution of bilaterality. Am. Zool. 38, 672–684.

Martindale, M.Q., Henry, J.Q., 1999. Intracellular fate mapping in a basal metazoan, the ctenophore *Mnemiopsis leidyi*, reveals the origins of mesoderm and the existence of intermediate cell lineages. Dev. Biol. 246, 243–257.

Martindale, M.Q., Finnerty, J.R., Henry, J.Q., 2002. The Radiata and the evolutionary origins of the bilaterian body plan. Mol. Phylogenet. Evol. 24, 358–365.

Martindale, M.Q., Pang, K., Finnerty, J.R., 2004. Investigating the origins of triploblasty: "mesodermal" gene expression in a diploblastic animal, the sea anemone *Nematostella vectensis* (phylum, Cnidaria; class, Anthozoa). Development 131, 2463–2474.

Martinelli, C., Spring, J., 2003. Distinct expression patterns of the two T-box homologues Brachyury and Tbx2/3 in the placozoan *Trichoplax adherens*. Dev. Genes Evol. 213, 492–499.

Medina, M., Collins, A.G., Silbermann, J.D., Sogin, M.L., 2001. Evaluating hypothesis of basal animal phylogeny using complete sequences of large and small subunit rRNA. Proc. Natl. Acad. Sci. U. S. A. 98, 9707–9712.

Miljkovic-Licina, M., Gauchat, D., Galliot, B., 2004. Neuronal evolution: analysis of regulatory genes in a first-evolved nervous system, the hydra nervous system. Biosystems 76, 75–87.

Miller, D.J., Hayward, D.C., Reece-Hoyes, J.S., Schotten, I., Catmull, J., Gehring, W.J., Callaert, P., Larsen, J.E., Ball, E.E., 2000. Pax genes diversity in the basal cnidarian *Acropora millepora* (Cnidaria, Antho-zoa): implications for the evolution of the Pax gene family. Proc. Natl. Acad. Sci. U. S. A. 97, 4475–4480.

Molkentin, J.D., Olson, E.N., 1996. Combinatorial control of muscle development by basic helix-loop-helix and MADS-box transcription factors. Proc. Natl. Acad.Sci. U. S. A. 93, 9366–9373.

Müller, P., Yanze, N., Schmid, V., Spring, J., 1999. The homeobox gene Otx of the jellyfish *Podocoryne carnea*: role of a head gene in striated muscle and evolution. Dev. Biol. 216, 582–594.

Müller, P., Seipel, K., Yanze, N., Reber-Müller, S., Streifwolf-Engel, R., Stierwald, M., Spring, J., Schmid, V., 2003. Evolutionary aspects of developmentally regulated helix–loop–helix transcription factors in striated muscle of jellyfish. Dev. Biol. 255, 216–229.

Naya, F.J., Wu, C., Richardson, J.A., Overbeek, P., Olson, E.N., 1999. Transcriptional activity of MEF2 during mouse embryogenesis monitored with a MEF2-dependent transgene. Development 126, 2045–2052.

Nielsen, C., 1995. Animal Evolution: Interrelationships of the Living Phyla. Oxford Univ. Press.

Nielsen, C., 2004. Trochophora larvae: cell-lineages, ciliary bands and body regions. 1. Annelida and Mollusca. J. Exp. Zool., Part B Mol. Dev. Evol. 302, 35–68.

Nielsen, C., Rostgaard, J., 1976. Structure and function of entoproct tentacles with a discussion of ciliary feeding types. Ophelia 15, 115–140.

Nosek, J., Tomaska, L., Fukuhara,, Suyama, Y., Kovac, L., 1998. Linear mitochondrial genomes: 30 years down the line. TIG 14, 184–188.

Odorico, D.M., Miller, D.J., 1997. Internal and external relationships of the Cnidaria: implications of primary and predicted secondary structure of the 5′-end of the 23S-like rDNA. Proc. R. Soc. London, Ser. B Biol. Sci. 264, 77–82.

Pabst, O., Herbrand, H., Arnold, H.H., 1998. Nkx2-9 is a novel homeobox transcription factor which demarcates ventral domains in the developing mouse CNS. Mech. Dev. 73, 85–93.

Patient, R.K., McGhee, J.D., 2002. The GATA family (vertebrates and invertebrates). Curr. Opin. Genet. Dev. 12, 416–422.

Peterson, K.J., Davidson, E.H., 2000. Regulatory evolution and the origin of the bilaterians. Proc. Natl. Acad. Sci. U. S. A. 97, 4430–4435.

Petroni, G., Spring, S., Schleifer, K.H., Verni, F., Rosati, G., 2000. Defensive extrusive ectosymbionts of Euplotidium (Ciliophora) that contain microtubuli-like structures are bacteria related to Verrucomicrobia. Proc. Natl. Acad. Sci. U. S. A. 97, 1813–1817.

Piraino, S., De Vito D., Schmich, J., Bouillon, J., Boero, F., 2004. Reverse development in Cnidaria. Can. J. Zool. 82, 1748–1754.

Podar, M., Haddock, S.H., Sogin, M.L., Harbison, G.R., 2001. A molecular phylogenetic framework for the phylum Ctenophora using 18S rRNA genes. Mol. Phylogenet. Evol. 21, 218–230.

Poinar, H.N., Stankiewicz, B.A., 1999. Protein preservation and DNA retrieval from ancient tissues. Proc. Natl. Acad. Sci. U. S. A. 96, 8426–8431.

Rees, W.J., 1966. The evolution of the Hydrozoa. In: Cnidaria and their evolution. Symp. Zool. Soc. Lond. 16, 199–222.

Revilla-i-Domingo, R., Davidson, E.H., 2003. Developmental gene network analysis. Int. J. Dev., 695–704.

Rieger, R.M., 1994. Evolution of the "lower" Metazoa. In: Bengtson, S. (Ed.), Early Life on Earth. Columbia Univ. Press, New York, pp. 475–492.

Rieger, R.M., Weyer, S., 1998. The evolution of the lower Metazoa: evidence from the phenotype. In: Müller, W.E.G. (Ed.), Molecular Evolution: Towards the Origin of Metazoa, Prog. Mol. Subcell. Biol., vol. 21. Springer, Berlin, pp. 21–43.

Salvini-Plawen, L., 1978. On the origin and evolution of the lower Metazoa. Zool. Syst. Evol.-Forsch. 16, 40–88.

Schmid, V., 1969. Zur Gametogenese von Podocoryne carnea M. Sars. Rev. Suisse Zool. 76, 1071–1078.

Schmid, V., Ono, S.I., Reber-Müller, S., 1999. Cell-substrate interaction in Cnidaria. Microsc. Res. Tech. 15 (44), 254–268.

Scholtz, G., 2004. Coelenterata versus Acrosomata—On the position of comb jellyfish (Ctenophora) in metazoan phylogeny. Sitz.ber. Ges. Nat.forsch. Freunde Berl. 43, 15–33.

Schuchert, P., 1993a. Trichoplax adherens (Phylum Placozoa) has cells that react with antibodies against the neuropeptide RFamide. Acta Zool. 74, 115–117.

Schuchert, P., 1993b. Phylogenetic analysis of the Cnidaria. J. Zool. Syst. Evol. 31, 161–173.

Schuchert, P., Reber-Müller, S., Schmid, V., 1993. Life stage specific expression of a myosin heavy chain in the hydrozoan Podocoryne carnea. Difference 54, 11–18.

Sedgwick, W., 1884. On the origin of metameric segmentation and some other morphological questions. Q. J. Microsc. Sci. 24, 43–82.

Seipel, K., Yanze, N., Schmid, V., 2004a. Developmental and evolutionary aspects of the basic helix–loop–helix transcription factors Atonal-like1 and Achaete-scute homolog 2 in the jellyfish. Dev. Biol. 269, 331–345.

Seipel, K., Yanze, N., Schmid, V., 2004b. The germ line and somatic stem cell gene Cniwi in the jellyfish Podocoryne carnea. Int. J. Dev. Biol. 48, 1–7.

Seipel, K., Eberhardt, M., Müller, P., Pescia, E., Yanze, N., Schmid, V., 2004c. Homologs of vascular endothelial growth factor and receptor, VEGF and VEGFR, in the jellyfish Podocoryne carnea. Dev. Dyn. 231, 303–312.

Sellers, J.R., 2000. Myosins: a diverse superfamily. Biochim. Biophys. Acta 1496, 3–22.

Simeone, A., 1998. Otx1 and Otx2 in the development and evolution of the mammalian brain. EMBO J. 17, 6790–6798.

Showell, C., Binder, O., Conlon, F.L., 2004. T-box genes in early embryogenesis. Dev. Dyn. 229, 201–218.

Slack, J.M., Holland, P.W.H., Graham, C.F., 1993. The zootype and the phylotypic stage. Nature 361, 490–492.

Smith, K.M., Gee, L., Blitz, I.L., Bode, H.R., 1999. CnOtx, a member of the Otx gene family, has a role in cell movement in Hydra. Dev. Biol. 212, 392–404.

Spicer, D.B., Rhee, J., Cheung, W.L., Lasser, A.B., 1996. Inhibition of myogenic bHLH and MEF2 transcription factors by the bHLH protein twist. Science 272, 1476–1480.

Spring, J., Yanze, N., Middel, A.M., Stierwald, M., Gröger, H., Schmid, V., 2000. Ancestral role of the mesoderm specification factor twist in the life cycle of jellyfish. Dev. Biol. 228, 363–375.

Spring, J., Yanze, N., Jösch, C., Middel, A.M., Winninger, B., Schmid, V., 2002. Conservation of Brachyury, Mef2, and Snail in the myogenic lineage of jellyfish: a connection to the mesoderm of Bilateria. Dev. Biol. 244, 372–384.

Stanley, G.D., Stuermer, W., 1983. The first fossil ctenophore from the lower Devonian of West Germany. Nature (London) 303, 518–520.

Stierwald, M., Yanze, N., Bamert, R.P., Kammermeier, L., Schmid, V., 2004. The Sine oculis/Six class family of homeobox genes in jellyfish with and without eyes: development and eye regeneration. Dev. Biol. 274, 70–81.

Storch, V., Welsch, U., 1974. Epithelialmuscular cells in Lingula unguis (Brachiopoda) and Branchiostoma lanceolatum (Acrania). Cell Tissue Res. 154, 543–545.

Tardent, P., 1978. Coelenterata, Cnidaria. In: Seidel, F. (Ed.), Morphogenese der Tiere. Gustav Fischer Verlag, Jena, pp. 83–289.

Technau, U., Bode, H.R., 1999. HyBra1, a Brachyury homologue, acts during head formation in Hydra. Development 126, 999–1010.

Technau, U., Scholz, C.B., 2003. Origin and evolution of endoderm and mesoderm. Int. J. Dev. Biol. 47, 513–539.

Valentine, J.W., Erwin, D.H., Jablonski, D., 1996. Developmental evolution of metazoan body plans: the fossil evidence. Dev. Biol. 173, 373–381.

Van de Vyver, G., 1993. Reproduction sexuée-embryologie. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Cnidaires, Cténaires, Vol. III, Fascicule 2. Masson, Paris, pp. 417–471.

Verma-Kurvari, S., Savage, T., Gowan, K., Johnson, J.E., 1996. Lineage-specific regulation of the neural differentiation gene MASH1. Dev. Biol. 180, 605–617.

Wade, M., 1993. Scyphozoan fossils: vendimedusae, cubomedusae, scyphomedusae, conulata. In: Grassé, P.-P. (Ed.), Traité de Zoologie. Cnidaires, Anthozoaires, Vol. III, Fascicule 3. Masson, Paris, pp. 923–942.

Weber, C., 1981. Structure, histochemistry, ontogenetic development, and regeneration of the ocellus of Cladonema radiatum Dujardin (Cnidaria, hydrozoa, Anthomedusae). J. Morphol. 167, 313–331.

Weber, C., Alder, H., Schmid, V., 1987. In vitro transdifferentiation of striated muscle to smooth muscle cells of a medusa. Cell Differ. 20, 103–115.

Weiler-Stolt, B., 1960. Ueber die Bedeutung der Interstitiellen Zellen fuer die Entwicklung der Fortpflanzung mariner Hydroiden. Roux' Arch. Entwicklungsmech. 152, 398–454.

Weiss, A., Leinwand, L.A., 1996. The mammalian myosins heavy chain gene family. Annu. Rev. Cell Dev. Biol. 12, 417–439.

Werner, B., 1963. Effect of some environmental factors on differentiation and determination in marine Hydrozoa, with a note on their evolutionary significance. Ann. N. Y. Acad. Sci. 105, 461–488.

Willmer, P., 1990. Invertebrate Relationships: Patterns in Animal Evolution. Cambridge Univ. Press, Cambridge, UK.

Wray, G.A., 2003. Transcriptional regulation and the evolution of development. Int. J. Dev. Biol. 47, 675–684.

Yanze, N., Gröger, H., Müller, P., Schmid, V., 1999. Reversible inactivation of cell-type-specific regulatory and structural genes in migrating isolated striated muscle cells of jellyfish. Dev. Biol. 213, 194–201.

Yanze, N., Spring, J., Schmidli, C., Schmid, V., 2001. Conservation of Hox/ParaHox-related genes in the early development of a cnidarian. Dev. Biol. 236, 89–98.

Yoshida, S., Ohbo, K., Takakura, A., Takebayashi, H., Okada, T., Abe, K., Nabeshima, Y., 2001. Sgn1, a basic helix–loop–helix transcription factor delineates the salivary gland duct cell lineage in mice. Dev. Biol. 240, 517–530.