Evolution of the Heart from Bacteria to Man

NANETTE H. BISHOPRIC

Department of Molecular and Cellular Pharmacology, University of Miami, Miami, Florida 33101, USA

ABSTRACT: This review provides an overview of the evolutionary path to the mammalian heart from the beginnings of life (about four billion years ago) to the present. Essential tools for cellular homeostasis and for extracting and burning energy are still in use and essentially unchanged since the appearance of the eukaryotes. The primitive coelom, characteristic of early multicellular organisms (~800 million years ago), is lined by endoderm and is a passive receptacle for gas exchange, feeding, and sexual reproduction. The cells around this structure express genes homologous to NKX2.5/tinman, and gradual specialization of this "gastroderm" results in the appearance of mesoderm in the phylum Bilateria, which will produce the first primitive cardiac myocytes. Investment of the coelom by these mesodermal cells forms a "gastrovascular" structure. Further evolution of this structure in the bilaterian branches Ecdysoa (Drosophila) and Deuterostoma (amphioxus) culminate in a peristaltic tubular heart, without valves, without blood vessels or blood, but featuring a single layer of contracting mesoderm. The appearance of Chordata and subsequently the vertebrates is accompanied by a rapid structural diversification of this primitive linear heart: looping, unidirectional circulation, an enclosed vasculature, and the conduction system. A later innovation is the parallel circulation to the lungs, followed by the appearance of septa and the four-chambered heart in reptiles, birds, and mammals. With differentiation of the cardiac chambers, regional specialization of the proteins in the cardiac myocyte can be detected in the teleost fish and amphibians. In mammals, growth constraints are placed on the heart, presumably to accommodate the constraints of the body plan and the thoracic cavity, and adult cardiac myocytes lose the ability to re-enter the cell cycle on demand. Mammalian cardiac myocyte innervation betrays the ancient link between the heart, the gut, and reproduction: the vagus nerve controlling heart rate emanates from centers in the central nervous system regulating feeding and affective behavior.

KEYWORDS: apoptosis; conduction system; evolution; heart development; mammals; metabolism; phylogeny; stem cells

FROM METEORITES TO METABOLISM (4.5–3.5 BILLION YEARS AGO)

At the root of the tree of life are the prokaryotes, which appeared approximately 3.8 billion years ago (BYA). The earth was emerging from the Hadean period, an extremely hot and volatile era characterized by incessant collisions, explosions, and

Address for correspondence: Prof. Nanette H. Bishopric, M.D., Prof. Pharmacology, Medicine, and Pediatrics, Dept. of Molecular and Cellular Pharmacology, University of Miami, P.O. Box 016189 (R-189), Miami, FL 33101, USA. Voice: 305-243-6775; fax: 305-243-6082.
nhb@chroma.med.Miami.edu

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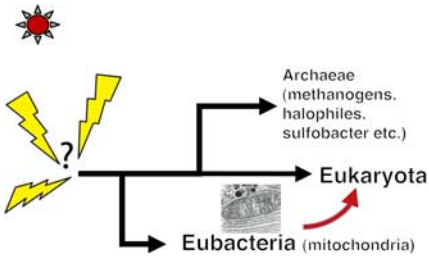

FIGURE 1. In the beginning. The creation of the Earth was followed rather quickly by the appearance of life, c. 4 billion years ago. Ingestion of one prokaryote by another leads to the first eukaryotic life form [in color in Annals Online].

volcanic eruptions (FIG. 1). Consequently, life is likely to have first appeared under conditions vastly different from those existing today. Research into modern-day microbial populations at extremes of heat and pressure may lead to a better picture of the properties of the first living organism. However, it is likely that this ancestor was a single cell, bounded by a protein–lipid membrane externally but without defined internal compartments segregating metabolic and reproductive functions.[1] This cell would have been highly heat tolerant and able to obtain energy and carbon from inorganic substrates. It would have been anaerobic, for there was no oxygen in the Hadean atmosphere. Based on analyses of organisms harvested from deep ocean volcanic vents, which are thought to replicate features of the Hadean environment, molecular hydrogen and sulfur may have served as electron donors and oxidized sulfur and CO_2 as electron acceptors for energy generation. From this early prokaryote branched the eubacteria and the so-called archaea, including the halophiles and methanogens. Comparative analysis of 16S rRNA sequences suggests that the archaeae may have evolved relatively little since then.

The appearance of enzymes, or catalytic proteins, was a critical and probably essential first step in the formation of living organisms. In the absence of oxygen, primitive monists utilized enzymes capable of accelerating chemical reactions that could generate energy through the breakdown of carbohydrates. These glycolytic enzymes have been highly conserved throughout subsequent evolution, as have been other eubacterial features, including HMG CoA reductases, required for cell membrane components, and ribosomes. Although well-developed cytoskeletons do not appear until later in evolution, membrane- and DNA-associated forms of actin and homologues of other cytoskeletal proteins, including tubulin, have been identified in the eubacteria.[2–4]

The appearance of photosynthetic cyanobacteria ~3.5 BYA eventually caused the appearance of oxygen in the atmosphere beginning about 2.8 BYA. This profound environmental change precipitated a number of new phenomena, including rust, oxidative stress, and the development of a capacity for oxygen metabolism in certain bacteria. The enzymes in this pathway were probably derived from similar enzymes used in the oxidation of sulfur, but with a considerably greater relative yield of energy per reducing equivalent.[5] This increase in bioenergetic efficiency provided the footing for a quantum expansion of biological complexity.

EUKARYOTES: THE ACCIDENTAL SYMBIONTS (2.1–1.8 BILLION YEARS AGO)

Just as today, the early prokaryotes lived in close proximity and frequently used each other as food. It is now generally believed that one such ingestion resulted not

in the breakdown of the eaten, but in the establishment of an endosymbiosis in which the meal, most likely a member of the alpha-proteobacteria, remained metabolically active within the organism that ate it: thus appeared the first mitochondria.[6] This hypothesis is supported by evidence from *Reclinomonas americana*, a single-celled organism whose mitochondria contain an almost intact alpha-proteobacterial genome. The alpha-proteobacteria are related to the rickettsia, and many species exhibit obligate or opportunistic intracellular symbiotic lifestyles.[7] Similarly, an endosymbiosis involving cyanobacteria in the ancestors of green plants likely resulted in the development of the chloroplast. The appearance of other bilamellate organelles, such as the lysosomes, may have come about in the same way.

The presence of multiple autonomously replicating mitochondria in this new lifeform meant a tremendous increase in energy generating capacity. Moreover, each of these mitochondria could realize a 20-fold increase in the energy yield from carbohydrates simply by facilitating a move from glycolytic to oxidative metabolism when oxygen was available. In what could be thought of as a downside to the relationship, the mitochondria-dependent apoptosis pathway could have developed from residual components of an ancient prokaryotic defense system.[8–10]

Even the eukaryotic nucleus may have an endosymbiotic origin, although this is more controversial.[11–15] Molecular evidence suggests that the eukaryotes resemble both the eubacteria and archaeae, as if they arose from a symbiosis between these two prokaryotes.[16] DNA- and RNA-processing functions appear to have come from the archaeae: both archaea and eukaryotes have genes for histones, but the eubacteria do not.[17] On the other hand, several genes involved in metabolism appear to have a eubacterial rather than archaeal origin.[18] One view is that archaeae may have taken up residence within bacteria and gradually lost the need for independent metabolic activity. An even more contentious suggestion is that viruses present in the primordial soup were responsible for the transfer to prokaryotes of genetic material within an envelope, the nucleus then being a sort of chronic viral infection.[19] Whatever its origin, the nucleus provides a separation between transcription (nuclear) and translation (cytosol), and hence an opportunity for greater RNA editing before protein translation is initiated.

PLANTS AND ANIMALS: A COMMON ORIGIN FOR MULTICELLULAR ORGANISMS?

Eukaryotes branched into more than 60 separate lines, including the green plants, red algae, diatoms, and protists. However, resolving the kinships among fungi, plants, and metazoans has been difficult[20] (FIG. 2). Molecular phylogeny of a number of ancient genes, including elongation factor 1a, support a sister relationship between the fungi and the metazoans, with a more distant kinship to the green plants. On this basis a new eukaryotic superfamily has been proposed, the Opisthokonts (meaning "flagellum in the rear"). This kingdom includes the fungi, collar flagellates (including *Giardia lamblia*) and our direct ancestors, the metazoans. In this model, our earliest common eukaryotic ancestor would have been capable of directed movement, facilitated not only by a new abundance of energy but also by the development of a cytoskeleton, from which the flagellum likely derived. Evolution

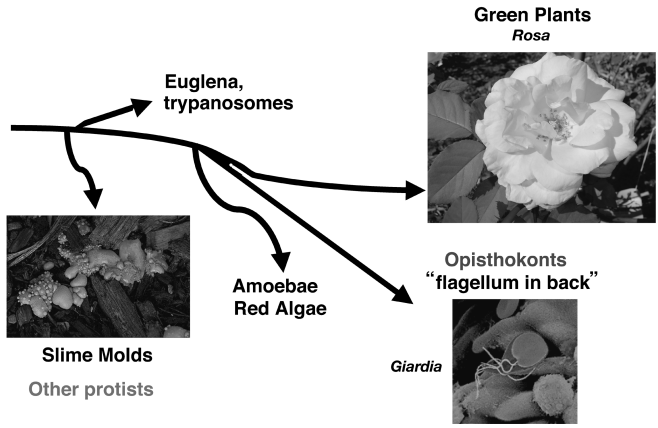

FIGURE 2. Divergence of the eukaryotes. Eukaryotes, still single-celled, split into more than sixty separate lines. The timing of plant divergence remains conjectural [in color in Annals Online].

of the cytoskeleton, and of a more pliable outer membrane, may have been driven by the need to eat other organisms.[21] The earliest cytoskeletal structures have been expanded into the cilia, mitotic spindles, and components of cytokinesis. The actomyosin filaments that permit cellular contraction are similar to those in budding yeast (*Saccharomyces cerevisiae*), which form an actomyosin-containing constriction ring during cell division.[22] This model suggests that green plants took a radically different evolutionary path in which movement was a low priority; meanwhile, traits we share with plants, such as multicellularity and tissue differentiation, are the result of convergent evolution rather than a common inheritance.

However, other lines of evidence, including analysis of a different set of genes, suggest a closer relationship between plants and metazoans that excludes the fungi. For example, plants and metazoans express homologues of p300/CBP acetyltransferases, but fungi do not.[23,24] Atrial natriuretic peptides have been identified in both metazoans and green plants, and serve to regulate solute flow through tissues in both groups.[25,26] GATA transcription factors, similar to those in metazoans, are present in plants and regulate transcription in response to light and nitrates.[27] MADS-box transcription factors are also found in plants, where they regulate aspects of development including flower formation.[28] The entire suite of G1-regulating proteins, including E2F and Rb, appear to be exclusive to the metazoans and green plants, as are the signals regulating the designation of totipotent stem cells.[29] These findings will need to be resolved in future models of the plant–animal–fungus trifurcation.

METAZOANS AND MULTICELLULARITY (800–700 MILLION YEARS AGO)

Some protists (algae, kelp) and many fungi exist as complexes of cells rather than as free-living organisms. However, multicellularity is a hallmark of metazoan life

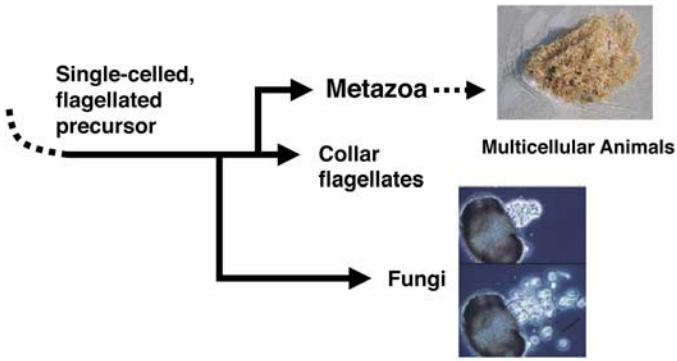

FIGURE 3. The opisthokonts, ancestors of multicellular life. A representative metazoan (a sponge) is shown [in color in Annals Online].

(FIG. 3). The most primitive extant metazoan phylum, Poriphera (sponges), as well as members of the sister phylum Cnidaria (corals) form motile, flagellated blastulae that migrate and eventually attach to substrate to form new multicellular organisms with two basic cell layers separated by a gelatinous matrix. New insights into the molecular origins of multicellular life, and the basal position of Poriphera in the animal kingdom, have been recently reviewed.[30,31] Most of the important fundamental features of multicellular life are present in Poriphera and simply undergo further specialization and refinement as evolution proceeds. Several of the more important innovations will be mentioned here.

Separation of Cell Layers

Sponges, like corals and jellyfish, are diploblastic: that is, they have only two embryonic cell layers, endoderm and ectoderm. They have a single central body cavity, or coelom, in which eating, gas exchange, and reproduction occur. The endodermal lining of this cavity is involved in three major functions: circulation of seawater (through ciliary action), nutrient absorption, and reproduction, through capture of sperm that may filter into the coelom. Subsequent evolutionary events will gradually separate these functions in higher metazoans, but this observation from Poriphera suggests that a single endodermal cell type was responsible for all three in the last common ancestor of all animals.

Matrix Molecules

An extracellular matrix is required to hold cells together, and collagen and beta-crystallin first appear in this context.[32] Collagen will go on to become the most abundant protein in the metazoan kingdom. In sponges, collagen synthesis has been shown to be regulated by a homologue of myotrophin, evidence of an early autocrine/paracrine loop directing cell growth, and analogous to the induction of cardiac hypertrophy by mammalian myotrophin.[33]

Cell–Cell Communication and Recognition

Disaggregated sponge cells can re-associate in a process that depends on a proteoglycan aggregation factor and its receptor, a membrane protein with immunoglobulin and scavenger receptor homologies.[34] The sponge possesses the ability to recognize and reject allografts from other sponges, and the grafted cells undergo programmed death with upregulation of a death domain–containing receptor homologous to Fas.[35] Related innovations thought to have arisen in Urmetazoan ancestors include cell–cell and cell-matrix adhesion molecules, epithelial tight junctions, an early immune system, and the allocation of pluripotent stem cells with surface markers resembling those on mammalian precursor cells.[36,37] Proteins with homology to Bcl-2 can be found in sponges,[38–41] showing that both immune recognition and apoptosis are phylogenetically ancient. Apoptotic signaling pathways are also found in plants,[42] strengthening the argument that all multicellular organisms are related from a common ancestor (see above).

Body Patterning Molecules

The body plans of the earliest multicellular animals, including Poriphera, do not include a head or a tail or indeed any axis of symmetry. Radial or axial body plan symmetry are likely to have appeared in an ancestor common to both the Cnidaria (e.g., corals, jellyfish, sea anemones, hydra) and Bilateria, from which mammals are descended (FIG. 4). It is this hydra-like ancestor that may have taken the first steps toward mesoderm as well.[43,44] As noted earlier, jellyfish are diploblastic, with outer and inner cell layers separated by a jellylike substance, the mesohyl; however, during

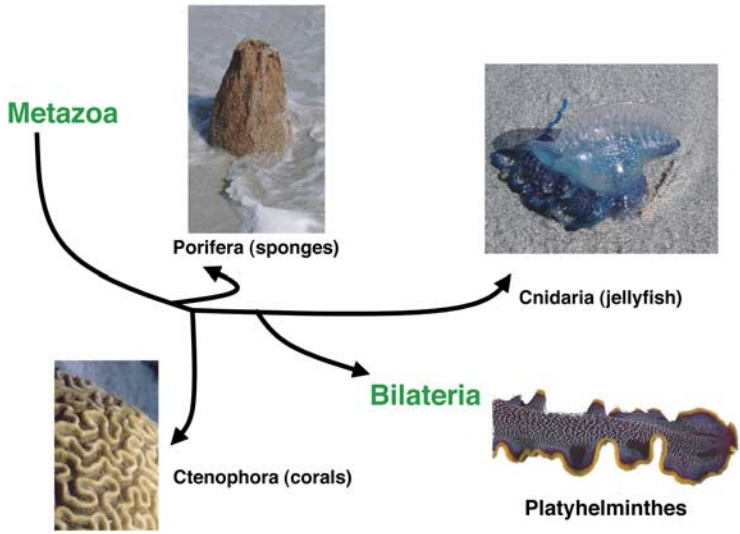

FIGURE 4. The first animals. Early branching of the metazoan family, of which Porifera are considered basal members [in color in Annals Online].

the medusa, or sexually reproductive, stage of the jellyfish life cycle, a number of specialized non-myoepithelial cells are found, including striated muscle cells, and sensory and nerve cells.[45] The striated muscle cells derive from a third germ layer that separates from the ectoderm early in medusa development,[46] possibly analogous to the middle germ layer of Bilateria, and express muscle-specific forms of troponin and myosin heavy chain. Further evidence that this third germ layer is a prototype of mesoderm is that it expresses the mesoderm-patterning genes Twist and Brachyury, as well as the muscle regulatory proteins MEF-2 and Snail.[45]

THE SYMMETRICAL BODY PLAN: BILATERIA (700–600 MILLION YEARS AGO)

All Bilateria are unequivocally triploblastic. At the root of this group are the flatworms, with later branches including the Ecdysoa, ancestors of the insects; the Lophotrochozoa, ancestors of annelids and mollusks; and the Deuterostomes. Our bilaterian ancestors were probably wormlike organisms with a recognizable front and back end (including a one-way intestine with anus), but lacking a recognizable head or eyes.[47] Patterning of the sagittal axis, and the development of a head with a separate opening to the environment, occurred with the radiation of the Bilateria and apparently coincident with the expansion of the Hox gene cluster.[48] Evidence suggests that a single prototype of these pattern-dictating genes underwent one or more rounds of tandem duplication around the time of the great Cambrian explosion, a time when a large increase in the number of life-forms appears in the archaeological record.[49] The Hox gene cluster dictates segmentation, headness, and tailness in the Bilateria,[50,51] and increasing copy number and divergence of these genes has been accompanied by increasing body plan complexity. In addition to the anterior–posterior axis, a clear dorsoventral axis becomes established sometime during bilaterian evolution.

The Tubular Heart

A critical step, the appearance of a single or paired heart primordium, occurred in a bilaterian ancestor—most likely prior to the divergence of Deuterostomes and Ecdysoa. Based on commonalities among the major branches of Bilateria (FIG. 5), this structure was most likely a tubular, pulsatile structure that lacked an enclosed vascular system but instead served to force fluid through pericellular interstices. Lacking chambers, septa, and valves, these early heart tubes probably did not drive unidirectional blood flow. During embryogenesis in insects, mollusks and annelids, this heart tube begins as an invagination from the gut, and continuity between the heart tube(s) and gut persists into the adult form. The myocytes investing the heart tube may have features of myoepithelium, vertebrate cardiomyocytes, and/or striated muscle, depending on the organism, and may have self-renewal properties.[52] The Drosophila heart contains additional features such as a cardioaortic valve and pericardial cells.

Whether the insect and vertebrate hearts evolved independently has been argued for nearly 200 years, largely on anatomic grounds: the insect heart tube is dorsal, while the vertebrate heart is ventral. Although it is possible that the emergence of

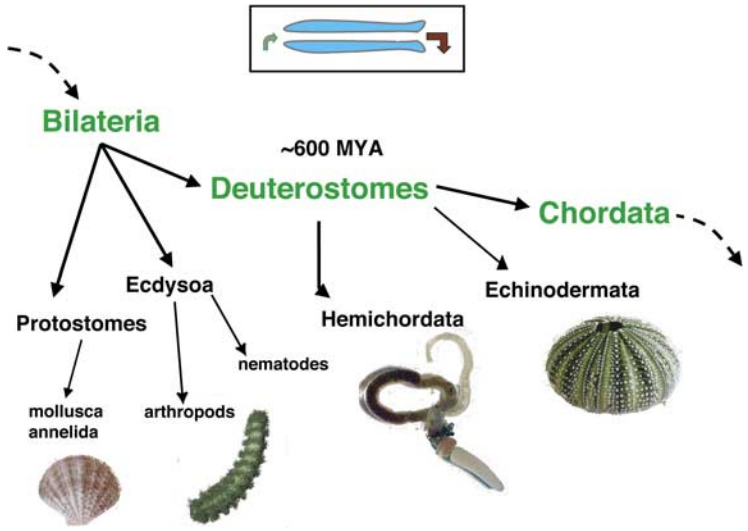

FIGURE 5. The two-ended body plan. Major branches of the Deuterostomes are shown. Two important model organisms, Drosophila and *C. elegans*, belong to the arthropods and nematodes, respectively. The diagram depicts the novel two-stoma body plan [in color in Annals Online].

insect and vertebrate hearts from non-cardiac mesoderm were separate events after the divergence of Deuterostomes and Ecdysoa, another point of view is that a simple longitudinal heart tube was present in the earliest bilaterians and subsequently underwent independent morphological and gene regulatory modifications. Functional and genetic, if not anatomic, evidence supports a close relationship between this primordial structure and the mammalian, chambered heart. The Drosophila heart tube is induced by expression of a homeotic gene, tinman, which was later found to have a mammalian homologue with the same function (Nkx2.5). Nkx homologues play similar roles in amphioxus and tunicates and have been cloned from octopus and cuttlefish.[53] Drosophila heart cells also express homologues of MEF2 and Hand, two genes involved in cardiogenic differentiation in vertebrates.[54–56] Orthologous gene regulatory patterns dictating nervous system development and anteroposterior patterning are also shared between Drosophila and vertebrates. These similarities can be explained by body plan inversion or structural relocations in the descendents of a common ancestor that already possessed a heart tube, notochord, and anteroposterior axis.[57] Interestingly, the single *Caenorhabditis elegans* MEF-2 homologue, *ceMEF2*, is dispensible for myogenesis, indicating that nematodes may have evolved alternative functions for this protein.[58]

THE TWO-ENDED ANIMAL: DEUTEROSTOMES (~600 MILLION YEARS AGO)

The Deuterostomes include the Echinodermata (e.g., sea urchins, starfish), Hemichordates (acorn worms) and Chordates. The Deuterostome body plan has a through-

gut, with two openings at the head and rear of the bilaterally symmetric body plan, in place of the single gastrovascular–reproductive opening of earlier metazoans. Creation of the new opening, the mouth, involves specialization of head structures that require expression of the patterning genes Otx, Forkhead, Brachyury, and Goosecoid, among others. These gene products dictate both the appearance of the anterior gut and of a dorsal cluster of nerve cells that replaces a diffuse net of nerves.[43,59]

With these promising innovations, the original Deuterostome is still thought to have been a headless, eyeless, worm-like creature similar to the modern acorn worm. This latter organism is an enteropneust ("gut-breather") suggesting that our common ancestor still lacked segregation of gas exchange and digestive functions. Appearance of the mouth marks the beginning of the separation of gut and vascular system and is a critical feature distinguishing Deuterostomes from the other two major branches of Bilateria: Ecdysoa (e.g., fruit flies), and Lophotrochozoa (e.g., annelid worms).

Deuterostome evolution coincides with the beginning of the multiplication and functional divergence of genes encoding components of the contractile proteins. Superimposition of molecular phylogenetic trees suggests that significant radiation of striated muscle actin, myosin, and troponin isoforms occurred during the Cambrian Explosion sometime between 570 and 540 million years ago (MYA). In particular, divergence of smooth and striated muscle isoforms of the EF-hand proteins (myosin essential light chains, cardiac myosin regulatory light chains, and tropinin C), as well as striated- and smooth-muscle forms of myosin heavy chain and actin, appear to have taken place during or before the appearance of the chordates.[60]

THE NERVOUS SYSTEM GETS ORGANIZED: CHORDATES (~550 MILLION YEARS AGO)

The Chordates are defined by the presence of a dorsal nerve cord and a muscular supporting notochord (FIG. 6). Three major chordate branches extant today are the Urochordates (tunicates), the Cephalochordates (e.g., amphioxus), and the Vertebrates, which have a segmented bony enclosure for the axial nervous system. Myotomes and skeletal muscle are well established in the chordates, although phylogenetic trees suggest that the myogenic factors MyoD and Myf arose earlier. The basal members of this family, the sea squirts, typically have a notochord only during the larval stage, which involutes after attachment and immobilization. Other new features in animals from this group include development of a filter-feeding pharynx or gill structures,[61] the first appearance of an organized liver (amphioxus), and complete self-containment of the vascular system.

Both amphioxus and tunicates have a tubular heart that contracts rhythmically. The direction of flow may alternate in isolated tunicate heart preparations (M. Morad, personal communication). As in earlier Deuterostomes, these vessels are centered on the gut and pharynx, although no longer continuous with it. Features unique to amphioxus are the presence of both dorsal and ventral vessels, several parts of which are contractile.[62] The notion that these indeed constitute a heart equivalent is supported by the identification of an amphioxus Nkx2.5 homologue (*AmphiNk2-tin*) that is initially expressed in the ventral vessel.[53] This heart lacks chambers, valves, or endothelial lining and consists entirely of myocardial cells.[52]

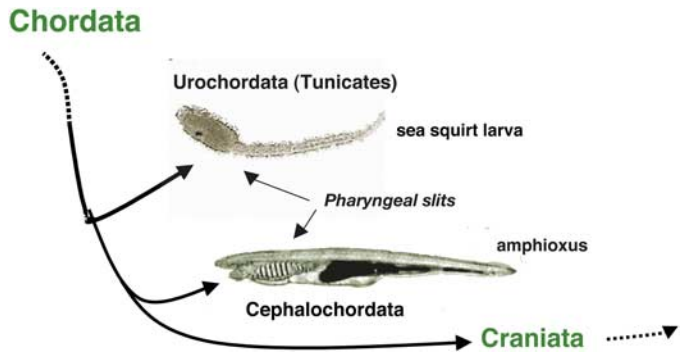

FIGURE 6. Chordates: the gut and gas exchange separate. Development of the enclosed vascular system, distinct from the gastrointestinal tract, appears first in Chordates. Tunicates (sea squirts) are basal members of the chordate family, as seen here in larval form. After anchoring to the sea floor, adult tunicates lose their nervous system, gills, and mobility, but retain a cardiovascular system [in color in Annals Online].

BRAINS AND HEARTS: THE VERTEBRATES (~500 MILLION YEARS AGO)

At the base of the Vertebrate branch are the hagfish and lampreys (FIG. 7). Hagfish (*Myxinidae*) have a partially calcified skull, but a cartilaginous vertebral column, so that they only just qualify as vertebrates. Their closed vascular system has several contractile components in series, including the portal vein heart and the systemic

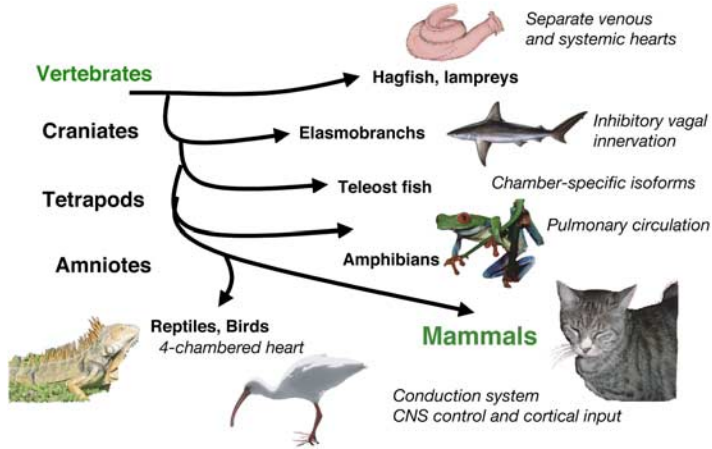

FIGURE 7. Some vertebrate innovations in cardiac morphology and function. Note the apical position of Felis domesticus [in color in Annals Online].

heart. The portal vein heart delivers blood to the gill vasculature and is roughly analogous to the right-sided cardiac chambers in birds and mammals. The Myxine portal vein heart and both atria express a natriuretic peptide.[63,64] Subsequent vertebrate evolution has produced many radical innovations in cardiac shape and function, including chambers and valves, separate respiratory and systemic circulations, endothermy, gap junctions, and the conduction system. A detailed discussion of each of these is beyond the scope of this review; the reader is referred to several excellent papers on general and specific aspects of vertebrate heart development.[65–67]

Endothermy and Energy Conservation

A recurring theme in vertebrate evolution is the development of adaptations to manage energy consumption and storage. Steps to improve bioenergetic efficiency, such as optimizing the efficiency of blood circulation, modifying metabolic and contractile protein functions under periods of stress, and providing central nervous system control over cardiac activity can be identified at various stages of vertebrate evolution. The development of endothermy in avians and mammals (and possibly therapods) conferred greater environmental adaptability and reproductive success[68] but also increased the metabolic cost of daily living.[69] This will have increased demand on the heart and cardiovascular system to deliver nutrients, as well as have increased the amount of effort needed to obtain nutrients from the environment. Many vertebrates undergo metabolic slowing during periods of limited resources, and some, including hagfish, can survive extended periods of anoxia. Similar adaptive methods, such as a switch to anaerobic glycolysis under hypoxia, can be observed in the mammalian myocardium.[70]

Isoform Specialization

Gene duplications likely facilitated many of the innovations of the vertebrate heart. The Devonian period (~420 MYA) saw a huge proliferation of fish species, a movement from marine into fresh water environments, and eventually the first scramble of vertebrates onto land (~300 MYA). The accompanying radiation of many genes encoding contractile and cytoskeletal elements, creatine kinase, and natriuretic peptides led to altered molecular properties for these proteins, and provided for functional specialization.[71–74] Cardiac actin and cardiac troponin C diverged from their skeletal counterparts prior to the frog–mammal divergence (350 MYA), while the three classes of cardiac, slow skeletal, and fast skeletal muscle isoforms diverged some time before the bird–mammal separation. Functional specialization is illustrated by trout cardiac TnC, which exhibits amino acid changes that confer increased calcium sensitivity, offsetting the desensitizing effect of cold and allowing cardiac function to be maintained at low water temperatures.[75] The acquisition of novel transcriptional regulatory properties for contractile proteins permits great plasticity in the vertebrate heart, including the ability to increase contractile protein mass rapidly.[76] Products of gene duplication may also acquire divergent regulatory properties: although cardiac and skeletal actin isoforms are tightly conserved at the protein level, their gene control regions are highly dissimilar.[77–80] Divergence at the promoter level provides for different levels of transcriptional response depending on the extracellular milieu, and for the recruitment of isoforms with specific adaptive properties during periods of increased demand.

Separation of the Pulmonary Circulation

Definitive organization of the original tubular heart into separate systemic and pulmonary circulations occurred sometime between separation of the teleost fish and amphibians (350–300 MYA).[81] Teleost fish hearts have only two chambers that serve both for oxygenation and tissue perfusion, connected by an atrioventricular valve, while the amphibian heart has two atria and a single ventricular chamber. In reptiles, a muscular ridge running from the apex to the base of the ventricle creates directed flow of oxygenated and deoxygenated blood. This ridge is small in turtles, and considerable mixing occurs, but in lizards and snakes, the ridge is larger and separation is more efficient.[82] Birds and mammals have complete atrial and ventricular septa, allowing separation between oxygenated and deoxygenated blood, and a full division of the systemic and pulmonary circulations. The importance of this divided and directional flow can be observed when the system is defective.[83]

Myocardial Regeneration (Or Not)

The ability to efficiently replace lost myocytes disappeared sometime around the appearance of warm-bloodedness. Unlike mollusks, arthropods, and amphibians,[52] mammals cannot generate significant numbers of new ventricular myocytes after birth, either through self-renewal or replacement. This loss has become a significant liability only very recently, with the appearance of *Homo sapiens sapiens* and the unique ability of Western societies to meet and exceed daily caloric requirements.[84] Recent, detailed studies have identified a pool of undifferentiated mononuclear precursor cells in human ventricular myocardium, identifiable by expression of one or more surface markers including *c-kit* and sca-1;[85–87] a second population of isl-1–positive precursor cells has recently been observed in the atria and right ventricular outflow tract.[88] The extent to which these cells resemble the various types of precursor cells identified in myocardial tissue from arthropoda to ascidians and whether myocardial regenerative capability will be re-created in humans are currently topics of intensive research.

Cell–Cell Communication

Connexins are proteins that form intracellular junctions that transmit several types of chemical and electrical signals. The appearance of connexin-based gap junctions parallels the increasing complexity and size of the vertebrate heart. A basal connexin has been reported in chordates and more than 20 separate connexin family members are found in mammals. Rapid communication through gap junctions is a critical factor allowing the vertebrate heart to contract in a rapid, synchronous manner, as distinct from the more peristaltic, wave-like contractions of invertebrate hearts.[89] Synchronous contraction contributes to biomechanical efficiency and was further promoted in vertebrate evolution by the appearance of a specialized electrical conduction system. The development of the major components of this system has been reviewed elsewhere[90,91]

Love and the Mammalian Heart

The process of gradual separation of the primitive body cavity into three distinct functional systems is nearly complete in mammals. The combined digestion-sex-

respiration organ has become the gastrointestinal tract, the reproductive system, and the heart. Clues to their ancient unity can be found in their early embryonic gene expression programs and differentiation cues.[92] Interestingly, central nervous system control over these functions has evolved in tandem, re-investing the heart with a role in reproduction. In hagfish, a single, unmyelinated nerve (the vagus) sends branches to the viscera, but there is no direct innervation of the heart. In lower vertebrates, this innervation couples cardiac function to perception of danger by inducing a state of immobilization.[93,94] Higher vertebrates utilize the well-known fight-or-flight response, which inhibits vagal immobilization and stimulates heart rate through secretion of sympathoadrenal hormones into the bloodstream. A recent evolutionary feature, specific to mammals, is the development of a myelinated vagus nerve with sources in the medullary nucleus ambiguus. Changes in vagal tone are critically responsible for rapid slowing and speeding of heart rate in mammals. At the same time, efferent pathways from the mammalian frontal cortex allow vagal tone to be influenced by perceptions of safety and desire for social proximity. The individual is thus able to become calm and receptive to interaction, which might otherwise be terrifying, and invoke death-feigning or flight. This function of the vagus nerve critically enables the behavior required for courtship, copulation, and sustained pair bonding.[94] The tendency to identify our hearts as the location of affection, passion, and constancy may therefore have a sound basis in mammalian neurophysiology. Is the latest chapter in cardiac evolution to be the development of love?

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REFERENCES

1. LAKE, J.A. 1989. Origin of the eukaryotic nucleus: eukaryotes and eocytes are genotypically related. Can. J. Microbiol. **35:** 109–118.
2. GARNER, E.C., C.S. CAMPBELL & R.D. MULLINS. 2004. Dynamic instability in a DNA-segregating prokaryotic actin homolog. Science **306:** 987–989.
3. LEWIS, P.J. 2004. Bacterial subcellular architecture: recent advances and future prospects. Mol. Microbiol. **54:** 1135–1150.
4. KRUSE, T., J. BORK-JENSEN & K. GERDES. 2005. The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. Mol. Microbiol. **55:** 78–89.
5. WEBSTER, K.A. 2003. Evolution of the coordinate regulation of glycolytic enzyme genes by hypoxia. J. Exp. Biol. **206:** 2911–2922.

6. GRAY, M.W., G. BURGER & B.F. LANG. 1999. Mitochondrial evolution. Science **283:** 1476–1481.
7. BATUT, J., S.G. ANDERSSON & D. O'CALLAGHAN. 2004. The evolution of chronic infection strategies in the alpha-proteobacteria. Nat. Rev. Microbiol. **2:** 933–945.
8. BLACKSTONE, N.W. & D.R. GREEN. 1999. The evolution of a mechanism of cell suicide. BioEssays **21:** 84–88.
9. AMEISEN, J.C. 2002. On the origin, evolution, and nature of programmed cell death: a timeline of four billion years. Cell Death Differ. **9:** 367–393.
10. KOONIN, E.V. & L. ARAVIND. 2002. Origin and evolution of eukaryotic apoptosis: the bacterial connection. Cell Death Differ. **9:** 394–404.
11. MANS, B.J., V. ANANTHARAMAN, L. ARAVIND & E.V. KOONIN. 2004. Comparative genomics, evolution and origins of the nuclear envelope and nuclear pore complex. Cell Cycle **3:** 1612–1637.
12. SERAVIN, L.N. 1986. [The origin of the eukaryotic cell. IV. The general hypothesis of the autogenous origin of eukaryotes]. Tsitologiia **28:** 899–910.
13. GOFSHTEIN, L.V. 1978. [Plant histones. Relevance to the evolution of prokaryotes to eukaryotes]. Biokhimiia **43:** 947–958.
14. GUPTA, R.S., K. AITKEN, M. FALAH & B. SINGH. 1994. Cloning of *Giardia lamblia* heat shock protein HSP70 homologs: implications regarding origin of eukaryotic cells and of endoplasmic reticulum. Proc. Natl. Acad. Sci. U.S.A. **91:** 2895–2899.
15. PENNISI, E. 2004. The birth of the nucleus. Science **305:** 766–768.
16. Hartman, H. & A. Fedorov. 2002. The origin of the eukaryotic cell: a genomic investigation. Proc. Natl. Acad. Sci. U.S.A. **99:** 1420–1425.
17. BAILEY, K.A., S.L. PEREIRA, J. WIDOM & J.N. REEVE. 2000. Archaeal histone selection of nucleosome positioning sequences and the procaryotic origin of histone-dependent genome evolution. J. Mol. Biol. **303:** 25–34.
18. MARTIN, W., H. BRINKMANN, C. SAVONNA & R. CERFF. 1993. Evidence for a chimeric nature of nuclear genomes: eubacterial origin of eukaryotic glyceraldehyde-3-phosphate dehydrogenase genes. Proc. Natl. Acad. Sci. U.S.A. **90:** 8692–8696.
19. BELL, P.J. 2001. Viral eukaryogenesis: was the ancestor of the nucleus a complex DNA virus? J. Mol. Evol. **53:** 251–256.
20. LANG, B.F., C. O'KELLY, T. NERAD, *et al*. 2002. The closest unicellular relatives of animals. Curr. Biol. **12:** 1773–1778.
21. NASMYTH, K. 1995. Evolution of the cell cycle. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **349:** 271–281.
22. WATTS, F.Z., D.M. MILLER & E. ORR. 1985. Identification of myosin heavy chain in Saccharomyces cerevisiae. Nature **316:** 83–85.
23. BORDOLI, L., M. NETSCH, U. LÜTHI, *et al*. 2001. Plant orthologs of p300/CBP: conservation of a core domain in metazoan p300/CBP acetyltransferase-related domains. Nucl. Acids Res. **29:** 589–597.
24. PANDEY, R., A. MULLER, C.A. NAPOLI, *et al*. 2002. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. Nucl. Acids Res. **30:** 5036–5055.
25. BILLINGTON, T., M. PHARMAWATI & C.A. GEHRING. 1997. Isolation and immunoaffinity purification of biologically active plant natriuretic peptide. Biochem. Biophys. Res. Commun. **235:** 722–725.
26. VESELY, D.L., W.R. GOWER & A.T. GIORDANO. 1993. Atrial natriuretic peptides are present throughout the plant kingdom and enhance solute flow in plants. Am. J. Physiol. **265:** E465–E477.
27. REYES, J.C., M.I. MURO-PASTOR & F.J. FLORENCIO. 2004. The GATA family of transcription factors in *Arabidopsis* and rice. Plant Physiol. **134:** 1718–1732.
28. NAM, J., C.W. DE PAMPHILIS, H. MA & M. NEI. 2003. Antiquity and evolution of the MADS-box gene family controlling flower development in plants. Mol. Biol. Evol. **20:** 1435–1447.
29. STILLER, J.W. 2004. Emerging genomic and proteomic evidence on relationships among the animal, plant and fungal kingdoms. Genomics Proteomics Bioinformatics **2:** 70–76.
30. KING, N. 2004. The unicellular ancestry of animal development. Dev. Cell **7:** 313–325.

31. MULLER, W.E., M. WIENS, T. ADELL, *et al*. 2004. Bauplan of urmetazoa: basis for genetic complexity of metazoa. Int. Rev. Cytol. **235:** 53–92.
32. KRASKO, A., I.M. MULLER & W.E. MULLER. 1997. Evolutionary relationships of the metazoan beta gamma-crystallins, including that from the marine sponge *Geodia cydonium*. Proc. R. Soc. Lond. B. Biol. Sci. **264:** 1077–1084.
33. SCHRODER, H.C., A. KRASKO, R. BATEL, *et al*. 2000. Stimulation of protein (collagen) synthesis in sponge cells by a cardiac myotrophin-related molecule from *Suberites domuncula*. FASEB J. **14:** 2022–2031.
34. BLUMBACH, B., Z. PANCER, B. DIEHL, *et al*. 1998. The putative sponge aggregation receptor. Isolation and characterization of a molecule composed of scavenger receptor cysteine-rich domains and short consensus repeats. J. Cell Sci. **111:** 2635–2644.
35. WIENS, M., A. KRASKO, B. BLUMBACH, *et al*. 2000. Increased expression of the potential proapoptotic molecule DD2 and increased synthesis of leukotriene B4 during allograft rejection in a marine sponge. Cell Death Differ. **7:** 461–469.
36. MULLER, W.E., M. WIENS, I.M. MULLER & H.C. SCHRODER. 2004. The chemokine networks in sponges: potential roles in morphogenesis, immunity and stem cell formation. Prog. Mol. Subcell. Biol. **34:** 103–143.
37. BOSCH, T.C.G. 2004. Control of asymmetric cell divisions: will cnidarians provide an answer? BioEssays **26:** 929–931.
38. WIENS, M., A. KRASKO, C.I. MULLER & W.E. MULLER. 2000. Molecular evolution of apoptotic pathways: cloning of key domains from sponges (Bcl-2 homology domains and death domains) and their phylogenetic relationships. J. Mol. Evol. **50:** 520–531.
39. ARAVIND, L., V.M. DIXIT & E.V. KOONIN. 1999. The domains of death: evolution of the apoptosis machinery. Trends Biochem. Sci. **24:** 47–53.
40. MITTLER, R., L. SIMON & E. LAM. 1997. Pathogen-induced programmed cell death in tobacco. J. Cell Sci. **110:** 1333–1344.
41. KAWAI, M., L. PAN, J.C. REED & H. UCHIMIYA. 1999. Evolutionally conserved plant homologue of the Bax inhibitor-1 (BI-1) gene capable of suppressing Bax-induced cell death in yeast(1). FEBS Lett. **464:** 143–147.
42. BALK, J., C.J. LEAVER & P.F. MCCABE. 1999. Translocation of cytochrome c from the mitochondria to the cytosol occurs during heat-induced programmed cell death in cucumber plants. FEBS Lett. **463:** 151–154.
43. MARCELLINI, S., U. TECHNAU, J.C. SMITH & P. LEMAIRE. 2003. Evolution of Brachyury proteins: identification of a novel regulatory domain conserved within Bilateria. Dev. Biol. **260:** 352–361.
44. LARTILLOT, N., M. LE GOUAR & A. ADOUTTE. 2002. Expression patterns of fork head and goosecoid homologues in the mollusc *Patella vulgata* supports the ancestry of the anterior mesendoderm across Bilateria. Dev. Genes Evol. **212:** 551–561.
45. SPRING, J., N. YANZE, C. JOSCH, *et al*. 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.
46. KUHN, A. 1910. Die Entwicklung der Geschlectsindividuen der Hydromedusen. Zool. Jahrbuch. **30:** 145–164.
47. BAGUNA, J. & M. RIUTORT. 2004. The dawn of bilaterian animals: the case of acoelomorph flatworms. BioEssays **26:** 1046–1057.
48. POPODI, E., J.C. KISSINGER, M.E. ANDREWS & R.A. RAFF. 1996. Sea urchin Hox genes: insights into the ancestral Hox cluster. Mol. Biol. Evol. **13:** 1078–1086.
49. ZHANG, J. & M. NEI. 1996. Evolution of Antennapedia-class homeobox genes. Genetics **142:** 295–303.
50. PETERSON, K.J., S.Q. IRVINE, R.A. CAMERON & E.H. DAVIDSON. 2000. Quantitative assessment of Hox complex expression in the indirect development of the polychaete annelid Chaetopterus sp. Proc. Natl. Acad. Sci. U.S.A. **97:** 4487–4492.
51. HART, C.P., A. FAINSOD & F.H. RUDDLE. 1987. Sequence analysis of the murine Hox-2.2, Hox-2.3 and Hox-2.4 homeo-boxes: evolutionary and structural comparisons. Genomics. **1:** 182–195.
52. MARTYNOVA, M.G. 2004. Proliferation and differentiation processes in the heart: muscle elements in different phylogenetic groups. Int. Rev. Cytol. **235:** 215–250.
53. HOLLAND, N.D., T.V. VENKATESH, L.Z. HOLLAND, *et al*. 2003. AmphiNk2-tin, an

amphioxus homeobox gene expressed in myocardial progenitors: insights into evolution of the vertebrate heart. Dev. Biol. **255:** 128–137.
54. KOLSCH, V. & A. PAULULAT. 2002. The highly conserved cardiogenic bHLH factor Hand is specifically expressed in circular visceral muscle progenitor cells and in all cells of the dorsal vessel during Drosophila embryogenesis. Dev. Genes Evol. **212:** 473–485.
55. BODMER, R. & M. FRASCH. 1999. Genetic determination of Drosophila heart development. *In* Heart Development. R.P. Harvey & N. Rosenthal, Eds.: 65–90. Academic Press. San Diego.
56. CRIPPS, R.M., T.L. LOVATO & E.N. OLSON. 2004. Positive autoregulation of the Myocyte enhancer factor-2 myogenic control gene during somatic muscle development in Drosophila. Dev. Biol. **267:** 536–547.
57. GERHART, J.C. 2000. Inversion of the chordate body axis: are there alternatives? Proc. Natl. Acad. Sci. U.S.A. **97:** 4445–4448.
58. DICHOSO, D., T. BRODIGAN, K.Y. CHWOE, *et al*. 2000. The MADS-box factor CeMEF2 is not essential for *Caenorhabditis elegans* myogenesis and development. Dev. Biol. **223:** 431–440.
59. TAGAWA, K., N. SATOH & T. HUMPHREYS. 2001. Molecular studies of hemichordate development: a key to understanding the evolution of bilateral animals and chordates. Evol. Dev. **3:** 443–454.
60. OTA, S. & N. SAITOU. 1999. Phylogenetic relationship of muscle tissues deduced from superimposition of gene trees. Mol. Biol. Evol. **16:** 856–867.
61. DOMINGUEZ, P., A.G. JACOBSON & R.P.S. JEFFERIES. 2002. Paired gill slits in a fossil with a calcite skeleton. Nature **417:** 841–844.
62. SIMOES-COSTA, M.S., M. VASCONCELOS, A. SAMPAIO, *et al*. 2005. The evolutionary origin of cardiac chambers. Dev. Biol. **277:** 1–15.
63. REINECKE, M., D. BETZLER & W.G. FORSSMANN. 1987. Immunocytochemistry of cardiac polypeptide hormones (cardiodilatin/atrial natriuretic polypeptide) in brain and hearts of *Myxine glutinosa* (Cyclostomata). Histochemistry **86:** 233–239.
64. ENDO, H., T.K. YAMADA, M. KUROHMARU & Y. HAYASHI. 1997. Structure of portal vein heart wall in the brown hagfish (*Paramyxine atami*). J. Morphol. **231:** 225–230.
65. CHEN, J.N. & M.C. FISHMAN. 1996. Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. Development **122:** 3809–3816.
66. FISHMAN, M.C. & K.R. CHIEN. 1997. Fashioning the vertebrate heart: earliest embryonic decisions. Development **124:** 2099–2117.
67. CHEN, J.N. & M.C. FISHMAN. 2000. Genetics of heart development. Trends Genet. **16:** 383–388.
68. HILLENIUS, W.J. & J.A. RUBEN. 2004. The evolution of endothermy in terrestrial vertebrates: Who? When? Why? Physiol. Biochem. Zool. **77:** 1019–1042.
69. SUAREZ, R.K., C.J. DOLL, A.E. BUIE, *et al*. 1989. Turtles and rats: a biochemical comparison of anoxia-tolerant and anoxia-sensitive brains. Am. J. Physiol. **257:** R1083–R1088.
70. WEBSTER, K.A. & N.H. BISHOPRIC. 1992. Molecular regulation of cardiac myocyte adaptations to chronic hypoxia. J. Mol. Cell. Cardiol. **24:** 741–752.
71. UDA, K., T. SUZUKI & W.R. ELLINGTON. 2004. Elements of the major myofibrillar binding peptide motif are present in the earliest of true muscle type creatine kinases. Int. J. Biochem. Cell Biol. **36:** 785–794.
72. KAWAKOSHI, A., S. HYODO, A. YASUDA & Y. TAKEI. 2003. A single and novel natriuretic peptide is expressed in the heart and brain of the most primitive vertebrate, the hagfish (*Eptatretus burgeri*). J. Mol. Endocrinol. **31:** 209–220.
73. FOCK, U. & H. HINSSEN. 2002. Nebulin is a thin filament protein of the cardiac muscle of the agnathans. J. Muscle Res. Cell Motil. **23:** 205–213.
74. AOUACHERIA, A., C. CLUZEL, C. LETHIAS, *et al*. 2004. Invertebrate data predict an early emergence of vertebrate fibrillar collagen clades and an anti-incest model. J. Biol. Chem. **279:** 47711–47719.
75. GILLIS, T.E. & G.F. TIBBITS. 2002. Beating the cold; the functional evolution of troponin C in teleost fish. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. **132:** 763–772.

76. ANDERSEN, J.B., B.C. ROURKE, V.J. CAIOZZO, *et al*. 2005. Postprandial cardiac hypertrophy in pythons. Nature **434:** 37–38.
77. BISHOPRIC, N.H. & L. KEDES. 1991. Adrenergic regulation of the skeletal alpha-actin gene promoter during myocardial cell hypertrophy. Proc. Natl. Acad. Sci. U.S.A. **88:** 2132–2136.
78. BISHOPRIC, N.H., R. GAHLMANN, R. WADE & L. KEDES. 1992. Gene expression during skeletal and cardiac muscle development. *In* The Heart and Cardiovascular System, 2nd ed., H.A. Fozzard *et al*., Eds.: 1587–1597. Raven Press. New York.
79. SARTORELLI, V., N.A. HONG, N.H. BISHOPRIC & L. KEDES. 1992. Myocardial activation of the human cardiac alpha-actin promoter by helix-loop-helix proteins. Proc. Natl. Acad. Sci. U.S.A. **89:** 4047–4051.
80. SLEPAK T.I., K.A. WEBSTER, J. ZANG, *et al*. 2001. Control of cardiac-specific transcription by p300 through myocyte enhancer factor-2D. J. Biol. Chem. **276:** 7575–7585.
81. SIMOES-COSTA, M.S., M. VASCONCELOS, A.C. SAMPAIO, *et al*. 2005. The evolutionary origin of cardiac chambers. Dev. Biol. **277:** 1–15.
82. WANG, T., J. ALTIMIRAS & M. AXELSSON. 2002. Intracardiac flow separation in an *in situ* perfused heart from Burmese python *Python molurus*. J. Exp. Biol. **205:** 2715–2723.
83. RISHNIW, M. & B.P. CARMEL. 1999. Atrioventricular valvular insufficiency and congestive heart failure in a carpet python. Aust. Vet. J. **77:** 580–583.
84. EATON, S.B., M. KONNER & M. SHOSTAK. 1988. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am. J. Med. **84:** 739–749.
85. BELTRAMI, A.P., L. BARLUCCHI, D. TORELLA, *et al*. 2003. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell **114:** 763–776.
86. URBANEK, K., F. QUAINI, G. TASCA, *et al*. 2003. Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. Proc. Natl. Acad. Sci. U.S.A. **100:** 10440–10445.
87. OH, H., S.B. BRADFUTE, T.D. GALLARDO, *et al*. 2003. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc. Natl. Acad. Sci. U.S.A. **100:** 12313–12318.
88. LAUGWITZ, K.L., A. MORETTI, J. LAM, *et al*. 2005. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature **433:** 647–653.
89. BECKER, D.L., J.E. COOK, C.S. DAVIES, *et al*. 1998. Expression of major gap junction connexin types in the working myocardium of eight chordates. Cell Biol. Int. **22:** 527–543.
90. THOMPSON, R.P., M. RECKOVA, A. DE ALMEIDA, *et al*. 2003. The oldest, toughest cells in the heart. Novartis Found. Symp. **250:** 157–174; discussion 174–176, 276–279.
91. SEDMERA, D., M. Reckova, A. DE ALMEIDA, *et al*. 2003. Functional and morphological evidence for a ventricular conduction system in zebrafish and *Xenopus* hearts. Am. J. Physiol. Heart. Circ. Physiol. **284:** H1152–H1160.
92. NAKAMURA, T. & M.D. SCHNEIDER. 2003. The way to a human's heart is through the stomach: visceral endoderm-like cells drive human embryonic stem cells to a cardiac fate. Circulation **107:** 2638–2639.
93. TAYLOR, E.W., D. JORDAN & J.H. COOTE. 1999. Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol. Rev. **79:** 855–916.
94. PORGES, S.W. 1998. Love: an emergent property of the mammalian autonomic nervous system. Psychoneuroendocrinology **23:** 837–861.