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The Vertebrate Body Axis: Evolution and Mechanical Function[1]

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SYNOPSIS. The body axis of vertebrates is an integrated cylinder of bones, connective tissue, and muscle. These structures vary among living and extinct vertebrates in their orientation, composition, and function in ways that render useless simplistic models of the selective pressures that may have driven the evolution of the axis. Instead, recent experimental work indicates that the vertebrate axis serves multiple mechanical functions simultaneously: bending the body, storing elastic energy, transmitting forces from limbs, and ventilating the lungs. On the biochemical level, research on human intervertebral discs has shown that collagens resist tension and torsion while proteoglycans bind water to resist compression. This molecular behavior predicts mechanical behavior of the entire joint, which, in turn helps determine the mechanical behavior of the vertebral column. The axial skeleton, in turn, is reconfigured by axial muscles that work by way of three-dimensional connective tissues that derive mechanical advantage for the muscle force by using the skin to increase leverage. Models may eventually help determine which evolutionary changes in the vertebrate body axis have had important functional and possibly adaptational consequences. Current reconstruction of the hypothetical stem lineage of early chordates and vertebrates suggests that the gradual mineralization of the vertebral elements, appearance of fin rays and new median fins, and transverse and then horizontal segmentation of the axial musculature are all features correlated with increases in swimming speed, maneuverability, and body size.

INTRODUCTION

> ". . . the nature of the [intervertebral] joint is different from every other that is met with in animal bodies, and there are many circumstances respecting it, which render it uncertain whether human ingenuity can ever make any resemblance to it, that can be applied to the purposes of mechanics."—Everard Home (1809)

Home's skepticism did not keep him from being the first to propose an answer—elastic energy storage—to what remains a central question in vertebrate biology: what is the mechanical function of the axial skeleton? However, the axial skeleton and its structure, function, or evolution, cannot be understood in isolation from the rest of the vertebrate body (Wainwright, 2000), and our goal is to present an integrated picture of the vertebrate body axis.

It is a particularly exciting time to study the vertebrate axis in light of recent discoveries which shed light on the origin of vertebrates, including the discovery of two new jawless fishes (Shu *et al.,* 1999) and another fish-like chordate (Chen *et al.,* 1999) in the Lower Cambrian (530 million years ago, Mya) of south China. These nearly-complete, soft-tissue fossils extend backward by some 60 million years, from the beginning of the Ordovician period (470 Mya; see Janvier, 1996), our knowledge of the early axial anatomy of vertebrates. Both *Myllokunmingia* and *Haikouichthys* (Shu *et al.,* 1999) show structures of the axis of interest to workers studying the function of living species—myosepta of complex three-dimensional shape (Van Leeuwen, 1999), notochords without hemal arches (Czuwala *et al.,* 2000; Hoff and Wassersug, 2000; Long *et al.,* 1998; Sinwell *et al.,* 1999), and a robust dorsal fin tapering to an apparently pointed caudal fin (Hoff and Wassersug,

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2000; Lauder, 2000). This spate of activity underscores a dilemma for paleontologists and evolutionary biologists—How can we understand vertebrate evolution unless we understand the function of axial structures?

Also propelling the study of axial function and evolution is recent work on the genetic control of axial patterning. On the cranial-caudal axis, which at least in ascidian chordates may be established by the maternal *posterior end mark* genes (Di Gregorio and Levine, 1998), *Hox* genes encode transcription factors that are expressed colinearly with axial phenotype in vertebrates (Burke *et al.,* 1995). *Hox* genes maintain and interpret positional information (for review see Holland and Garcia-Fernandez, 1996). Duplication of *Hox* clusters in some vertebrate clades has been implicated in the evolution of ''axial complexity,'' a term usually left undefined. The recent discovery of twice as many clusters in teleost fish compared to mammals has caused some to reconsider (Amores *et al.,* 1998). Rather than discarding the hypothesis that the number of *Hox* genes is proportional to axial complexity, however, we propose that axial complexity in fishes has not been fully appreciated. Upon inspection, one finds complex three-dimensional tendon systems (Willemse, 1959, 1972; Westneat *et al.,* 1993; Gemballa, 1995), numerous and variable bones within the axial musculature (Gemballa and Britz, 1998; Mooi and Gill, 1995), helical muscle fiber trajectories (Alexander, 1969), skin acting as an exotendon (Wainwright *et al.,* 1978; Long *et al.,* 1996), and muscle generating a radial bulging stress (''pressure;'' see Westneat *et al.,* 1998) and stiffening the body in flexion (Long, 1998; Czuwala *et al.,* 1999). This complexity is not surprising if one considers that most teleosts are obligate axial swimmers (Nelson, 1994). Confusion about axial complexity highlights a predicament for geneticists and evolutionary biologists—How can we understand the genetic control of the vertebrate axis unless we understand the function and diversity of axial structures?

Strategic Questions

In spite of, or perhaps because of, the relative dearth of information on the vertebrate body axis, a groundswell of interest and activity is underway. In the next sections, we attempt to review and integrate work being done in three kinds of research programs. We are cheered to note that the distinction between these programs, caused by historical inertia in graduate training and in extramural funding, is blurring as researchers join forces to tackle ''interdisciplinary'' projects. Thus is it with an acknowledgement of the artificiality of these categories that we present three on-going research programs and the strategic questions they address:

(1) Integration of structure and function. What is the relation between axial structures and their mechanical functions at each spatial scale? How do the structures at the smallest scale (protein and ultrastructure) influence mechanical function at the largest (body bending, locomotion)?

(2) Integration of mechanical functions. How do the different mechanical functions of the axial muscle, skeleton, connective tissues, and skin interact to produce the coordinated, high-power behaviors characteristic of locomoting vertebrates? How are the locomotor mechanics integrated with other mechanical functions, such as lung ventilation? Are there other mechanical functions of axial tissues?

(3) Analysis of the evolution of structure and function. What mechanical features of the vertebrate axis have been modified over time? How have those modifications influenced the evolutionary history of different vertebrate lineages?

DISCUSSION

Integration of Function and Structure

Compared to the body axis of any other vertebrate clade, we know more about the integrated function and structure of the mammalian vertebral column—its fundamental biochemical, structural and mechanical properties—because of the potential for biomedical applications in particular and because of our innate interest in mammals in general. In humans, researchers have sought to identify the bases for congenital abnormalities like scoliosis, mechanical failure associated with eccentric strains or trauma, and degenerative changes accompanying aging. Biochemical analysis of the extracellular matrices comprising the intervertebral discs search for molecular explanations underlying physicochemical properties that determine biomechanical function (Maroudas, 1980). In humans and other species, including marine mammals, morphological studies of the vertebrae, soft skeletal tissues, and their functional relationships seek an understanding of how the spine and associated structures form a functional biomechanical unit (Gaudin and Biewener, 1992; Hukins and Meakin, 2000; Long *et al.,* 1997). Biomechanical studies have investigated the mechanical properties of individual structures, the properties of units containing more than one structure, and the spine as a whole (Gal 1992; 1993*a, b*; Hukins and Meakin, 2000; Iatridis *et al.,* 1999; Long *et al.,* 1997).

At the smallest spatial scale, the functional structures of the mammalian intervertebral disc (IVD) are the fibrillar assemblies, interfibrillar matrix macromolecules, and particularly proteoglycans and hyaluronic acid, whose relative composition and organization are related to injuries and congenital defects that cause back problems (see Eyre, 1979). In the mammalian IVD, collagen fibrillar composition and organization is quite complex, starting with a disperse network of thin, type II or cartilage collagen fibrils present in the gel-like nucleus pulposus at the center of the IVD. Here collagen accounts for 4 to 25% of the dry mass of the tissue. Circumscribing the nucleus laterally in the transverse plane, the annulus fibrosus connects neighboring vertebrae and limits the volume of the IVD. The annulus fibrosus is composed of lamellae built from thick, linearly arrayed, collagen fibrils that vary in their relative proportion of the two collagen gene products, types I and II. Near the medial margin of the annulus, adjoining the nucleus pulposus, nearly all of the collagen is type II. The proportion of type I collagen, which is found in mammalian ligaments and tendons, increases in the outer lamellae, reaching 100% at the external margin. The functional significance of the two separate collagen phenotypes, I and II, remains unclear in spite of our general understanding of the functional role of collagen fibers in the annulus fibrosus in constraining swelling pressure in the nucleus and resisting torsion and bending (Hukins and Meakin, 2000). Is the pattern of differentially expressed collagen phenotypes indicative of functional specialization within the extracellular matrix of the IVD?

The function of collagen within the IVD can only be understood with regard to the co-existing proteoglycans. In the nucleus pulposus, a proteoglycan called aggrecan, which also occurs in the interfibrillar matrix of cartilage, exists in high concentrations and generates the structure's gel-like properties. Aggrecan is a huge macromolecule composed of a core protein with approximately 100 sulfated glycosaminoglycan chains, giving rise to a high negative fixed charge density. The concentration of aggrecan, coupled with its fixed charge density and associated counterions, produces a large swelling pressure that can be developed because of the stiffness of the collagen fibrils in the annulus fibrosus and the cartilaginous end plates of the vertebrae. When the IVD is externally loaded, the aggrecan-rich nucleus pulposus acts hydrostatically, evenly distributing pressure to the surrounding tissues. Loss of aggrecan in the degenerating disc results in the loss of the hydrostatic function of the nucleus, and thus the end plates and annulus fibrosus are exposed to high point pressures, with the probability of disc failure (see Urban *et al.,* 2000). Thus it is clear that aggrecan, collagen, and the high hydrostatic pressure they generate are essential to normal IVD function and vertebral column mechanics.

Is this mosaic of collagens and aggrecans also found in the IVD and notochords of non-mammalian vertebrates? Aside from sporadic reports of collagen typing in shark vertebrae (*e.g.,* see Rama and Chandrakasan, 1984), few studies have applied contemporary biochemical analyses to axial tissues of other vertebrates. Without this information, we are precluded from understanding the functional relation between collagen and proteoglycan chemistry and the biomechanical properties of species that exhibit specialized vertebral mechanics.

Headway is being made, however, in the two living outgroup taxa to jawed vertebrates, the lampreys (Order Peteromyzontiformes, sister taxon to gnathostomes; see Janvier, 1996) and the hagfishes (Order Myxiniformes, sister taxon to vertebrates). The notochord sheath of the marine lamprey, *Petromyzon marinus,* is composed of type II collagen fibrils, similar in composition and structure to those of mammalian cartilage (Eikenberry *et al.,* 1984; Potter and Welsch, 1992; Brodsky *et al.,* 1994). In contrast, the major fibrillar component in the notochordal sheath of hagfish, *Mxyine glutinosa,* appears to be distinct from the fibril-forming collagens of lamprey notochord, mammalian intervertebral discs or cartilage (Koob *et al.,* unpublished). Given that the notochords of hagfish resist bending in a manner analogous to that of the notochords of white sturgeon, *Acinpenser transmontanus* (compare Long *et al.,* 1998 to Long, 1995), and assuming that sturgeon notochordal sheaths are collagenous (Schmitz, 1998a), this distinction establishes that similar functional units can be attained with distinct matrix assemblages. We need more detailed biochemical analyses of a range of vertebral tissues in order to develop hypotheses about the evolution and adaptation of connective tissues for specific biomechanical needs of the vertebrate axis.

The smallest bending element in the human vertebral column is often considered to be the ‘‘motion segment,’’ an intervertebral disc and its contiguous superior and inferior vertebrae (Hukins and Meakins, 2000). This arrangement is clearly not the only one capable of meeting the mechanical demands of a body loaded in compression and bent to various degrees along the body axis. The core of the hagfish notochord, which accounts for over 95% of the volume, contains giant, vacuolated cells and no extracellular matrix (Welsch *et al.,* 1998). In marked contrast, the nucleus pulposus in mammalian IVDs contains very few cells and is predominated by a well-hydrated extracellular matrix. Within the cells of the hagfish notochordal cores are large vacuoles bounded by a dense array of intermediate filaments (Fig. 1). Cell membranes are extensively interconnected by numerous and regularly spaced desmosomes. The cell-based core of the hagfish notochord is a hydrated gelatinous material often referred to as ‘‘jelly.’’ It does not resemble cartilage in any form, but closely approximates the gel-like structure of the nucleus pulposus. A peculiar aspect of the notochord core is that it derives its physical properties through a chemical means distinct from that in the nucleus pulposus. This is remarkable considering that the notochord is the putative developmental and phylogenetic ancestor of the nucleus pulposus.

Biochemical analyses showed that while the notochord sheath contains four proteoglycans, one of which is an aggrecan-like

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FIG. 1. Morphology and ultrastructure of hagfish notochord. A and B are illustrations of the notochord in *Myxine* sp. reproduced from Cole (1905 and 1907). A. Diagram of the notochord in situ showing large vacuolated cells in the core surrounded by the fibrous sheath (Cole, 1907). On the left side of the diagram, spinal nerves are shown emanating from the spinal chord dorsal to the notochord. B. Transverse section of the notochord and sheath taken from the mid-body region (Cole, 1905). The elastica externa (el.ext.) bound the outer perimeter of the notochord separating the sheath from the thick collagenous fibers of the myosepta (sk. 1.). The relatively small cells of the chordal epithelium (ch. ep.) abut against the inner aspect of the sheath. Note that the sheath contains no cells. Vacuolated cells (chrodal cells - ch. c.) predominate the core jelly. C. thick section from the mid-body of the hagfish *Myxine glutinosa* showing the relative size and position of the notochord. The notochord core is translucent. D. Electron micrograph of the chordal epithelium and sheath (nt. sh.). E. Cells in the notochord core with giant vacuoles dominating the cytoplasm. While the vacuoles appear empty in this section, other sections revealed condensed polyanionic material stained with cuprolinic blue. The vacuoles likely are the compartment that contains the material responsible for the fixed charge density and swelling properties. The box indicates the area shown at higher magnification in F. F. Neighboring cells are interconnected by a dense array of desmosomes. Intermediate filaments form a network around the vacuoles. Electron micrographs were kindly provided by Dr. John Trotter.

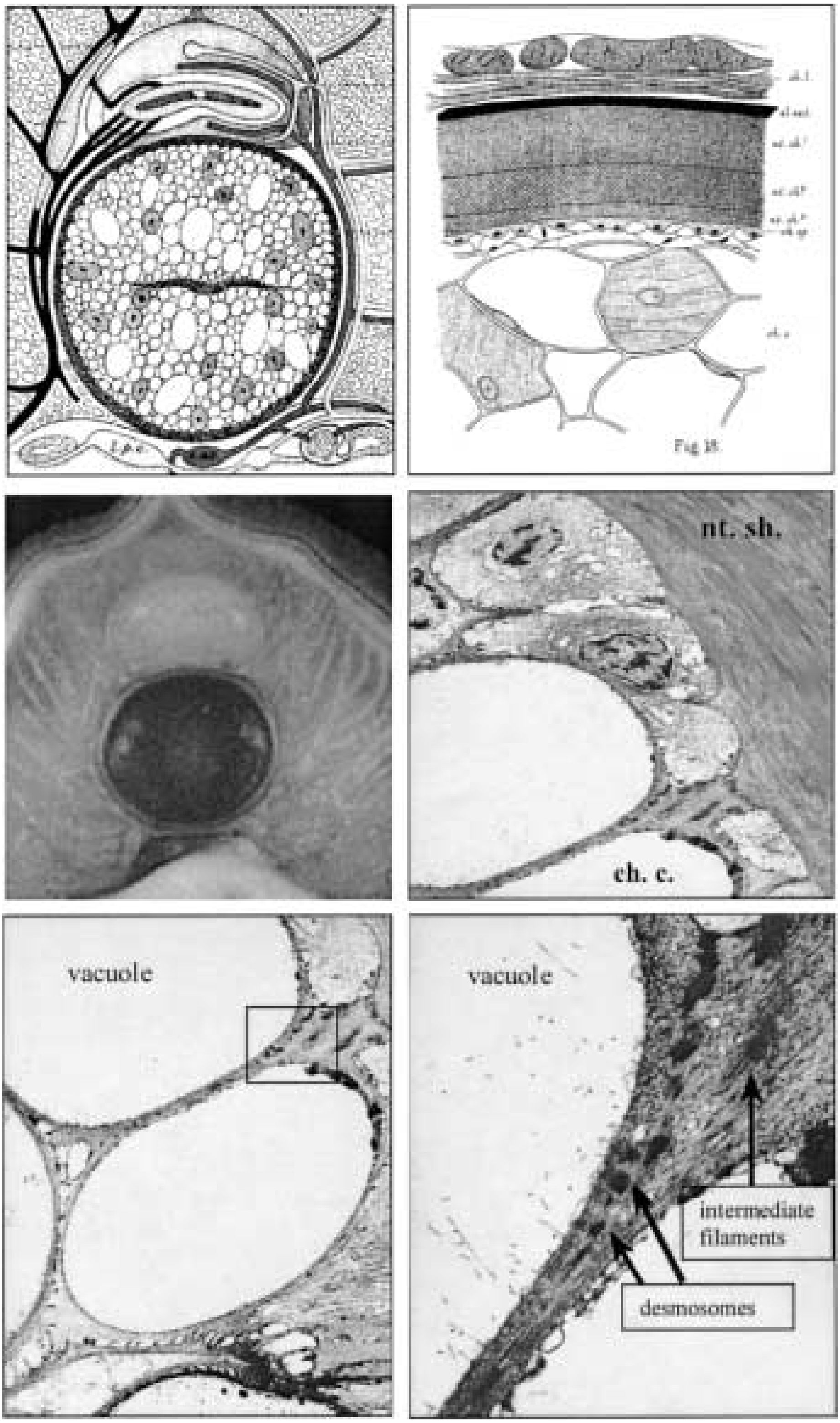
Fig. 18.
nt. sh.
ch. c.
vacuole
vacuole
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molecule, these molecules are limited to the sheath and the core does not contain proteoglycans of any kind (Koob *et al.,* 1994). Yet the hagfish notochord has a high fixed charge density comparable the mammalian nucleus pulposus, is likewise osmotically active, and in swelling tests performs like the nucleus pulposus (Kielstein *et al.,* 1996). The core of the notochord exerts a swelling pressure on the fibrous sheath, presumably imparting hydrostatically controlled structural stiffness, just as the nucleus pulposus exerts its hydrostatic pressure on the annulus fibrosus (Fig. 2). The hagfish notochord has accomplished a similar feat as the nucleus pulposus of mammals but with different elements.

Unconstricted notochords are also possessed by the adults of a variety of jawed fishes (see Goodrich, 1930), including hexanchiform sharks, sturgeons, paddlefish, living lungfishes, and coelocanths. Like the hagfish notochord, unconstricted notochords in the shortnose sturgeon, *Acipenser brevirostrum,* and African lungfish, *Protopterus annectens,* are composed of large vacuolated cells with little or no extracellular matrix (Schmitz 1998a; 1998b). In a teleost fish with vertebral centra, the yellow perch *Perca fluvescens,* the intervertebral joint contains two compartments, one of which contains populations of cells identical to the notochord cells of hagfish, sturgeon and lungfish, and another which apparently is fluid filled (Schmitz, 1995). We might suppose that these notochordal tissues in bony fish operate much like the hagfish notochord; however, without compositional data and biomechanical tests conducted at physiological strains and strain rates (but see Long, 1992), we are left to speculate (see Laerm, 1976, Symmons, 1979; Lauder, 1980; Schmitz, 1995).

We know more about the mechanical function of the notochord in the embryos of amphibians, where osmotically-driven changes in internal pressure cause the notochord to straighten and, in so doing, straighten and lengthen the embryo (Adams *et al.,* 1990). Function of fiber-wound, pressurized bodies has been predicted with only limited success from theory (Alexander, 1987). Experiments have proven more informative—cylindrical models show that with fiber angles greater than 55° to the long axis, increases in internal pressure stiffen, lengthen and straighten the notochord model (Koehl *et al.,* 2000). At angles less than 54°, increases in internal pressure drive shortening and bending. Mechanical stability is reached if the angle is equal to 54°; fiber angles converge on this value as pressure increases and cylinders shorten or lengthen (Koehl *et al.,* 2000). Beyond immediate conclusions related to the amphibian embryo, these studies are important in expanding our understanding of potential mechanical functions of the notochord not directly related to locomotion.

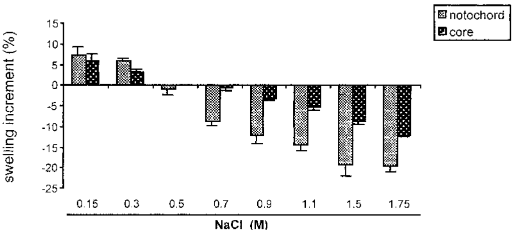

FIG. 2. In response to changes in external osmolarity, the notochord and notochordal core of *Myxine glutinosa* swell or shrink. For tests on the intact notochord specimens, excised 2 cm long segments ligated with 00 silk were weighed, then incubated at 4°C for 24 hr in 1 mM NaH_2PO_4, pH 7.0 containing the indicated concentrations of NaCl, after which the specimens were weighed again. Swelling is expressed as the percentage change in wet weight. For the core samples, tissue was excised from isolated notochords and extracted in 6 M guanidine-HCl, 0.5 M Na acetate, pH 6.0 for 24 hr at 4°C. The extract was centrifuged at 27,000 × g for 30 min, and the supernate containing solubilized components was collected. The supernate was exhaustively dialyzed against 1 mM NaH_2PO_4 to remove guanidine. One ml aliquots of the dialyzed extract were loaded into dialysis tubing, weighed, dialyzed against 1 mM NaH_2PO_4 containing the indicated NaCl concentrations as above, and then weighed. Changes in wet weight greater than that of dialyzed control salt solutions are expressed as a percentage increase or decrease. N = 5 for each NaCl concentration; error bars show S.D. The osmolarity of the 0.5 M NaCl solution at 949 mmol/kg approximates that of 1070 mmol/kg in the blood of *M. glutinosa.* Data adapted from Koob *et al.,* (1994) and Kielstein *et al.,* (1996).

For students of the mammalian axial skeleton, orthopaedic research over the past 20 years has systematically investigated the proximate causes of the development and maintenance of the composition and biomechanical properties of skeletal tissues.

Researchers in this field focus on the direct effects of mechanical loads on cellular metabolism, extracellular matrix biosynthesis and organization both *in vitro* and *in vivo* (Buckwalter and Grodzinsky, 1999). In recent studies the focus has been directed towards the physical and molecular mechanisms by which cells transduce mechanical signals into chemical messages that then regulate biosynthesis and organization of matrix components (Ishara *et al.,* 1997; Matsumoto *et al.,* 1999). The overall objective is to determine exactly how the skeletal connective tissues adapt to their particular mechanical requirements during development and how these properties are regulated and maintained during normal activity. For vertebral structures, the nucleus pulposus has been the principal tissue subjected to these types of analysis. *In vitro* data show that hydrostatic loads, one form of physical load to which the disc is continually subjected, influence biosynthesis of aggrecan (Ishara *et al.,* 1996). Not only was stimulation of aggrecan synthesis observed, but inhibition also occurred under specific loading regimes. These observations indicate that the ultimate biomechanical property of the nucleus pulposus, and thereby the properties of the disc as a whole, is continually regulated during the lifetime of the adult organism. These results also suggest that genetic regulation of the composition and organization of axial connective tissues can be modified by mechanical inputs thereby adapting the tissues to particular biomechanical functions.

The vertebrate axial skeleton is much more than a series of vertebrae coupled with the connecting intervertebral disc tissues. In most vertebrates, chondrichthyans being the principal exception, an extensive and structurally complex ligamentous network joins neighboring vertebrae and, in some cases, spans more than one intervertebral joint (for fishes, see Symmons, 1979). The properties of these ligaments govern, in part, displacement between vertebrae and ultimately the flexural stiffness of the whole spine to a greater or lesser degree depending on their structural and mechanical properties, which can vary substantially between specific ligaments and among species. As with intervertebral discs, most contemporary information focuses on mammalian systems, particularly as they relate to the human condition. Hukins and Meakin (2000) describe the differing structures and mechanical properties of the longitudinal ligaments, ligamenta flava, interspinous and supraspinous ligaments, and their impact on the biomechanical properties of the spine. Each of these ligaments has unique mechanical properties compared to the others, and as such, limit or allow flexure of the spine.

The contribution of axial ligaments to stabilization of the vertebral column in most vertebrates has long been recognized, but poorly characterized, perhaps due to the general perception that ligaments are fairly uniform and inert skeletal structures. Functional analyses of exactly how specific ligaments act during normal activity are lacking for most taxa. The same can be said for the zygopophyseal joints. Emerging observations, however, while all too few in number for any detailed comparative analysis, are sufficient to clearly show that ligaments have been adapted to specific vertebral function. Peruse virtually any biomechanics text and you will find a description of the ligamentum nuchae in ungulates, usually recruited to illustrate the biomechanical properties of elastin dominated tissues. Hukins and Meakin (2000) pointed out that the ligamenta flava, which contains twice as many elastic fibers as collagen fibers, are highly extensible, due to the nonlinear arrangement of the collagen fibers, allowing large deformations without damage. These simple exemplars show that all axial ligaments are not created equally. Without functional analyses of axial ligaments, the basis for flexural stiffness in any particular vertebrate axis is wanting. This is also true for the vertebral processes and zygopophyseal joints. A case in point is the vertebral column in dolphins. Long *et al.,* (1997) have shown that both the ligaments and the spinal processes, coupled with IVD size, are crucial for providing variations in bending properties along the spine that relate directly to locomotor performance.

The function of the vertebrate backbone relies on an array of tissues, with variable

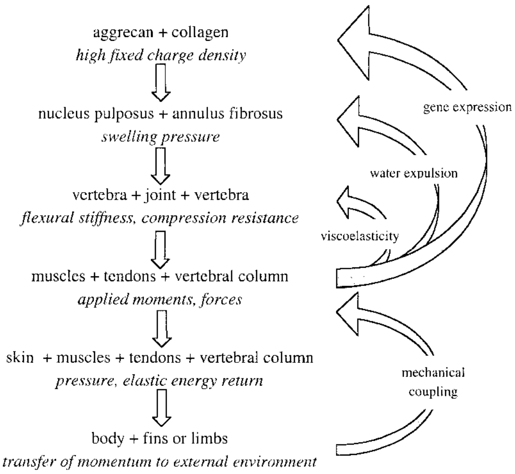

FIG. 3. In mammalian axial systems, structures at the smallest size scale (aggrecan and collagen) produce a mechanical property (*high fixed charge density*) that at the next level (nucleus pulposus and annulus fibrosus) produces another mechanical property (*swelling pressure*), and so forth. The properties at some levels (*applied moments, forces, transfer of momentum*) feedback, via the mechanism shown by the curved arrows, to alter the structures and properties at a lower level. See text for explanation and sources.

composition and structure integrated into a multitude of configurations. In a generalized paradigm, the flexibility of neighboring vertebrae is constrained by the following: the size and configuration of the intervertebral discs, the fibrous tissues, *i.e.*, the annulus fibrosus and its analogues that directly connect the centra, the properties of the osmotically driven nucleus pulposus, the ligaments that join vertebral processes, and the size, configuration, and interaction of the vertebral processes themselves. In developing hypotheses about the evolution of the vertebrate axis and its functional properties, not only must we consider the overall biomechanics of the spine, but we must also consider the contribution of individual units, their composition, structure, and functional properties.

These intriguing results from orthopaedic research give us the opportunity to present a model of the structure and function of the mammalian axial system integrated from the molecular to the behavioral level (Fig. 3). We find it particularly useful that the feedback between levels demands that integrated models of the vertebrate axis recognize and explain the following: (1) the mechanical behavior at one level is not independent of that at other levels and (2) feedback is mechanical and has both mechanical and genetic effects. The lack of independence between and among levels means that if one is interested in understanding, for example, how the control and dynamics of a particular muscle in the system impact particular whole-body movements, then the system and its component parts must be simultaneously and interactively modeled (Daniel, 1995; Katz and Jordan, 1997).

Integration of Mechanical Functions

The body axis of vertebrates, like other animal cylinders, is subjected to internal and external forces that cause parts of it to bend, stretch, shorten, twist, and shear (Wainwright, 1988). From a mechanical viewpoint, these movements act to transfer momentum from the body to the environment. Momentum is the product of mass and velocity, and as animals change shape they add velocity to the mass of the external medium. The rate at which momentum is transferred is force, and thrust is the portion of that total force working to propel the animal (locomotion) or, if the animal is stationary, to propel the environment (ventilation of lungs or movement of sediments).

The body movements that transfer momentum begin internally as muscle contraction. Contractions, and their effect on other structures, are not simple to model because muscles are not simple, linear engines (Wakeling and Johnston, 1999*b*; Rome *et al.*, 2000; Swank and Rome, 2000). The force that a vertebrate striated muscle cell (called a muscle "fiber") generates depends on its contractile units' ("sarcomeres") instantaneous length, instantaneous velocity, level of activation, previous contractile history, and the load against which it is contracting (see Brown and Loeb, 1998; Daniel and Tu, 1999). The load against which a muscle acts depends, in turn, on the instantaneous state of the coupled mechanical system mentioned in the previous section (Fig. 3). Current work has focused on describing three other aspects of this integrated system: (1) the dynamic (strain- and strain-rate-dependent) passive mechanical properties of the structures in series and in parallel with muscle, (2) the external fluid or ground-reaction forces acting over or on the surface of the body, and (3) the inertia of the body. Coupled models that include all four systems have been attempted in invertebrates (Jordan, 1994; Daniel, 1995) and in fish using axial undulations (Carling *et al.*, 1998; Cheng *et al.*, 1998; Pedley and Hill, 1999; Librizzi *et al.*, 1999). None have yet recovered the modeled animal's full range of locomotor performance or behavior.

These models may be limited because they fail to implement non-shortening functions of muscle such as the ability to modulate (1) stiffness and damping (Meyerhofer and Daniel, 1990; Biewener, 1998; Long, 1998), (2) radial bulging stress (''pressure'' see Wainwright *et al.*, 1978; Westneat *et al.*, 1998), and (3) speed of the internal force transmission (Root and Long, 1997). In addition, muscle force cannot be accurately modeled without coupling the contractile unit to the passive structures within and surrounding the muscle (Daniel and Tu, 1999). Within the sarcomere, the myosin filaments responsible for generating contractile force are arrayed transversely in lattices or superlattices (for review see Luther et al. 1995) and are joined in parallel by titan filaments (Labeit and Kolmerer, 1995). Titan filaments of more than one micrometer in length span one-half of the sarcomere, linking Z disc to M line, giving myofibrils their intrinsic elasticity and maintaining their highly-ordered ultrastructure (for review see Labeit and Komerer, 1995). In turn, muscle fibers, composed of myofibrils in parallel, must be attached to each other since they do not span the entire length of the whole muscle in mammals. Muscle fibers appear to overlap with tapered ends in parallel, connected by an extracellular matrix dominated by collagen fibrils (Trotter, 1993). The net force generated by this network of fibers is then transmitted to collagen fibers of tendons by way of the interdigitated myotendinous junction at their interface; the forces are transferred by shear loading (Spierts *et al.*, 1996).

Other short-comings of integrated models are structural and neural. When the arrangement of the lateral axial musculature is modeled as nearly-constant with changes in axial position, then the influence of the change in the orientation and moment arm of the muscular force vectors are not explicitly treated (Ekeberg, 1993). By assuming that the entire lateral musculature is simultaneously active in fast-starting fish, muscular moment arms (Wakeling and Johnston, 1998, 1999*a*) and vector orientation (Czuwala *et al.*, 1999) have shown to vary regionally along the body axis and temporally as the fast-start progresses. Regional and temporal variation in bending moments and power production have also been predicted to occur during steady undulatory swimming in fish (Cheng and Blickhan, 1994; Rome and Swank, 2000; Swank *et al.*, 2000). In all models, the timing of muscular bending moments is dependent on the pattern of neural activation, which involves central pattern generation coupled to sensory feedback from stretch receptors (Ekeberg, 1993; Miller and Sigvardt, 1998, 2000; Sigvardt and Miller, 1998). But neural activation patterns alone are not sufficient to explain muscle function in vertebrates, such as fish, with complex segmentation of the axial muscle (Jayne and Lauder, 1995). Indeed, models of myomeric function that rely on complete segment activation and simple traveling waves of activation may be misleading, because fish have the ability to use parts of the complex myomere for distinct functions (Thys, 1997) or to only use a portion of the myomere for some swimming behaviors (Jayne and Lauder, 1995). In fish, the functional consequences of myomere shape and myoseptal arrangement are likely to be important in force transmission and moment generation along the axis (Westneat *et al.*, 1993; Van Leeuwen, 1999).

The lateral axial muscle may have additional mechanical roles in amphibians, where it retains transverse segments while displaying medio-lateral layering in adults. The muscle layers of the lateral hypaxial muscle may be specialized, by virtue of their differences in proximity to the vertebral column and preferred fiber angle, for pressurizing, bending, or twisting the body axis (Brainerd and Simons, 2000). In addition, axial muscle in adult amphibians may work to maintain posture, transmit locomotor forces generated by the contact of the limbs with the substrate, and generate mechanical work for burrowing (O'Reilly *et al.*, 2000).

In amphibian tadpoles, however, the function of the axial muscles appear to be for lateral bending alone (Hoff and Wassersug, 2000). More complex is the pattern of neural activation, which can be activated in a standing or traveling wave depending on the tadpole's swimming behavior (Hoff and Wassersug, 2000). In addition to oscillating the tail, axial muscle also acts to oscillate the head, producing a large yawing motion that computational-fluid-dynamic models show is necessary for efficient thrust production in swimming tadpoles (Lui *et al.*, 1997).

As suggested by the arrangement of muscle fiber-to-fiber connections and myotendinous junctions discussed above, when we examine force transfer between structures at the microscopic level we tend to find shear loading (Wainwright, 1988). Shear is a force that attempts to slide two parallel surfaces past one another (Wainwright *et al.*, 1976). Thus muscles themselves operate as shear inducers, sliding thin filaments (actin polymers) past thick (myosin polymers). Within discrete tendons or ligaments, shear of fibers and fluid past each other may account for mechanical behavior known as creep and stress-relaxation (Purslow *et al.*, 1998). Shear loading may also explain the bending mechanics of the interspinous ligaments running axially between the zygopophyses of the neural spines of dolphin vertebrae (Long *et al.*, 1997). Thus shear loading mechanisms may play a central role in integrative models of the vertebrate body axis. Just how pervasive shear-loading mechanisms are at the macroscopic scale remains an open question.

At the gross level, we find that tensile loads are transmitted within long tendons and, when these structures are present, they act to sum the serially-generated forces for focal application on a propulsive element. In cetaceans and tunas, such longitudinal tendons connect axial muscle to fluke or caudal fin, and, in combination with laterally-placed and helically-wrapped connective tissues in the skin, act to increase the displacement advantage and moment arm of the contracting muscle (Pabst, 2000). The skin of tunas and dolphins, and probably longnose gars as well (Long *et al.*, 1996), thus acts as a retinaculum, preventing the force trajectory from bowstringing and losing mechanical advantage (Pabst, 2000). Given the terrestrial intermediates in the evolution of cetaceans (Fish, 1996), this is a remarkable case of convergence in axial systems.

Another important principle needed to integrate mechanical functions is the property of resonance in dynamically bending structures (Timoshenko *et al.*, 1974). If the locomotor reconfigurations of the body axis can be driven at the natural frequency of the body, then the costs of that bending can be minimized, leaving muscle force available for external transfer and conversion to thrust (Blight, 1976, 1977; Long and Nipper, 1996). This tuning of the body's natural frequency and the timing of muscle activation has been described in swimming scallops (DeMont, 1990) and jellyfish (DeMont and Gosline, 1988). In the more structurally complex vertebrate body, muscles have the capacity to alter body stiffness to match the natural frequency to that of the tailbeat in eels, *Anguilla rostrata* (Long, 1998). When modeled as a flexible beam with a physiologically-realistic elastic modulus (Oxner *et al.*, 1993), the swimming tadpole larva of frogs have a predicted resonant frequency (3 Hz) that corresponds to the preferred tailbeat frequency of real tadpoles of the same size and species (Hoff and Wassersug, 2000). During fast starts in sunfish, *Lepomis gibbosus,* a mechanical model that treats the body as an axially-loaded column predicts that muscles actively alter flexural stiffness (Czuwala *et al.*, 1999). In this case, a transient increase in body stiffness may act to rapidly accelerate the body wave of bending. Since the speed of a bending wave is proportional to the square root of the structure's stiffness (Timoshenko *et al.*, 1974), any change in stiffness can have important consequences for resonant behavior and for the rate at which forces are transmitted.

Analysis of the Evolution of Structure and Function

> ". . . if ancestral craniates were filter-feeders, how is it that features connected with locomotion (notochord, elaborate nervous system, segmented muscle blocks) are what make craniates distinctive?"—Henry Gee (1996)

The evolution of early craniates (vertebrates + hagfishes, Janvier, 1996) remains a puzzle because changes in axial structure do not always correlate with changes in habitat or behavior (for caveats see Lauder, 1995). Parsimony reconstructions of the hypothetical ancestor of craniates yield a filter feeding, obligate axial locomotor using body undulations to swim and burrow (Gee, 1996). If the putative ancestor of chordates was a sessile filter feeder, then how can we explain the changes in the body axis of the hypothetical stem lineage of vertebrates that take place *before* a change in feeding habits? In this section, we address this specific question first. We then discuss the general issue of the mechanical consequences, if any, that the changes in axial structure generate in the evolution of vertebrates.

Analysis of evolutionary function has been codified by Brandon (1990) in his description of ideal adaptation explanations. When testing "how-possibly" adaptation scenarios of the prior appearance of features, investigators reconstructing historical events must assume that the novel character state in question (1) had arisen because of selection, (2) was heritable, and (3) was from a population with simple genetic and selective structures. What is left to test is (1) the *polarity* of the states (which state came first?) and (2) the *ecological consequences* of the change in character state (how does the new state alter mechanical function in the environment?). Polarity is obtained from phylogenetic reconstruction and the ecological consequences can be tested with biomechanical models that causally link structural changes with changes in mechanical function (Kingsolver and Koehl, 1985; Brandon, 1990; Amundson and Lauder, 1994).

To explore the polarity of structure changes for the body axis of early vertebrates, we reconstructed character state changes in the stem lineage of the hypothetical ancestor to chordates to the hypothetical ancestor of tetrapods and ray-finned fishes (Fig. 4). In this phylogenetic framework (Fig. 4A), the changes in the axial skeleton of hypothetical stem vertebrates have the following polarity (ancestral → derived): notochord (node 1) → notochord with neural arches (node 4) → vertebral column with centra and hemal arches (node 5). The changes in the lateral musculature have the following polarity: unsegmented (node 1) → transversely segmented with V-shape (node 2) → horizontally segmented into epaxial and hypaxial muscle (node 5). The changes in the external body form have the following polarity: post-anal tail (node 1) → caudal fin with cartilaginous fin rays (node 3) → dorsal and anal fins (node 4) → caudal fin epicercal, two dorsal fins (node 5). Polarity of the changes in habitat, swimming and feeding behaviors can likewise be reconstructed (Fig. 4B). The changes in habitat have the following polarity: marine pelagic/benthic (node 1) → pelagic only (node 5). The changes in swimming behaviors have the following polarity: axial undulatory swimming (node 1) → axial undulatory swimming/burrowing (node 2) → axial and median fin undulatory swimming (node 4) → axial carangiform undulatory swimming (node 5). The changes in feeding behaviors have the following polarity: filter feeding (node 1) → active predatory/scavenging adult (node 3). Please note that these character state changes should be treated with caution since homology, in some cases, is still unclear, in spite of recent genetic tests of axial identities (see Holland and Fernandez, 1996). For example, developmental and phylogenetic information suggests to some that homology cannot be supported for vertebral centra (compare Schaeffer, 1967; Panchen, 1977; Laerm, 1979; Gardiner, 1983; Gardiner and Schaeffer, 1989).

In order to predict possible functional consequences of the changes in axial struc-

FIG. 4. In living chordates, origin of novel axial structures (A) and related behaviors (B) in the hypothetical stem lineage (nodes 1 to 6). Arrows indicate first appearance of trait. The branching patterns and characters are based on Schaeffer (1967), Nelson (1994), Gee (1996), Janvier (1996), Stokes and Holland (1998), and S. A. Wainwright (personal communication). The character states of the hypothetical ancestors were reconstructed using the median-state rule (Lundberg and McDade, 1990) employing simple parsimony (for caveats see Cunningham *et al.*, 1998). Note that if we were to include fossils, some features (hemal arches and paired fins) shown here as evolving in the common ancestor of gnathostomes would be reconstructed as having evolved earlier, in the common ancestor of Petromyzontiformes (or the monophyletic clade including this Order) + Gnathostomata (Janvier, 1996).

ture (Fig. 4A), we correlated structural change with changes in habitat, swimming behavior, and feeding mode (Fig. 4B). The results of this correlation (Table 1, first three right-hand columns) are very general, and extreme caution must be exercised in drawing conclusions, since structures not listed may be causally related to the changing behavior and vice versa. With that in mind, description of the correlated changes may at least provide fodder for experimental or theoretical tests of the evolutionary associations.

Alternatively, the functional consequenc-

TABLE 1. *Correlations between structural and behavioral changes in the early stem lineage of Chordates and Vertebrates.*[1]

Node[2]	Structural changes	Behavioral changes	Possible mechanical changes	Predicted evolutionary functions
1	• notochord • muscular post-anal tail • dorsal nerve tube	• pelagic undulating larvae • benthic sessile filter-feeding adult	• notochord replaces circular and transverse muscle needed to control axial length • more muscle available for lateral bending	• swimming larvae disperse farther
2	• V-shaped segmented axial muscles • dorsal hollow nerve tube • gills with blood vessels	• axial undulatory swimming and burrowing adults	• regional control of body bends • active metabolic scope increased	• increased maneuverability and performance
3	• caudal fin with cartilaginous rays • braincase • myelinated nerves	• active predatory, scavenging adults	• stiffer caudal fin transfers more momentum, more thrust	• faster swimming speeds
4	• neural arches • bony fin rays • caudal fin ray muscles • dorsal and anal fins • sensory-line neuromasts	• undulating median fins	• internal support for new propulsive fin • body stiffer in bending	• wider range of locomotor maneuvers • faster swimming speeds • larger body size due to increased thrust power
5	• vertebral centra • hemal arches • horizontal septa in axial muscle • muscular paired fins • epicercal tail • two dorsal fins	• pelagic carangiform swimming	• stiffen body for faster rate of bending • greater forces to new fins	• faster steady swimming • greater accelerations during fast starts
6	• discrete tendons • lungs			

[1] Please note that the "possible mechanical changes" and "predicted evolutionary functions" listed below are based on extrapolations from the structural changes alone, are purely speculative, and require careful testing by way of biomechanical models and experiments. See Figure 4 and text for citations.

[2] The nodes are the hypothetical ancestors in the stem lineage leading from the common ancestor of Chordates to that of Actinopterygians and Sarcopterygians (see Fig. 3).

es of changes in axial morphology can be independently predicted from biomechanical studies (see Lauder, 1995 for caveats). Adding skeletal elements stiffens the axis, a change which will increase the natural frequency of bending (Long and Nipper, 1996), permit higher tailbeat frequencies (Timoshenko *et al.,* 1974), and hence generate more locomotor power (Wu, 1977). With stiff neural and hemal spines inclined to pass over intervertebral joints, bending of the joint bends the spines (Videler, 1993), a mechanical situation that stores and delivers elastic energy (C. A. Pell, personal communication) and may act to increase fast-start accelerations (Lauder, 1980). Transverse segmentation of muscle permits point loads on backbone via connective tissue myosepta (Clark, 1964) and hence regional specialization along the axis that might provide hydrodynamic power anteriorly (Cheng and Blickhan, 1994), force for active stiffening posteriorly (Long, 1998), or summation and transmission of the force from many segments to the caudal fin (Westneat *et al.,* 1993; Van Leeuwen, 1999; Pabst, 2000). Horizontal segmentation into epaxial and hypaxial sections might permit further regional specificity for raising the neurocranium during prey strike (Thys, 1997). In terms of external body form, as the caudal fin gains stiffness with cartilaginous fin rays, it increases the thrust forces that can be transferred from body to water (Lauder, 2000). Acquisition of an epicercal (or heterocercal) tail corresponds with the transition to stiffer-bodied carangiform swimming (Table 1); in sharks the heterocercal tail generates a thrust vector with a downward pitching angle (Ferry and Lauder, 1996; Lauder, 2000). With the addition of dorsal and anal fins, the body gains transfer efficiency (Weihs, 1989) by reducing added mass while increasing body depth.

Interesting evolutionary changes in the structure and function of the vertebrate axis are not limited to early vertebrates. In nonmammalian tetrapods, the evolution of body axis appears to be constrained, in part, by the simultaneous and mechanically exclusive requirements for lateral body bending and lung ventilation (Carrier, 1987). In archosaurs, the two functions are decoupled by the evolution of a novel ventilatory mechanism in which the gastralia, the pubic bones, the ischiotruncus muscle, and the hypaxial musculature combine to depress the floor of the abdomen (Carrier and Farmer, 2000). In birds, the loss of gastralia and the consolidation of the trunk into a more compact and tightly linked skeleton appears to have changed the ventilation mechanisms to one that uses the rocking of the bird's large sternum (Carrier and Farmer, 2000). In mammals, the motion of the body axis and internal organs during terrestrial locomotion appears to have been co-opted to help drive lung ventilation (Bramble and Jenkins, 1994; Carrier, 1996). Coupling of locomotion and ventilation, a common feature in tetrapods, may in part be explained by the conservative nature of the axial skeleton. Compared to appendicular skeletal elements, elements of the axis in mammals show less variability in length-standardized size (Cullinane, 2000). Whether this lack of variation is caused by stabilizing selection or developmental constraint remains to be tested.

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