Differentiation of the Cardiac Outflow Tract Components in Alevins of the Sturgeon Acipenser naccarii (Osteichthyes, Acipenseriformes): Implications for Heart Evolution

Alejandro Guerrero,1 José M. Icardo,2 Ana C. Durán,1 Alejandro Gallego,1 Alberto Domezain,3 Elvira Colvee,2 and Valentín Sans-Coma1*

1Department of Animal Biology, Faculty of Science, University of Málaga, 29071 Málaga, Spain
2Department of Anatomy and Cell Biology, University of Cantabria, 30911 Santander, Spain
3Department R&D Piscifactoría “Sierra Nevada”, Camino de la Piscifactoría 2, 18313 Ríofrío, Granada, Spain

ABSTRACT Previous work showed that in the adult sturgeon an intrapericardial, nonmyocardial segment is interposed between the conus arteriosus of the heart and the ventral aorta. The present report illustrates the ontogeny of this intermediate segment in Acipenser naccarii. The sample studied consisted of 178 alevins between 1 and 24 days posthatching. They were examined using light and electron microscopy. Our observations indicate that the entire cardiac outflow tract displays a myocardial character during early development. Between the fourth and sixth days posthatching, the distal portion of the cardiac outflow tract undergoes a phenotypical transition, from a myocardial to a smooth muscle-like phenotype. The length of this region with regard to the whole outflow tract increases only moderately during subsequent developmental stages, becoming more and more cellularized. The cells soon organize into a pattern that resembles that of the arterial wall. Elastin appears at this site by the seventh day posthatching. Therefore, two distinct components, proximal and distal, can be recognized from the fourth day posthatching in the cardiac outflow tract of A. naccarii. The proximal component is the conus arteriosus, characterized by its myocardial nature and the presence of endocardial cushions. The distal component transforms into the intrapericardial, nonmyocardial segment mentioned above, which is unequivocally of cardiac origin. We propose to designate this segment the “bulbus arteriosus” because it is morphogenetically equivalent to the bulbus arteriosus of teleosts. The present findings, together with data from the literature, point to the possibility that cells from the cardiac neural crest are involved in the phenotypical transition that takes place at the distal portion of the cardiac outflow tract, resulting in the appearance of the bulbus arteriosus. Moreover, they suggest that the cardiac outflow tract came to be formed by a bulbus arteriosus and a conus arteriosus from an early period of the vertebrate evolutionary story. Finally, we hypothesize that the embryonic truncus of birds and mammals is homologous to the bulbus arteriosus of fish. J. Morphol. 260:172–183, 2004.

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KEY WORDS: heart; conus arteriosus; bulbus arteriosus; Acipenseriformes; Osteichthyes

It is generally accepted that the most anterior component of the primitive vertebrate heart is the conus arteriosus, a contractile chamber whose walls consist largely of cardiac muscle overlying an elastic fibrous coat (Gegenbauer, 1866; Santer, 1985; Zummo and Farina, 1989). A well-developed conus arteriosus is present in the adult heart of the elasmobranchs (Gegenbauer, 1866, 1891, 1901; Stöhr, 1876; Kisch, 1930; Parsons, 1930; Santer, 1985; Sans-Coma et al., 1995), lungfishes (Lankester, 1879; Boas, 1880a; Robertson, 1914), coelacanths (Anthony et al., 1965; Millot et al., 1978), and primitive actinopterygians such as the polypteriforms (Parsons, 1930), acipenseriforms (Parsons, 1930; Icardo et al., 2002a,b), and lepisosteiforms (Parsons, 1930).

In the halecomorph Amia calva and in primitive teleost species belonging to the genera Albula, Pterothrissus, Megalops, and Tarpon, the cardiac outflow tract (OFT) is composed of two distinct portions: a posterior, myocardial conus arteriosus and an anterior, nonmyocardial bulbus arteriosus (Stannius, 1846; Boas, 1880b; Senior, 1907abc; Parsons, 1930; Satchell, 1991). In most teleosts, however, the conus arteriosus has classically been considered to be very reduced in size or even absent (Smith, 1918; Santer, 1985; Satchell, 1991; Farrell and Jones, 1992), a fact which is concomitant with the remarkable develop-
ment of the bulbus arteriosus in the phylogenetically advanced bony fishes. The bulbus arteriosus of teleosts is an intrapericardial, elastic chamber that connects the conus arteriosus with the ventral aorta, or the ventricle with the ventral aorta when the conus arteriosus might be absent. Histologically, the bulbus arteriosus is organized into two layers that contain myofibroblasts, smooth muscle cells, collagen, and elastin (Priede, 1976; Santer, 1985; Satchell, 1991), distributed in species-specific patterns (Icardo et al., 1999a,b, 2000a,b).

The evolutionary origin of the bulbus arteriosus has been the subject of a still unresolved controversy. Several authors believe that the bulbus is a backward extension of the ventral aorta into the pericardial cavity (Boas, 1901; Bridge, 1904; Krause, 1923; Grodzinski, 1938; Bertin, 1958; Parker and Haswell, 1962; Weichert and Presch, 1975; Lawson, 1979). The observations of other authors (Parsons, 1930; Licht and Harris, 1973; Priede, 1976) are best understood if it is assumed that the bulbus arteriosus originates as a modification of the anterior part of the conus arteriosus, i.e., that the bulbus is of cardiac origin (Yamauchi, 1980; Farrell and Jones, 1992).

Icardo et al. (2002a,b) reported that the ventral aorta of the sturgeon *Acipenser naccarii* consists of two segments, one located within the pericardial cavity and the other above this cavity. The intrapericardial segment, interposed between the conus arteriosus and the extrapericardial aortic segment, displays a discontinuous internal elastic lamina lining the endocardium, a middle layer of smooth muscle cells, and an epicardial covering. The smooth muscle cells are organized into an inner longitudinal layer, a middle circumferential layer, and an external longitudinal layer. In contrast, the extrapericardial aortic segment shows a continuous subendocardial elastic lamina and a thick layer of circumferentially oriented smooth muscle cells. The outer longitudinal cell layer is less developed, while the inner longitudinal layer is mostly absent. In addition, a distinct intima-like layer is located between the inner endocardia and the smooth muscle cells. These structural differences between the two aortic portions indicate that the intrapericardial segment constitutes a transitional segment, the embryonic origin of which might differ from that of the extrapericardial ventral aorta (Icardo et al., 2002b).

The existence of this transitional, intrapericardial segment between the conus arteriosus and the extrapericardial ventral aorta in the sturgeon heart is undoubtedly of great interest from an evolutionary viewpoint. Knowledge of its formation might throw some light on the controversy concerning the evolutionary origin of the bulbus arteriosus. In this regard, it should be emphasized that sturgeons belong to the chondrosteans, a primarily Paleozoic and Triassic fish assemblage that constitutes the first stage of actinopterygian evolution (Colbert, 1955; Carroll, 1988). On this basis, we conducted a study to illustrate the ontogeny of the cardiac OFT of *Acipenser naccarii*, an autochthonous species of both the Adriatic sea and the Iberian Peninsula (Garrido-Ramos et al., 1997) which presumably inhabited the whole European Mediterranean basin (Domezain et al., 2003; Robles et al., 2003). The main goals were: 1) to describe the development of the transitional segment lying between the conus arteriosus and the extrapericardial ventral aorta, and 2) to gather new data that may contribute to a better understanding of the evolution of the heart within the lineage of actinopterygian fish.

**MATERIALS AND METHODS**

**Animals**

The sample examined consisted of 178 alevins of *Acipenser naccarii* obtained from the Sierra Nevada Fishery at Riofrío, Granada, Spain. The age of the alevins ranged from 1–24 days posthatching (dph). They were overanesthetized in 0.04% MS222 (tricaine methane sulfonate, Sigma Chemical Co., Poole, UK) and measured. In this context, it should be noted that the total length (TL; in mm) and the age (A; in days) of the present specimens were highly correlated (TL = 0.843 A + 8.507, R² = 0.977). The hearts of 141 alevins were examined using histological, histochemical, and immunohistochemical techniques; another 21 were studied by scanning electron microscopy, and the remaining 16 by means of semithin sections and transmission electron microscopy.

**Histological and Histochemical Techniques**

The alevins were fixed by immersion in MAW fixative (methanol:acetone:water, 2:2:1; ratio of fixative to tissue volume = 80:1) and embedded in Paraplast (Sigma). Serial sections of the heart, transversely, longitudinally, or sagittally cut at 10 μm, were stained with Delafield’s hematoxylin-eosin or Mallory’s trichrome stain for a general assessment of the histological components of the heart. In addition, the orcein-HCl method was used for the specific detection of elastin.

**Immunohistochemical Techniques**

Transverse, longitudinal, and sagittal sections of the heart, obtained following the protocol described above, were stained with monoclonal antibody MP20 (Developmental Studies Hybridoma Bank, University of Iowa) against a myosin heavy chain epitope, or with monoclonal anti-β-actin AC-15 (Abcam, UK) against the N-terminal of the β-isoform of actin. It should be noted that the use of monoclonal antisemooth muscle α-actin (Sigma, clone 14) was unsuccessful.

The sections were dewaxed in xylene, hydrated in an ethanol series, and washed in Tris-phosphate buffered saline (TPBS, pH 7.8). Endogenous peroxidase activity was quenched by incubation with 3% hydrogen peroxide in TPBS for 30 min. After washing with TPBS, nonspecific binding sites were saturated for 1 h with 10% sheep serum and 1% bovine serum albumin in TPBS plus 0.5% Triton X-100 (SBT). Sections were washed with TPBS and then incubated for 18 h in the primary antibody diluted in SBT. Control slides were incubated in SBT only.

After incubation, the sections were washed in TPBS (3 × 5 min), incubated for 1 h at room temperature in biotin-conjugated antimouse IgG (Sigma) diluted 1:100 in TPBS, washed again, and incubated for 1 h in ExtrAvidin conjugate (Sigma) diluted 1:150 in TPBS. Peroxidase activity was developed with Sigma Fast 3,3′-diaminobenzidine tablets according to the instructions of the
and a middle layer of highly cellularized mesenchyme tissue (Fig. 2b,c). Histological changes in the outer epithelium included, successively, loss of the myocardial organization, formation of a cuboidal cell monolayer, and transformation into a flattened epithelium. The transient presence of large intracellular lipid droplets was characteristic of this area. These histological changes occurred between days 4–5 dph, and were observed first in the left side of the OFT (Fig. 2c,d). The mesenchyme tissue of the MF20-negative distal portion of the OFT (Fig. 2a) was continuous with the cardiac jelly proximally, and with the extracardiac mesenchyme distally. In contrast, the MF20-positive portion of the OFT maintained its previous histological organization. The only change detected was the presence of a small number of cells in the cardiac jelly space (compare Fig. 2a, 2b).

In alevins aged 6 dph, the cardiac chambers had nearly occupied their definitive position (Fig. 1c). The sinus venosus and the atrium were located dorsally with regard to the ventricle and the OFT, and the ventricle occupied a caudal position. The MF20-negative distal portion of the OFT (Fig. 3c) showed positive immunoreactivity against the β-actin antibody (Fig. 3d), thereby suggesting a differentiation of mesenchyme cells into smooth muscle cells at this site. Cells in this segment were densely arranged (Fig. 2e). Large masses of extracellular material, presumably collagen, accumulated in the extracellular matrix, the density of this matrix being higher than that of the cardiac jelly. On the other hand, β-actin-positive cells were also present in the developing walls of the ventral aorta and aortic arches (Fig. 3d).

In alevins age 7–8 dph, the heart acquired the final anatomic configuration (Fig. 1d). Concomitantly, a major reorganization took place in the distal portion of the OFT (Fig. 2f,g). Cells became massively arranged along the longitudinal OFT axis, elastic fibers appeared in the extracellular matrix, and a distinct epicardial layer could be recognized (Fig. 2g). In addition, melanocytic cells appeared under the epicardium of this OFT segment down to the myocardial level (Fig. 2g). Meanwhile, proximal

**RESULTS**

In alevins age 1–3 dph, the heart displayed a simple tubular configuration (Fig. 1a). It consisted of four chambers: the OFT, the ventricle, the atrium, and the sinus venosus, arranged in a craniocaudal sequence. The conoventricular sulcus marked the boundary between the OFT and the ventricle. The atrioventricular sulcus separated the ventricle from the atrium. On the whole, the heart adopted the shape of a “C,” the convexity of which was directed toward the right side of the body. Histologically, the heart was a uniform structure composed of two cellular layers, the endocardial endothelium and the myocardium, separated by cardiac jelly (Fig. 2a). The entire myocardial layer showed positive immunoreactivity for MF20 (Fig. 3a) up to the cranial limit of the pericardial celomic cavity.

In alevins age 4 dph, the distance between the caudal limit of the OFT and the cranial wall of the atrium had shortened due to the ascent of the atrium. Meanwhile, the ventricle had partially moved towards its definitive midline position (Fig. 1b). In these specimens, the cardiac OFT retained its positive immunoreactivity against the MF20 antibody, except for a short, distal portion, which became MF20-negative (Fig. 3b). Semithin sections revealed that this distal portion, which had presumably lost its myocardial phenotype, consisted of an inner endothelial layer, an outer epithelial layer, and a middle layer of highly cellularized mesenchyme tissue (Fig. 2b,c). Histological changes in the outer epithelium included, successively, loss of the myocardial organization, formation of a cuboidal cell monolayer, and transformation into a flattened epithelium. The transient presence of large intracellular lipid droplets was characteristic of this area. These histological changes occurred between days 4–5 dph, and were observed first in the left side of the OFT (Fig. 2c,d). The mesenchyme tissue of the MF20-negative distal portion of the OFT (Fig. 2a) was continuous with the cardiac jelly proximally, and with the extracardiac mesenchyme distally. In contrast, the MF20-positive portion of the OFT maintained its previous histological organization. The only change detected was the presence of a small number of cells in the cardiac jelly space (compare Fig. 2a, 2b).

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**Fig. 1. Development of the heart of the sturgeon, Acipenser naccarii. SEM. a: 2 dph. The heart shows a “C” shape. Bending is more pronounced at the outflow tract (OFT) and ventricle (V) than at the atrium (A). The sinus venosus (SV) is a midline structure. The conoventricular and the atrioventricular sulci are indicated by arrows. b: 4 dph. The ventricle is moving toward the midline. The atrium is ascending to occupy a position to the left of the outflow tract. e: 6 dph. The ventricle is a midline structure. The atrium has shifted to occupy a dorsolateral position with regard to the outflow tract. The double arrow indicates the MF20-negative distal portion of the outflow tract. d: 10 dph. The heart has acquired the adult configuration. The double arrow indicates the nonmyocardial segment of the cardiac outflow tract. Note that the relative craniocaudal length of the heart decreases as the folding into an S-shaped structure progresses. Scale bars = 100 mm.**
Figure 1
and distal endocardial cushions developed in the myocardial portion of the OFT, became populated by cells, and cushion excavation began (Fig. 2e–h). Histogenesis proceeded through subsequent developmental stages, the distal OFT portion acquiring an aortic-like organization (Fig. 2i). In agreement with the histological findings, at the 7th dph, orcein staining detected for the first time the presence of elastin in the ventral aorta, aortic arches, and distal, nonmyocardial portion of the cardiac OFT (Fig. 3e).
Thereafter, the amount of elastin increased gradually in all of these cardiovascular structures (Fig. 3f).

From the 9th to the 24th dph, the distal portion of the cardiac OFT continued to be unreactive to MF20 (Fig. 3g) and reactive to β-actin (Fig. 3h). When viewed from the outside, this nonmyocardial portion appeared as a relatively short, narrow ring connected to the ventral aorta at the anterior limit of the pericardial cavity (Fig. 1d). In contrast, the proximal, myocardial portion of the OFT was much more developed in length and width. The boundary between the two portions was roughly annular (Fig. 1d).

Finally, it should be noted that we detected no apoptotic cells or macrophages in the developing cardiac OFT, either in serial semithin sections or in ultrathin sections.

**DISCUSSION**

Differentiation of Cardiac Outflow Tract Components

MF20 antimyosin antibody staining shows that the entire cardiac OFT of *Acipenser naccarii* displays a myocardial character during early development. Yet its distal portion loses MF20-positive immunoreactivity at the 4th dph and becomes β-actin-positive by the 6th dph. This indicates that this region undergoes a phenotypic transition, from a myocardial to a smooth muscle-like phenotype. The length of this region with regard to the whole cardiac OFT increases only moderately during subsequent developmental stages, becoming more and more cellularized. Furthermore, the cells soon organize into a pattern that resembles that of the arterial wall. According to the present findings, the distal OFT segment continues to show β-actin-positive immunoreactivity at least until the 24th dph. Elastin appears at this site by the 7th dph. From then on, the amount of elastin increases progressively.

Therefore, two distinct components, proximal and distal, can be recognized from the 4th dph in the cardiac OFT of *Acipenser naccarii*. The proximal component is the conus arteriosus, characterized by its myocardial nature and presence of endocardial cushions from which the conus valves derive. The distal component displays a nonmyocardial character and, presumably, transforms into the transitional (or intermediate) segment, intercalated between the conus arteriosus and the ventral aorta, as described by Icardo et al. (2002a,b) in adult sturgeons. The present data on the formation of this transitional segment supports the notion of Icardo et al. (2002b) that its morphogenetic origin diverges from that of the ventral aorta, a fact which may explain the structural differences between them in the adult heart (see Icardo et al., 2002a,b).

Several of the morphogenetic events reported herein are similar to those observed during the development of the bulbus arteriosus of the zebrafish (Hu et al., 2000). In this teleost species, the anticipated bulbus arteriosus displays MF20-positive immunoreactivity, revealing similar characteristics to those of the myocardium. At 4 weeks postfertilization, the bulbus loses its positive MF20 staining, showing a smooth muscle phenotype. According to Hu et al. (2001), this positive/negative MF20 staining transition during early development might be attributed to removal of OFT cardiomyocytes through apoptosis, or to a replacement of the smooth muscle cells from the head mesenchyme. In the sturgeon alevins examined, we were unable to detect any sign of apoptosis in the distal portion of cardiac OFT during the period in which it loses its primary myocardial condition. This diverges from the morphogenetic mechanisms that govern the remodeling of the myocardial cardiac OFT in birds, where myocyte apoptosis plays a fundamental role (Watanabe et al., 1998).

In *Acipenser naccarii*, the subendothelial space of the ventral aorta, and that of the nonmyocardial distal portion of the cardiac OFT, is populated early in development by a considerable number of mesenchyme cells that soon become organized into an arterial-like pattern. The morphogenetic pattern clearly diverges from that followed by the cells populating the endocardial cushions, which presumably originate from the cushion endocardium. This sug-
Figure 3
gests that the mesenchyme populating the distal portion of the OFT does not originate in situ, but proceeds from another morphogenetic source.

It is well known that in birds and mammals the cranial neural crest gives rise to ectomesenchyme that contributes to the septation of the cardiac OFT and to the arterIALIZATION of the aorta, aortic arches, and pulmonary artery. In fact, the presence of cells of neural crest origin in the OFT coincides with the loss of the myocardial character of the distal portion of the OFT and the acquisition by the mesenchyme cells of a smooth muscle phenotype (Bockman et al., 1987; Nishibatake et al., 1987; Hiruma and Hirokawa, 1992; Yablonka-Reuveni et al., 1995, 1998; Bergwerff et al., 1996, 1998; Waldo et al., 1998, 1999; Jiang et al., 2000).

Knowledge on the contribution of the neural crest to the development of the fish vascular system concerns a very limited number of teleost species. In members of the genus *Xiphophorus*, cells proceeding from the neural crest are involved in the formation of the vasculature of the gill arches (Sadaghiani and Vieikind, 1990). The segmental series of the pharyngeal arches of the zebrafish is formed by two migratory cell types, namely, neural crest and paraxial mesoderm cells (Schilling and Kimmel, 1994). In this latter species, neural crest cells participate in structuring the bulbus arteriosus, ventricle, atrioventricular junction, and atrium (Sato and Yost, 2003). After reaching the heart, neural crest-derived cells intermingle with the myocardium, adopting a cardiomyocyte cell lineage (Li et al., 2003; Sato and Yost, 2003).

It has been demonstrated that the neural crest cells that populate the bulbus arteriosus of the zebrafish proceed from different cellular groups of both the medial and lateral cardiac neural crest (Sato and Yost, 2003). Yet the spectrum of morphogenetic actions performed by these cells is still uncertain. They were adduced as possible precursors of the chondrocytes of the cartilaginous deposits occurring in the bulbus arteriosus of adult teleosts (Blanco et al., 2001). The melanocytes that appear in the embryonic cardiac OFT of the sturgeon are apparently neural crest-derived elements. Interestingly, the present observations suggest that the distal portion of the OFT becomes populated by cells proceeding from an extracardiac source at the time at which the switch from a myocardial to a smooth muscle-like phenotype takes place. This points to the possibility that neural crest cells might participate in the remodeling of the distal segment of the cardiac OFT, a question that merits further investigation.

### Implications for Heart Evolution

Gegenbaur (1866) introduced the name “conus arteriosus” to designate the myocardial segment located between the ventricle of the elasmobranch heart and the ventral aorta. He retained the ancient term “bulbus arteriosus” to indicate the nonmyocardial chamber interposed between the ventricle and the ventral aorta, or between the conus arteriosus and the ventral aorta in actinopterygian fishes. Gegenbaur’s nomenclature was adopted by numerous authors. Parsons (1930), however, used the name “conus arteriosus” to designate the morphologically anterior portion of the primitive cardiac tube, including the nonmyocardial bulbus arteriosus of the actinopterygians. At the same time, Goodrich (1930) argued that bulbus cordis should be applied to the anterior embryonic cardiac chamber throughout the crania, reserving the name conus arteriosus for the adult muscular, contractile chamber, derived from the bulbus cordis of fish and amphibians. More recently, De la Cruz et al. (1999) claimed that the term conus arteriosus should be reserved to indicate a region or segment present only in the embryonic heart, whereas the mature, anterior chamber of all vertebrate hearts should be called bulbus cordis. Nonetheless, the viewpoint that prevails in textbooks is that the cardiac OFT of both the elasmobranchs and primitive actinopterygians consists of a myocardial conus arteriosus, whereas in advanced actinopterygians it is mainly composed of a nonmyocardial bulbus arteriosus (Parker and Haswell, 1962; Weichert and Presch, 1975; Lawson, 1979; Johansen and Burggren, 1980; Hildebrand, 1982; Young, 1983; Nadal, 2001; Kardong 2002). The evolutionary switch from a cardiac OFT formed by a patent conus arteriosus, furnished with several rows of valves, to an OFT composed of a well-developed bulbus arteriosus and a reduced or even absent conus arteriosus, with a single row of valves, has been the subject of numerous conjectures.

The reduction in size of the conus arteriosus in teleosts has been implicitly assumed as a consequence of the progressive development of the bulbus arteriosus. The disappearance of the conus arteriosus in the adult heart of several teleost species has been attributed to its incorporation into the proximal end of the bulbus arteriosus or, much more...
frequently, to its condensation into the ventricle (Smith, 1918).

Boas (1901) hypothesized that, evolutionarily, the bulbus originated from the proximal part of the ventral aorta, which later extended backwards to replace the conus arteriosus of the elasmobranchs. This hypothesis relied on the fact that the histology of the bulbus is undoubtedly more arterial than cardiac in nature. Some years later, Senior (1909) showed that in the shad, *Alosa sapidissima*, the bulbus develops within the pericardial cavity. Nonetheless, Grodzinski (1938) pointed out that the bulbus cannot be regarded as a cardiac chamber, because it lacks cardiac muscle. This view was adopted by numerous subsequent authors (Bridge, 1904; Krause, 1923; Grodzinski, 1938; Bertin, 1958; Parker and Haswell, 1962; Weichert and Presch, 1975; Lawson, 1979).

Parsons (1930) disputed Boas’ hypothesis, considering that all segments of the cardiac tube within the pericardial cavity belong morphologically to the heart. He stated that the bulbus arteriosus of teleost fish is simply part of the original conus arteriosus, i.e., the preventricular portion of the primitive cardiac tube. The histological and biochemical studies of the bulbus arteriosus carried out by Licht and Harris (1973) in the carp, *Cyprinus carpio*, illustrated that the bulbus is basically distinct from the ventral aorta, a fact that led to the assumption that the bulbus arteriosus is an intrinsic part of the teleost heart derived, probably, from the primitive conus arteriosus (Priede, 1976; Yamauchi, 1980; Satchell, 1991; Farrell and Jones, 1992).

The present findings in *Acipenser naccarii* substantiate that the intrapericardial segment lying between the conus arteriosus and ventral aorta, described by Icardo et al. (2002a,b) as a transitional segment, is not a backward extension of the ventral aorta, but derives from embryonic cardiac OFT. Our findings, together with those reported by Hu et al. (2000), strongly suggest that the transitional segment of the sturgeon and the bulbus arteriosus of teleosts are homologous structures. This conclusion relies on the fact that the two structures originate from the distal portion of the cardiac tube following similar morphogenetic steps, namely: a first step characterized by the expression of a myocardial phenotype, a second step consisting of the loss of the myocardial phenotype, and a third, longer, histogenetic step, in which each of these structures acquires its definitive tissue organization.

It is currently accepted that both the embryonic and adult heart of elasmobranchs is composed of five myocardial compartments: the sinus venosus, atrium, atrioventricular canal, ventricle, and conus arteriosus (Gallego et al., 1998; Franco et al., 2002). However, Parsons (1930) reported that in several elasmobranch species, the muscular portion of the conus arteriosus in the adult heart does not as a rule extend as far as the cranial pericardial boundary. In such cases the distal portion of the conus consists of a tissue resembling that of the wall of the ventral aorta beyond the pericardial cavity. To our knowledge, no further attention has been paid to this noncontractile, distal portion of the elasmobranch conus arteriosus, either from the morphological or from the embryological viewpoint. Yet from Parsons’ descriptions it can be inferred that this nonmyocardial portion is morphologically equivalent to the transitional segment of the adult sturgeon heart.

From the preceding data we conclude that a true bulbus arteriosus exists in primitive actinopterygian fishes, i.e., the sturgeons; it corresponds to the secondarily nonmyocardial, distal portion of the cardiac OFT that Icardo et al. (2002b) referred to by using the term “intrapericardial transitional segment”. Moreover, we presume that a bulbus arteriosus also exists in the elasmobranchs. The anatomical configuration of the cardiac OFT of the most primitive living craniate chordates, i.e., the hagfishes and lampreys, differs between authors. Some of them consider that the cardiac OFT of the adult cyclostomes (Kent, 1978; Randall and Davie, 1980; Nadal, 2001; Kardong, 1998) and ammocoete larva (Daniel, 1934) is composed of a contractile conus arteriosus. Others (Fontaine, 1958; Santer, 1985) state that it consists of a noncontractile bulbus arteriosus, containing smooth muscle cells and elastic fibers (Yamauchi, 1980).

In our opinion, the bulbus appeared in an as-yet undetermined, early period of the craniata (vertebrate) evolutionary story. Thus, the cardiac OFT came to be formed by two components: 1) a secondarily nonmyocardial, distal component, the bulbus arteriosus; and 2) a myocardial, proximal component, the conus arteriosus, which supports the endocardial cushions that give rise to the cardiac OFT valves. Within the lineage of teleosts, the bulbus arteriosus evolved into a well-developed chamber, splitting into a wide range of structural variants (Icardo et al., 1999a,b, 2000a,b).

The exact significance of the bulbus arteriosus in elasmobranchs and primitive actinopterygian remains uncertain. In teleosts, it is classically accepted that the bulbus acts as a passive elastic reservoir or “Windkessel” (Von Skramlick, 1935) during the cardiac cycle, with particular importance in maintaining the ventral aorta blood flow during ventricular diastole (Johansen, 1962; Priede, 1976; Satchell, 1991; Bushnell et al., 1992; Jones et al., 1993). Yet recent work has shown that the teleost bulbus may exhibit a wider variety of physiological and behavioral patterns than previously believed (Icardo et al., 1999a,b, 2000a,b). In contrast, the possible significance of the conus arteriosus in teleosts has been disregarded. However, it has recently been reported that the gilthead seabream, *Sparus auratus*, a phylogenetically advanced teleost species, possesses a distinct conus arteriosus formed by a compact, well-vascularized myocardium, which
seems to be actively implicated in the performance of the conus valves (Schib et al., 2002). The discrimination between the conal and ventricular myocardia and the histomorphological characterization of the conus in this species was made possible by employing a combination of different microscopic techniques. This suggests that the existence of a conus arteriosus in other teleosts might have been overlooked because of the use of inappropriate methods. Therefore, we consider that the assumed disappearance of the conus in teleosts is a question that should be reviewed.

The next questions that should be asked are whether the existence of these two segments within the heart OFT is exclusive to fishes, and whether they are present in other vertebrate taxa. In birds and mammals the nomenclature used to describe the developing OFT diverges widely between authors (see Pexieder, 1995, for an extensive review of the literature). However, it is generally accepted that the embryonic OFT consists of two components, a proximal conus and a distal truncus. The two segments are invested by myocardium, develop within the pericardial cavity, and become furnished with opposing endocardial cushions or ridges (Kramer, 1942; Shaner, 1962; Van Mierop et al., 1963; Icardo et al., 1990; Icardo and Manasek, 1991; Manner, 2000). Division of the OFT involves fusion of apposing cushions and the development of the aortopulmonary septum. The latter is a horseshoe-shaped structure whose two limbs penetrate the truncal cushions down to the semilunar valve level (Icardo, 1985). As division of the truncus takes place, the truncus loses its myocardial nature and is later transformed into the proximal parts of the aorta and the pulmonary artery. The conus retains its myocardial investment, provides support for the developing arterial valves, and ends up as part of the ventricular outflows (see Icardo et al., 1990, and Icardo and Manasek, 1991, for reviews). The mechanisms involved in myocardial regression of the truncus are not yet clear, but apoptosis (Hurle et al., 1977), dedifferentiation (Argüello et al., 1978), and translocation of the entire septation complex towards the ventricle (Thompson et al., 1983) have been suggested (see Icardo, 1990, for a review). Thus, we believe that the embryonic truncus of birds and mammals is homologous to the fish bulbous arteriosus. This segment is myocardial in nature initially, loses its myocardial investment later, and finally adopts an arterial or arterial-like structure. In addition, the initial segment of the arterial trunks in humans is invested by the serous pericardium and, therefore, contained within the pericardial cavity. On the other hand, the embryonic conus of birds and mammals appears to be homologous to the fish conus arteriosus. This segment maintains its myocardial nature, supports the outflow valves, and constitutes the ventricular outflow. Whether the embryonic conus remains as a distinct segment in the adult heart or blends with the ventricular mass, and whether the entire OFT is septated or not, are, most probably, minor questions. It seems reasonable that, once a particular structure has appeared in evolution, and demonstrates a real survival or adaptive advantage, it should be maintained through successive evolutionary steps. Thus, it obeys the principle of universality (see Opitz and Clark, 2000). It is also reasonable that specific modifications are necessary to adapt that particular structure to meet changing physiological requirements. For instance, the relation between OFT septation and the beginning of air breathing is obvious. The OFT of the vertebrate heart appears to constitute a morphogenetic unit which becomes phylogenetically adapted to other parts of the heart and to the rest of the body in order to accomplish specific functions. This fits well with recent findings indicating that the myocardium of the conotruncus arises from a secondary heart field cranial to the initial area of fusion of the heart primordia (Waldo et al., 2001).

### LITERATURE CITED


A. GUERRERO ET AL.


Senior HD. 1907b. Teleosts with a conus having more than one row of valves. Anat Rec 4:83–84.

Senior HD. 1907c. Note on the conus of Megalops cyprinoides (Broussonet). Biol Bull 12:378–379


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