FORMATION OF THE SCAPULAR PART OF THE PECTORAL GIRDLE IN ANURAN LARVAE

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The development of the scapular part of the pectoral girdle was traced from the limb bud stage to the metamorphosis climax in three anuran species of different families: *Rana temporaria*, *Bufo bufo*, and *Bombina bombina*. It was found that the scapular part originates from two independent anlagen — the “upper-scapular” and the “lower-scapular”. These anlagen fuse together forming a unified cartilaginous element at the stage of young (nonhypertrophied) cartilage. The boundary between the definitive scapula and the supra-scapula passes within the lower anlage. This boundary is a synchondrosis arising under the influence of m. interscapularis. The ratio of the scapula and the suprascapula length is determined by the place of m. interscapularis formation.

Key Words: Anura, development, scapula, suprascapula, synchondrosis, pectoral girdle.

INTRODUCTION

In Anura the scapular part of the pectoral girdle comprises the bony scapula and suprascapular cartilages with the overlying cleithrum. Attention of authors, who studied its development, was chiefly attracted by the genesis and growth of the dermal ossification — the cleithrum (Goette, 1877; Gegenbaur, 1895; Schmalhausen, 1917; de Villiers, 1922). The cleithrum appears in ontogenesis rather late — just before the metamorphosis, when the scapula and the suprascapular cartilage have already got the proportions that are maintained in adults. Braus (1909) investigated the development of the pectoral girdle starting from the formation of the forelimb buds to the beginning of the metamorphosis in yellow-bellied toad (*Bombina variegata*). Unfortunately, he did not provide a detailed description because of insufficiency of material. Thus the formation and the early developmental stages of the cartilaginous elements of the anuran pectoral girdle have not been well studied yet. The aim of this work was to investigate early stages of development of the scapular part of the anuran pectoral girdle.

MATERIAL AND METHODS

Were studied species belonging to three anuran families (Ranidae, Bufonidae, and Discoglossidae): common frog (*Rana temporaria* L.), common toad (*Bufo bufo* L.), fire-bellied toad (*Bombina bombina* L.). Eggs of common frog and common toad were collected in the vicinities of St. Petersburg (Russia). Eggs of fire-bellied toad were obtained in laboratory from breeders taken from the natural habitat in Belgorod region (Russia) in spring, 1998. Tadpoles of three species were reared in laboratory using standard techniques (see Dabagian and Sleptsova, 1975; Ivanova and Pyastolova, 1979; Lang, 1988/89). Tadpoles were sacrificed in Bouin’s solution and then were embedded in paraffin with using standard methods. Larvae of all the species studied were staged according to the normal tables of *Rana temporaria* (see Dabagian and Sleptsova, 1975). We used specimens from the limb bud stage till the end of the metamorphosis. Totally, 51 specimens of *Rana temporaria*, 44 specimens of *Bufo bufo*, and 22 specimens of *Bombina bombina* were studied histologically. Serial paraffin histological sections of 7–13 mm thickness were made in three standard planes and the slides were stained by hematoxylin of Delafild and eosin using standard methods. Besides that the histological
sections through the larvae of *Bombina bombina* stained by hematoxylin and eosin, which were made by V. G. Borkhvardt in 1991 were reexamined. Nomenclature for muscles, nerves and blood vessels was used according to Gaupp (1896, 1899), and Nozdrachev and Polyakov (1994).

**RESULTS**

**Topography of organs in the anterior part of the tadpole body in *Rana temporaria, Bufo bufo, and Bombina bombina*** (Fig. 1)

The anterior part of the body has similar topography of organs in all three species studied. It is embraced by vast operculum, which contains gills and limb buds, ventrally and laterally. A big paired pronephros is situated in the anterior part of the body laterally from pharynx and lungs. The pronephros occupies second and third segments of the body between ear capsule and abdomen. Its upper margin lying on the level of notochord and the lower one reaches the level of abdomen. Laterally the pronephros contacts the integument and vast lymphatic sacs, lying under the skin. There is some free space remained between the upper margin of the pronephros and the lower edge of myomeres.

**Development of the scapular part of the pectoral girdle**

*Rana temporaria*

**Stages 39 – 40.** The forelimb buds arise from the body of larvae at the level of the lower part of the pronephros. They are filled with dense mesenchyme tissue. The space between the pronephros and the base of the limb bud is filled by loose mesenchymal cells. The same loose mesenchyme is situated above the pronephros on the distance of one and a half of a segment from the ear capsule back to the lymphatic heart (Figs. 1, 2a). Rare mesenchymal cells occur along the lateral surface of the pronephros between the pronephros and integument. Thick nervus spinalis II goes along the caudal part of the pronephros lateral surface reaching the base of the limb bud. Very thin n. spinalis II passes in the close vicinity of n. spinalis II but more caudally. Before entering the limb bud it fuses with n. spinalis II forming plexus brachialis. One branch of pl. brachialis embraces the pronephros from below and separates the mesenchyme of the limb from the mesenchyme of the body. The other branch enters the upper-anterior part of the limb bud.
(Fig. 2b). Not far from the latter a big blood vessel — arteria subclavia — goes into the forelimb.

**Stages 41 – 42.** Cell condensation of the humerus appears in the limb. An aggregation of mesenchymal cells lies above the humerus, near the fore edge of the limb bud base. It is referred hereafter as the “lower-scapular” one. The mesenchymal condensations of the prospective coracoids lie under the humerus level. Having entered the limb bud, the branch of the plexus brachialis separates the lower-scapular condensation from the mesenchyme, which lies under it.

**Stage 43 – 44.** The deposition of cartilaginous matrix begins in the humerus anlage. The lower-scapular cell condensation stretches upward embracing the pronephros lower part in a shape of a crescent. The m. dorsalis scapulae is formed outside the upper half of the lower-scapular cell condensation. Mesenchyme continues to accumulate between the pronephros and the myomeres, opposite the second myoseptum.

**Stage 44.** The lower-scapular condensation elongates upwards but does not reach the upper part of the pronephros. Chondrification starts within the lower part of the latter. The glenoid cavity is formed at the place of contact of the humerus and the cartilaginous anlagen of the girdle (Fig. 2c). A cell condensation appears between the pronephros and myomeres slightly forward to the second transverse myoseptum at the level of the dorsal edge of the notochord. It is named here the “upper-scapular” condensation.

**Stage 45.** The lower-scapular cell condensation becomes larger. Its cartilaginous lower part is elongated vertically. Chondrification starts at the mid-height level of the upper-scapular condensation. Mesenchyme condensates along the lateral surface of the pronephros between the lower- and the upper-scapular cell condensations. Presumptive m. dorsalis scapulae goes along the whole length of the lower-scapular condensation. Its cells are at the stage of myoblasts.

**Stage 46.** The lower-scapular cartilage goes upward above the pronephros and incurves inside the body of larvae at a straight angle. The upper-scapular cartilage is elongated. The lower- and the upper-scapular cartilages are separated by a dense mesenchymal layer. Both cartilages are similar in length (Fig. 3a). The extension of these cartilages along longitudinal body axis (the width of cartilage) is approximately equal. M. dorsalis scapulae extends from the humerus to the upper part of the lower-scapular cartilage. Its cells begin to be organized in myotubes.

**Stage 47.** Two cartilaginous anlagen — the lower- and the upper-scapular — are fused, but the border between them is distinguishable as a very young cartilage region, marked by the insertion of
Fig. 3. Transverse sections through the forepart of the body of *Rana temporaria* larvae at stages 46 (a), 47 (b), 48 (c), and 54 (d). Abbreviations: *clt* cleithrum; *pch* perichondrium; *m.d.sc* m. dorsalis scapulae; *m.isc* m. interscapularis; *sc* scapula; *ssc* suprascapula; *sz.A* subzone of irregularly arranged cells; *sz.B* subzone of cells organized in parallel horizontal layers; *z.h.cr* zone of hypertrophied cartilage; *z.sc* zone of small cells. See Figs. 1 – 2 for other abbreviations. The arrow shows the zone of dense mesenchyme between the upper- and the lower-scapular cartilages (a) and the border between the upper- and the lower-scapular cartilages at the place of the m. dorsalis scapulae attachment (b). Square brackets indicate approximate ranges of zones and subzones (c, d). Scale bar: 180 μm (a – c), 105 μm (d).
the m. dorsalis scapulae to the upper margin of the lower-scapular cartilage (Fig. 3b). The unified cartilage is a plaque, which continues almost vertically from the glenoid cavity to the mid-level of the spinal cord. In transverse sections, the plaque has two medio-lateral bends (in the shape of zigzag). In frontal sections, the lower part of the plaque is thick and consists of 7–8 cells, preserving a roundish shape. The upper part of the plaque is foliaceous and extends from the middle part of the first myomere up to one third of the second myomere. It consists of 2–3 cells. A part of the former lower-scapular cartilage, from the glenoid cavity to the point of its bending, is covered by perichondrium. This part corresponds to the prospective scapula. The m. interscapularis appears internally to the lower-scapular cartilage at the point of its bending. Cells of the m. interscapularis are spindle-shaped (Fig. 3b).

Stages 48–49. The border between the lower- and the upper-scapular cartilages is not visible. The structure of the unified cartilage is vertically differentiated. There are two areas of hypertrophied cartilage within this skeletal element. One of the areas is large and is situated in scapula; the other is small and is situated near the former upper-scapular cartilage. Between these areas there is a wide zone of small-cell cartilage, which contains two subzones. The first one (named subzone A hereafter) consists of the irregularly arranged cells and takes the place of the lower-scapula bend. The second one, lying above the first (named subzone B hereafter), is formed by cells organized in parallel horizontal layers. The ratio of the scapula dorsal-lateral length to the length of the suprascapula is about 1. The attachment of the m. dorsalis scapulae expands upwards and goes up to the two thirds of suprascapula length. The m. interscapularis starts from the middle part of the scapula and goes to the lowest one third of the suprascapula; it is formed by myotubes. The cleithrum appears below the m. dorsalis scapulae near the front edge of the scapular lateral surface in the region of the hypertrophied cartilage (Fig. 3c).

Stages 49–51. The scapula is completely covered by perichondral bone. The entire surface of the suprascapular cartilage is covered by perichondrium. The process of hypertrophy is expanded to the subzone B, where the arrangement of cells is still regular. The suprascapula curves up above the myomeres. The cleithrum has a shape of a narrow band and goes upwards along the suprascapularis lateral surface from its basement up to two thirds of its length.

Stages 52–54. Blood vessels penetrate the central part of the scapula and the cartilage begins to be destroyed. Bone marrow fills the whole scapula by the stage 54. The suprascapular cartilage grows up in the medial direction above myomeres. Having widened, it stretches from the middle part of ear capsule up to the central part of the third muscular segment. The cartilage area consists of the irregularly arranged cells and still exists between the scapula and suprascapula. Above this area cells are hypertrophied, but show a regular pattern (they are packed in horizontal layers) (Fig. 3d). The growing cleithrum almost reaches the top of the suprascapula covering two thirds of its width and turns on its medial side. Bone marrow appears within the cleithrum. The m. interscapularis is attached to the lowest third of suprascapula and also to the middle part of the scapula where the place of its attachment is slightly sloped in caudal direction.
**Bufo bufo**

In general, the time of appearance of the pectoral girdle elements in tadpoles of common toad corresponds to those of common frog. However, all the events in the development of the scapular part in *Bufo bufo* pass on one stage earlier. Contrary to common frog, length ratio of the scapula to suprascapula vary from about 1,0 to 1,7 at the same stage (Fig. 4). The unified cartilage plaque in the region of prospective scapula is just slightly thicker than the suprascapular part. The thickness is greater due to the hypertrophy of cells rather than to their number. Both the scapula and the suprascapula are two — three cells in thickness. The disproportion appears early between the scapular and the suprascapular lengths along the longitudinal body axis (suprascapula is much wider than scapula).

**Bombina bombina**

The sequence of formation of the pectoral girdle elements in fired-bellied toad is similar to that in common frog, but cartilage in all cell condensations appears one stage later as to compared to common frog. However, m. interscapularis is formed earlier than in common frog (at the stage 46), when the lower- and the upper-scapular cartilages are separated by a loose mesenchyme layer (Fig. 5a). Afterward the development of tadpoles of fire-bellied toad is synchronized with that of common frog. The lower- and the upper-scapular cartilages fuse at the stage 47. At this time the former is about twice as longer as the upper-scapular cartilage (Fig. 5b). The border between the cartilages is fully disappeared by the stage 49. The upper part of the unified cartilaginous plaque is very thin and consists of 2 – 3 cells. The plaque’s lower part is thicker and ellipse-shaped in cross section, consisting of 7 – 8 cells. Contrary to common frog, the scapula is very short in fire-bellied toad. The ratio of the scapula to the suprascapula lengths varies from 1 to 4,5. The m. interscapularis remains rather short. It is attached to scapula in the area of glenoid cavity. The other end of the muscle is attached to suprascapula central zone by its anterior margin and slightly above the deepest bending site by its posterior margin. At the end of the metamorphosis the scapula is covered by perichondral bone, the destruction of cartilage does not take place. The cleithrum is represented by a narrow bone band at the frontal margin of the suprascapula and, therefore, its shape differ from that in adult fire-bellied toads.

**DISCUSSION**

Braus (1909) described two mesenchymal condensations within the scapular part of pectoral girdle in yellow-bellied toad: one of them is situated near the base of the forelimb bud and another — slightly below the second and the third myomeres. Our results confirm these data. The mesenchyme in the anterior part of the body is irregularly arranged in all three species studied: in some regions it is almost absent, while in others there are some cells aggregations. Some of them are situated in the basement of the forelimb bud and also one is situated above the pro-
nephros (Fig. 2b, c). Some authors stated that mesenchyme is first to condense in the areas of limited space (see Borkhvardt, 1982; Borkhvardt and Kovalenko, 1985; Kovalenko, 1986). Mesenchymal cells condensate in places, where organs bend or where the wedge-shaped spaces are formed between the contacting structures (Kovalenko, 1992). In all the species studied the pronephros is relatively large during the whole premetamorphic development. It occupies the whole space between the ear capsules and abdominal cavity. Myomeres in these species are wider dorsally in the anterior part of the body, but they sharply became narrow towards notochord, retiring from the integument (Fig. 1). Thus, at the notochord level there is a space triangular in section, where the upper-scapular cell condensation is formed. The other condensation appears in the basement of the forelimb bud. The formation of the latter condensation is probably connected with existence of the thick n. brachialis and a. subclavia (it is arteria centralis after entering in the limb bud), which enter the basement of the limb bud. They separate the upper part of mesenchyme in the limb bud base (Fig. 2b). Borkhvardt (1994, 1996) denoted a morphogenetic role of blood vessels in the formation of the endochondral skeletal elements. He suggested that blood vessels may break up the skeletogenous mesenchyme of limb bud into some portions and thereby can determine the position of the prospective skeletal elements. Mesenchymal cells aggregate forming a dense condensation between the pronephros and the limb base (the “lower-scapular” cell condensation). The lateral surface of the pronephros adjoins quite closely to the large sub-surface lymphatic cavities; i.e., the free space between the pronephros and the integument is almost absent. In turn, it might be the cause of absence of mesenchymal cell condensation in this region. Mesenchymal cells accumulate in areas, where there is enough free space. However, there is only a broad band of loose mesenchyme between the lower- and the upper-scapular cell condensations (along the lateral surface of the pronephros). Chondrification starts in both the lower- and the upper-scapular mesenchymal cell condensations simultaneously. The lower-scapular cartilage grows upwards along the lateral surface of the pronephros; the upper-scapular cartilage grows towards the latter (Fig. 5a). At the stages 46 – 47 these cartilages fuse into a single element.

In adults of all investigated species the cleithrum overlaps the largest part of the suprascapula including the lower part of its lateral surface that contacts with the bony scapula. The entire cleithrum-suprascapula complex is separated from the latter by a pronounced borderline. Anatomically this border looks like a band of nonmineralized hyalin cartilage, represented by a zone of small-celled cartilage in histological sections (Figs. 3d, 6a, 6b). In his scheme of Anura pectoral girdle structure, Gaupp (1896) termed the border between the scapula and the suprascapula as synchondrosis. Braus (1909) supposed that the bridge of hyaline cartilage that arose between ossified scapula and suprascapula covered by cleithrum has a physiological character of articular ligament between both hard parts — synchondrose.

According to Akaevskiy (1968), synchondrosis is a confining connection between two skeletal elements formed by fibrillar or hyaline cartilage. Synchondrosis provides a considerable fastness of con-
confining articulations like syndesmosis or amphiarthrosis. But the existence of a greater mobility can lead to formation of the true joint like diarthrosis (see Epstein, 1940; Nikolaev, 1954; Ponomareva-Astrakhan'tseva, 1954; Kabardin, 1963). At the same time, the immobility of the skeletal elements leads later to formation of the synostosis (immobile articulation). Short ligaments, connecting contacting bones, induce gradual ossification, which leads to the coalescence of both bones in a united bony element (Nikolaev, 1954). Experiments on immobilization of musculature in a chicken at early developmental stages also showed that in absence of mobility the formation of articulations did not take place (Murray and Drachman, 1969; Sullivan, 1974).

In our case, the formation of synchondrosis takes place within the unified cartilage just in the zone of mobility providing by contractions of m. interscapularis. However, the joint is situated not between the different cartilaginous anlagen, but within the frames of one of them — the lower-scapular one. Thus, suprascapula is the product of fusion of two anlagen: the lower- and the part of the upper-scapular ones.

The length ratio of scapula to suprascapula may vary greatly in different species of amphibians. Schmalhausen (1917) examined changes of the ratio of the bony scapular part to the cartilaginous suprascapula (cleithral ossification covering the suprascapula) in phylogenesis. He concluded that in progressive evolution of the bone in anurans girdle, the scapula gradually replaces the suprascapular ossification further upwards. In common frog (Rana) and common toad (Bufo) the scapula is a little bit smaller than suprascapula, but in fired-bellied toad (Bombina) the scapula is very small (Fig. 6a, b). The length ratio of the scapula to suprascapula does not depend on initial proportions of the lower- and the upper-scapular cartilages. So, the lower-scapular cartilage is about twice longer than the upper-scapular one in fire-bellied toad at the stage of fusing of the upper- and the lower-scapular cartilages. However, the border between the scapula and suprascapula is determined earlier — with the appearance of the m. interscapularis, which is attached to the sides of the deepest part of the lower-scapular cartilage bending (Fig. 5a, b). Thus, scapula occurs to be short, as the proportions once appeared at the cartilaginous stage are maintained up to the definitive condition. The lower- and the upper-scapular cartilages are of about the equal length in common frog and in common toad (Fig. 3b). However, during
the formation of the interscapular muscle proportions of scapula and suprascapula are changed. In common toad, the length of the scapula becomes about twice shorter than suprascapula, in fire-bellied toad, the disproportion is more considerable: scapula is almost five times as shorter as suprascapula (Figs. 4, 5b). Thus, the proportions of the scapular part are defined early in ontogenesis — already at the unified plaque stage or even earlier (e.g., in fire-bellied toad). It is possible, that these proportions are determined by the site of interscapular muscle attachment.

CONCLUSIONS

The scapular part of the anuran pectoral girdle develops from two independent cartilaginous anlagen. At first two cartilages fuse into a single cartilaginous element with no trace of the border, and then two new elements — scapula and suprascapula — separates from this unified cartilage. The border between them forms not at the line of fusion of the upper- and the lower-scapular cartilages, but within the former lower-scapular cartilage. The length ratio of scapula to suprascapula is determined at the unified cartilage stage by the place of attachment of the m. interscapularis to the pectoral girdle, where the new border will form.

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