

Research Articles

Morphological integration in the cranium during anuran metamorphosis

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Summary. We examined the role of thyroid hormone in mediating morphological integration between cranial cartilage and bone during anuran metamorphosis. Exogenous T_3 applied to premetamorphic tadpoles (*Bombina orientalis*) via intracranial implants of plastic micropellets precociously induced typical metamorphic changes in both tissues, but also dissociated the relative timing of developmental events between them. Morphological integration between the two primary cranial tissues is achieved in part by each tissue responding independently to endocrine factors and does not reflect a tight developmental coupling between them.

Key words. Morphological integration; skull; Anura; thyroid hormone; metamorphosis.

Mechanisms of morphological integration are among the most important, albeit poorly understood, organizational processes in development¹⁻⁷. An outstanding example of morphological integration is the suite of changes that comprise cranial metamorphosis in anuran amphibians. During metamorphosis in these vertebrates, the cartilaginous larval skull is dramatically transformed by a combination of proliferation and resorption of existing cartilages and the formation of new ones; bone, which predominates in the adult skull, also appears for the first

time. Moreover, the events by which cartilaginous tissues transform and bony tissues differentiate occur in a precise temporal sequence that achieves a high degree of integration both among components of a given tissue type and between bone and cartilage.

Endocrine factors, particularly thyroid hormone (TH), play a predominant role in mediating morphological changes during amphibian metamorphosis⁸. Thyroid hormone is also known to affect the differentiation, growth, and remodeling of skeletal tissues in both am-

niotes⁹⁻¹² and amphibians¹³⁻¹⁵. In this report, we examine the role of TH in mediating morphological integration between cranial cartilage and bone during metamorphosis in the oriental fire-bellied toad, *Bombina orientalis*, a morphologically primitive anuran native to Korea. Quantitative data are drawn from both descriptive analyses of the timing of cartilage and bone development during natural metamorphosis and experimental analyses of skeletal development following intracranial administration of exogenous TH. They reveal that a) metamorphic changes in both cartilage and bone are mediated by TH, b) many changes in the two tissues are not tightly coupled developmentally, and c) the normal developmental relation between cartilage and bone, particularly the ontogenetic sequence of changes involving both tissues, in part reflects independent response of each tissue to TH.

Materials and methods

Premetamorphic tadpoles, stages 28/29, 30/31, and 32/33¹⁶, were obtained from laboratory crosses among wild-caught adults. Procedures for animal care, surgery, and anaesthesia are as described earlier^{17,18}. T₃ (3,3',5-triiodo-L-thyronine) was administered via a single, 0.25 ± 0.05 mg, plastic (Elvax 40P) micropellet implanted unilaterally within the dermis medial to the right eye^{18,19}. Three treatment dosages (2.5, 0.25, 0.025 µg) and one control dosage (0 µg) were used. In choosing T₃ we relied on evidence that it, and not T₄ (thyroxine), has the major role in inducing metamorphosis in amphibian tissues²⁰. Following preservation in 10% neutral-buffered formalin, specimens were prepared as whole mounts cleared and differentially stained for bone and cartilage²¹. Degree of ossification was expressed as the bone index, which equalled the total number of bones visible in whole mounts (maximum = 17); paired bones counted as one-half point for each side. Cartilage transformation was quantified by examining six characters that change substantially during metamorphosis, viz. cornu trabeculae, suprarostal cartilage, mandible (Meckel's and infrarostal cartilages), palatoquadrate cartilage, ceratohyal cartilage, and ceratobranchial cartilages²². For each character, three morphological states were defined: 0, larval; 1, transitional; and 2, postmetamorphic. The six individual character state values for each specimen were summed to give a single score, the cartilage index, with a range from 0 (larva) to 12 (metamorphosed froglet).

Results and discussion

Both skeletal tissues responded positively to hormone administration (table). Response of cartilage generally was dosage-dependent but not implant-stage-dependent ($p < 0.05$)²². Response of osteogenic tissues generally was both dosage-dependent and implant-stage-dependent ($p < 0.05$), the latter corresponding precisely to the

Effect of exogenous T₃ on cranial metamorphosis, 8 days after hormone administration

Dosage (µg T ₃)	Implant stage	Cartilage index (± SE)	N	Bone index (± SE)	N
0 (control)	28/29	0 ± 0	7	0 ± 0	6
	30/31	0.1 ± 0.14	7	0 ± 0	6
	32/33	0 ± 0	7	0 ± 0	6
0.025	28/29	0.8 ± 0.40	6	0 ± 0	6
	30/31	0.7 ± 0.33	6	0 ± 0	6
	32/33	0 ± 0	7	0 ± 0	6
0.25	28/29	5.4 ± 0.37	7	0 ± 0	6
	30/31	6.3 ± 0.18	7	0.2 ± 0.16	6
	32/33	5.4 ± 0.48	7	0.3 ± 0.21	6
2.5	28/29	8.0 ± 0.44	7	0 ± 0	6
	30/31	7.4 ± 0.53	7	0.7 ± 0.33	6
	32/33	8.1 ± 0.40	8	1.7 ± 0.33	6

presence or absence of cranial ossification centers at the time of hormone administration^{17,18}. For both cartilage and bone, morphological changes induced by exogenous T₃ were typical of those occurring during natural metamorphosis (figs 1, 2)^{18,22,23}.

While both cranial tissues responded positively to exogenous T₃, the rate of response differed between them. Specifically, cartilage proliferation and resorption proceeded much more quickly and extensively than bone formation, especially at higher dosages (table; figs 2, 3B). Consequently, in many T₃-treated tadpoles recovered after 8 days, cartilage transformation was far advanced, yet bone formation was in its earliest stages – the exact reverse of the temporal pattern of bone and cartilage development during the initial phase of natural metamorphosis (figs 1, 3A). In extreme cases of such T₃-induced dissociation, the natural sequence of cartilage transformation was nearly three-fourths complete while no bone was visible (fig. 2B). In other words, the normal temporal relation (and morphological integration) between cartilage and bone development was disrupted by exogenous T₃, although the changes within each tissue proceeded normally. We interpret these results to indicate that metamorphosis of both cranial cartilage and bone is mediated by T₃. Furthermore, we conclude that the morphological and temporal integration between these tissues which characterizes natural metamorphosis is in part the result of each tissue responding independently to this hormonal influence, and is not the result of tight developmental coupling between them.

Among the most remarkable aspects of vertebrate organogenesis is the precision with which disparate tissues come together to form complex, functional structures. Previous studies of a wide variety of taxa have identified the important role played by local interactions among skeletal and non-skeletal components in mediating both differentiation and subsequent growth and remodeling of cranial tissues²⁴⁻²⁶. In this study, we have identified an important, complementary role played by a systemic factor, thyroid hormone, in effecting morphological integration between cranial cartilage and bone during anuran metamorphosis. This result underscores

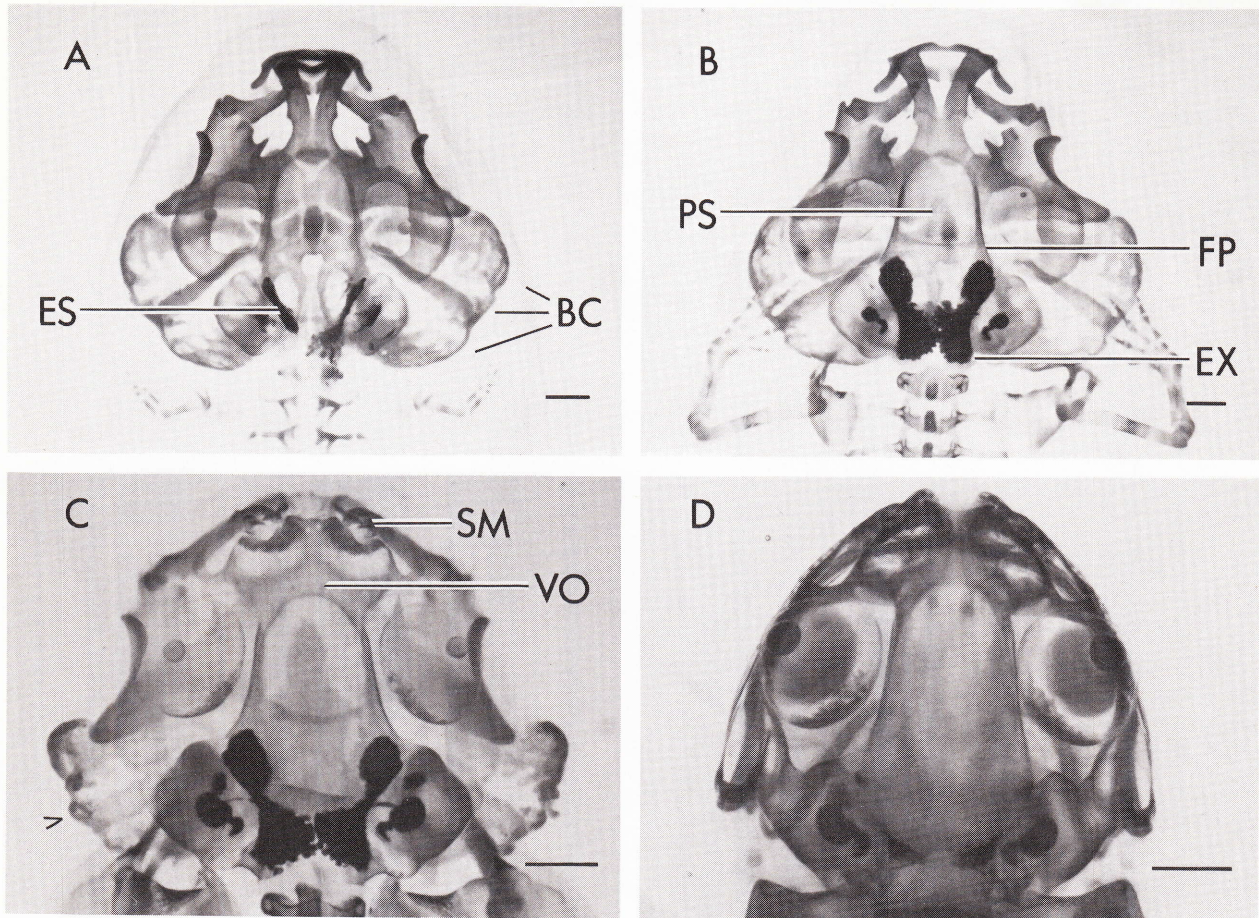


Figure 1. Skulls of naturally metamorphosing *Bombina orientalis* cleared and differentially stained for bone and cartilage; dorsal views. *A* Premetamorphic tadpole, stage 36. All cartilages are in the larval configuration; no bone is visible. BC, branchial cartilages. ES, calcified endolymphatic sacs. *B* Midmetamorphic tadpole, stage 39. Three bones are now visible – the median parasphenoid (PS) and paired frontoparietals (FP) and exoccipitals (EX) – but cartilages are essentially unchanged. *C* Mid-met-

morphic tadpole, stage 43. Additional bones have appeared (bone index = 7), such as the paired septomaxillae (SM) and vomers (VO). Cartilage transformation is one-half complete (cartilage index = 6); note, for example, partial resorption of the branchial cartilages (arrow). *D* Post-metamorphic froglet, stage 46. Nearly the entire adult complement of bones is present (bone index = 14), and the transformation of cartilage is virtually complete (cartilage index = 12). Scale bar: 1 mm.

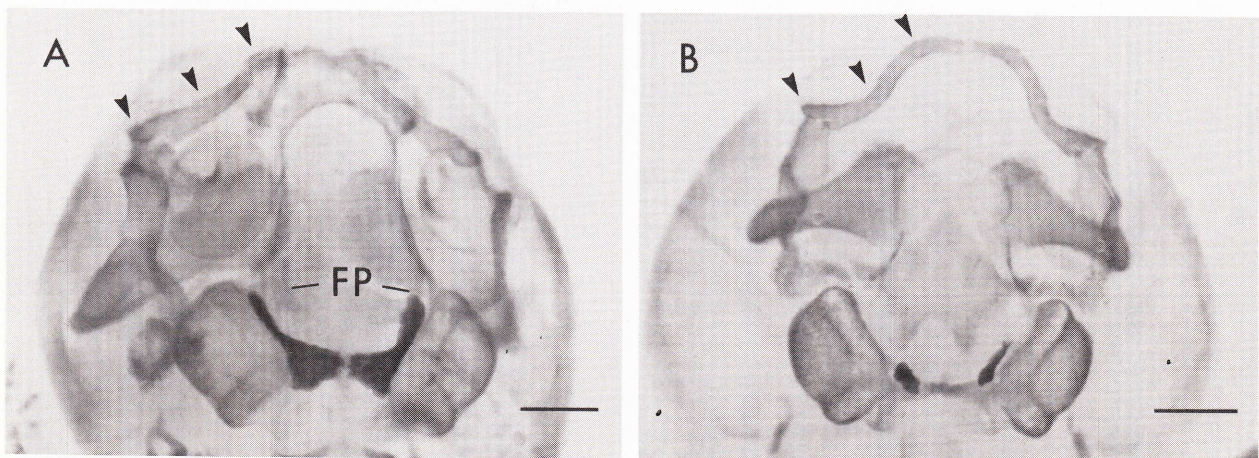


Figure 2. Skulls of T_3 -treated tadpoles recovered after 8 days; dorsal views. Dosage equals $2.5 \mu\text{g}$. *A* Implant stage 32/33. *B* Implant stage 28/29. Cartilage transformation is advanced in both specimens (cartilage index equals 8 and 7, respectively). Note, for example, the virtual absence of larval branchial cartilages and extensive remodeling of the lower jaw (arrows) (cf. fig. 1 A). Slight ossification of frontoparietals (FP) in *A* is the most extensive observed in any T_3 -treated specimen (bone index = 3;

exoccipitals and parasphenoid cannot be seen at this magnification); most specimens lacked bone entirely, as in *B*. Resorption of the calcified endolymphatic sacs (labeled in fig. 1, ES) in response to exogenous T_3 also is characteristic of natural metamorphosis (cf. figs 1, 2). Cranial morphology in all control specimens was virtually unchanged from the larval state (fig. 1 A). Scale bar: 1 mm.

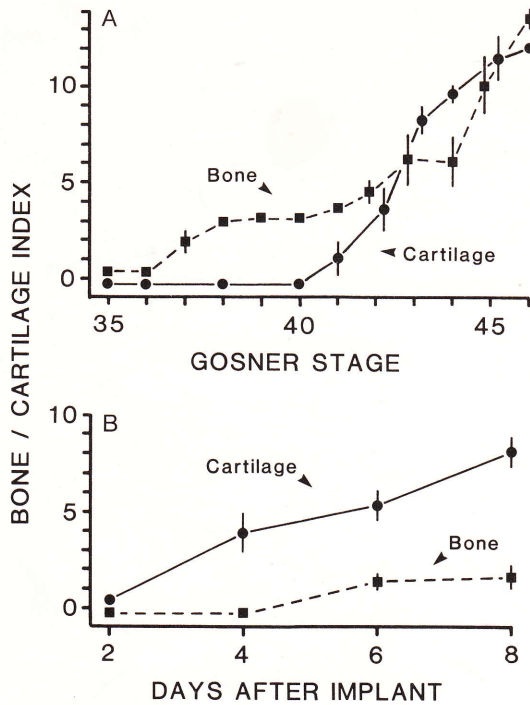


Figure 3. Relative timing of cranial cartilage and bone development. *A* During natural metamorphosis, bone formation initially precedes cartilage transformation (stages 37–40). *B* Following T₃ administration, events in the two tissues are dissociated and the natural sequence is inverted: cartilage transformation now proceeds in advance of bone formation. Ossification, but not cartilage transformation, is most advanced at this treatment regime (dosage, 2.5 µg; implant stage 32/33); the difference between bone and cartilage indexes after 8 days is even larger at lower dosages and earlier implant stages (table). Values denote means ± 2 SE.

the necessity to consider both local and systemic factors in analyses of cranial development and evolution¹².

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