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Type II collagen distribution during cranial development in *Xenopus laevis*

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Abstract. Epithelially expressed type II collagen is thought to play a prominent role in the embryonic patterning and differentiation of the vertebrate skull, primarily on the basis of data derived from amniotes. We describe the spatiotemporal distribution of type II collagen in the embryonic head of the African clawed frog, *Xenopus laevis*, using whole-mount and serial-section immunohistochemical analysis. We studied embryos spanning Nieuwkoop and Faber (1967) stages 21–39, a period including cranial neural crest cell migration and ending immediately before the onset of neurocranial chondrogenesis. *Xenopus* displays a transient expression of type II collagen beginning at least as early as stage 21; staining is most intense and widespread at stages 33/34 and 35/36 and subsequently diminishes. Collagen-positive areas include the ventrolateral surface of the brain, sensory vesicles, notochord, oropharynx, and integument. This expression pattern is similar, but not identical, to that reported for the mouse and two bird species (Japanese quail, domestic fowl); thus epithelially expressed type II collagen appears to be a phylogenetically widespread feature of vertebrate cranial development. Consistent with the proposed role of type II collagen in mediating neurocranial differentiation, most collagen-positive areas lie adjacent to subsequent sites of chondrogenesis in the neurocranium but not the visceral skeleton. However, much of the collagen is expressed after the migration of cranial neural crest, including presumptive chondrogenic crest, seemingly too late to pattern the neurocranium by entrapment of these migrating cells.

Key words: Amphibia – Type II collagen – Prepattern – Cartilage – Skull

Introduction

Despite intense study over the last 100 years, the mechanisms underlying the development of the vertebrate skull remain poorly known (Hall and Hanken 1985; Thorogood and Tickle 1988). One focus of contemporary studies is the degree to which the extracellular environment may mediate the patterning and/or differentiation of cranial skeletogenic cells (Thorogood 1987, 1988). Extracellular matrix components that may play a patterning role during cranial morphogenesis include type IV collagen (Xu et al. 1990), keratan sulfate-containing proteoglycans (Heath and Thorogood 1989), and especially epithelially expressed type II collagen (CII).

While CII is typical of cartilage in the adult, it has a wider embryonic distribution, including expression by epithelial cells. The procollagen peptide of epithelially expressed CII is translated from an mRNA containing an additional exon not found in cartilage CII mRNA. It is unknown, however, how this difference affects synthesis or regulation of expression of CII (Nah and Upholt 1991; Ryan and Sandell 1990). Prior to chondrogenesis, epithelially expressed CII is transiently expressed at times that coincide with matrix-mediated tissue interactions involved in promoting chondrogenesis, and it is largely localized at sites within the head where neurocranial cartilages will later form (Croucher and Tickle 1989; Fitch et al. 1989; Thorogood et al. 1986; Wood et al. 1991). Thus, it has been proposed that epithelially expressed CII may affect cranial patterning by entrapping skeletogenic cells in neurocranial regions, and mediate chondrogenic differentiation of the neurocranial mesenchyme (Thorogood et al. 1986; Thorogood 1988).

To date, the role of CII in neurocranial development has been evaluated only in the laboratory mouse (Mammalia), and in the Japanese quail and domestic fowl (Aves). Previous studies of the embryonic expression of CII in *Xenopus* document mRNA expression and regulation of collagen synthesis with respect to mesodermal specification (Bieker and Yazdani-Buicky 1992; Su et al. 1991), but the potential role of CII in amphibian skull development has not been explored. We focus on the detailed spatiotemporal expression pattern of CII in order to evaluate its role in neurocranial development as proposed by Thorogood (Thorogood et al. 1986; Thorogood

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1988). Accordingly, CII must be expressed at sites where the neurocranium will form in order to affect skeletal differentiation; it must be expressed before neural crest migration is completed in order to pattern neural crest-derived cartilages. Prior to chondrogenesis in *Xenopus*, the distribution of CII generally corresponds to the eventual location of many neurocranial cartilages – a spatial pattern of expression that is similar, although not identical, to that observed in other taxa. However, at least in the region of the presumptive anterior neurocranium, CII expression begins after the end of cranial neural crest cell migration. These data support the view that epithelially expressed CII is a phylogenetically widespread feature of vertebrate cranial development, and are consistent with the proposed role of CII in mediating neurocranial differentiation. However, they do not corroborate the hypothesized role in neurocranial patterning by entrapment of migratory neural crest cells.

Materials and methods

Animal care and embryos

Adult *Xenopus laevis* were purchased from Xenopus 1 (Ann Arbor, Mich.) and maintained in large aquaria. Embryos were obtained by artificial fertilization, dejellied in 2% aqueous cysteine hydrochloride (pH 7.9–8.1) and maintained in 20% Ringer's or 10% Holtfreter's solution (Klymkowsky et al. 1987). Embryos were reared at 15–24°C and fixed in Dent fixative [1 part dimethyl sulfoxide (DMSO): 4 parts methanol; Dent et al. 1989] at stages 21–39 (Nieuwkoop and Faber 1967) for at least 24 h before immunohistochemistry.

Immunohistochemistry

The distribution of type II collagen was examined using serial-section and whole-mount immunohistochemical methods (Dent et al. 1989; Klymkowsky and Hanken 1991). Briefly, specimens were bleached in 10% hydrogen peroxide for 1–10 days (the longer time period was necessary to bleach the pigmented retina completely), rinsed in TRIS-buffered saline (TBS), and incubated overnight at room temperature in a monoclonal mouse anti-chicken type II collagen antibody ($II_6B3/15A4$; Linsenmayer and Hendrix 1980) diluted 1:100 in newborn calf serum supplemented with 5% DMSO and 0.1% thimerosal. They were then washed thoroughly and incubated overnight in HRP-conjugated goat anti-mouse antibody (Bio-Rad) diluted 1:1,000 in supplemented serum. Specimens were washed again, then reacted with 0.5 mg/ml diaminobenzidine (Sigma, St. Louis, Mo.) and 0.02% H_2O_2 in TBS for 1–2 h. Specimens were either dehydrated in methanol and cleared in BABB (1 part benzyl alcohol : 2 parts benzyl benzoate) as whole mounts, or dehydrated in ethanol, embedded in Paraplast, cut as 6-μm serial sections, and counterstained with Eosin Y (Table 1).

Specificity of the type II collagen antibody was evaluated by examining staining in the absence of the primary antibody and by comparing the staining pattern of anti-CII with those of two other mouse monoclonal antibodies: anti-fast skeletal muscle myosin antibody F59, and the anti-acetylated α-tubulin antibody 6–11 B-1 (Chu and Klymkowsky 1989). In the absence of primary antibody, there was no non-specific staining, except for occasional staining of the superficial surface of the epidermis and in the lumens of the optic vesicles and brain ventricles (as described by Dent et al. 1989). The staining patterns with anti-myosin and anti-tubulin were distinctly different from anti-CII.

Microscopy and photomicrography

Specimens were examined using either a Wild M8 (whole mounts) or a LEITZ Dialux 22 (serial sections) microscope. Photographs were taken on Kodak T-Max film (EI 100 or 400) using a Wild MPS55 Photoautomat and tungsten illumination with a Wratten no. 11 filter.

Results

During embryogenesis of *Xenopus laevis*, type II collagen (CII) is localized in several regions within the head: notochord, brain, special sensory vesicles, oropharynx, and integument. Temporal changes in the distribution of CII are described below by region, based primarily on analysis of serial sections and supplemented with observations of whole mounts. Often, only a few of the specimens of a given stage were stained at the earliest appearance of CII

Table 1. Developmental stages and number of specimens examined. Key features of cranial neural crest migration and chondrogenic differentiation are taken from Nieuwkoop and Faber (1967), and Sadaghiani and Thiébaud (1987)

Stage	Key features	Serial sections	Whole mounts
21	Late neural tube, crest migration underway	1	3
22	Migration of cranial neural crest	5	2
23		3	3
24		3	2
25	Neural crest in neurocranial region	4	2
26	Neural crest penetrating visceral arches	3	7
27		2	3
28		5	5
29/30		4	3
31		7	2
32	Crest invading optic and auditory vesicles	5	2
33/34		8	5
35/36	Pre-cartilaginous anlage in visceral arches	4	1
37/38		7	2
39		5	1

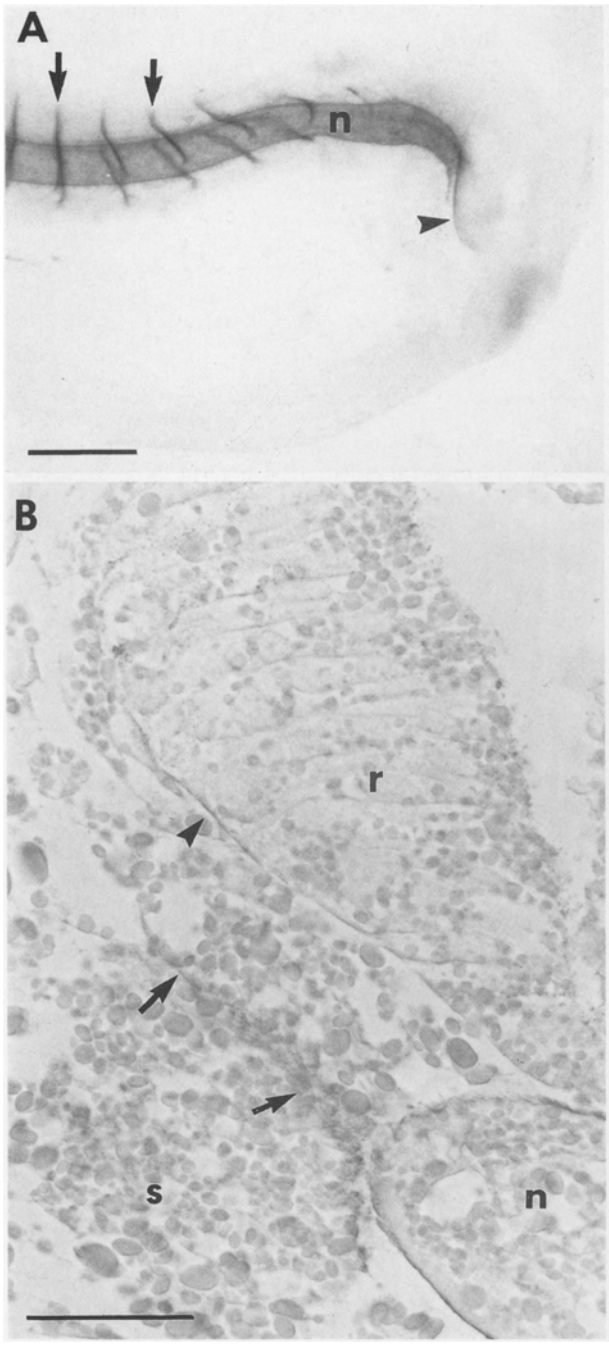

Fig. 1. A Lateral view of the head of a stage 26 whole-mount embryo stained for CII. In this and all subsequent whole mounts, anterior is to the right. Strong staining surrounds the notochord (*n*) and marks the intersegmental boundaries of the somites (*arrows*). Faint staining is detectable on the ventrolateral surface of the brain (*arrowhead*). *Bar* 250 μm. **B** Transverse section through the rhombencephalon (*r*) of a stage 26 embryo. The sheath of the notochord (*n*) is well stained; faint staining also lines the ventrolateral surface of the rhombencephalon (*arrowhead*) and an intersegmental boundary (*arrows*) of an adjacent somite (*s*). *Bar* 50 μm

in a particular structure. This may be due to variability in the staining procedure, but more probably reflects developmental variation within a stage. By the subsequent stage, most or all of the specimens showed staining.

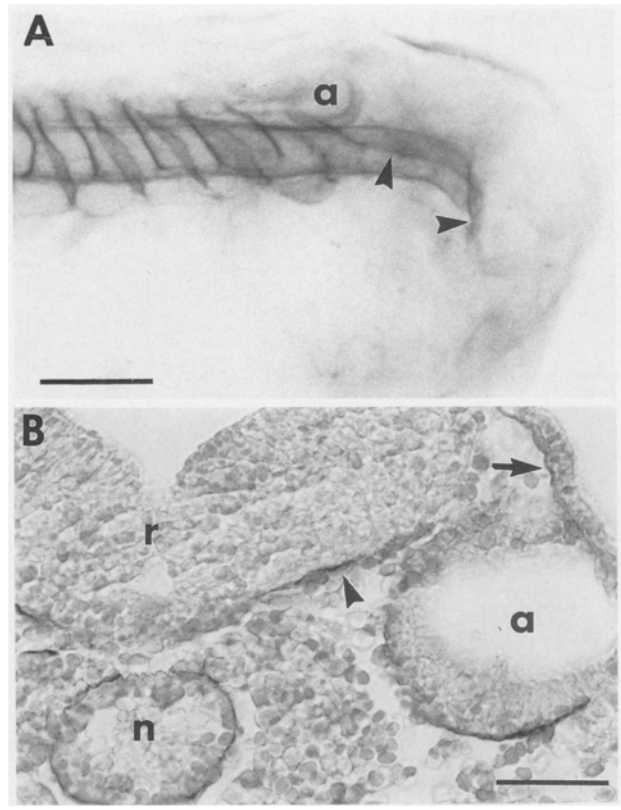

Fig. 2. A Lateral view of the head of a stage 32 whole-mount embryo. Staining of the notochord, intersegmental boundaries, auditory vesicles (*a*), and ventrolateral surface of the brain (*arrowheads*) is visible. *Bar* 250 μm. **B** Transverse section through the rhombencephalon (*r*) of a stage 32 embryo. CII surrounds the notochord (*n*), the ventrolateral surface of the rhombencephalon (*arrowhead*), the auditory vesicle (*a*), and the dermis (*arrow*). *Bar* 50 μm

Notochord

CII is detectable in the head at the earliest stage examined, stage 21, when faint staining is found on the sheath of the rostral notochord. Notochordal staining is more intense in the trunk, where it also lines the intersegmental boundaries and the medial walls of the somites. At stage 22, notochordal staining generally is more intense, although still faint rostrally; somitic staining is also more intense. By stage 26, the anterior notochord is strongly stained from the rostral tip caudad into the trunk (Fig. 1). From stages 29/30 to 35/36, the anterior notochord is stained more intensely than in postcranial regions (Figs. 2, 3, 4). At stages 37/38 and 39, only the rostral tip of the notochord stains strongly; weak staining occurs caudally from the level of the rhombencephalon (Fig. 5).

Brain

CII staining of the brain occurs on the basal surface of the neural epithelium. Stain is first visible at stage 22, when it lines the ventrolateral surface of the rhombencephalon. At stage 23, separate staining is also visible on the ventrolateral surface of the rostral mesencephalon.

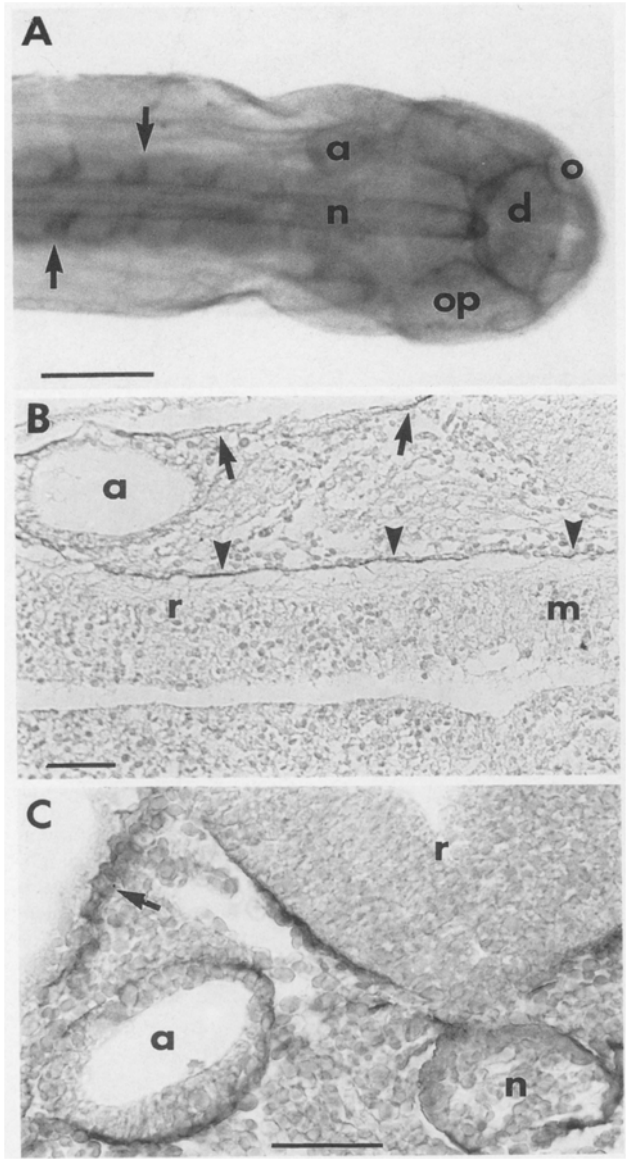

Fig. 3. A Dorsal view of a stage 33/34 whole-mount embryo. Stain lines the intersegmental boundaries (*arrows*), the notochord (*n*), the ventrally deflected diencephalon (*d*), the auditory vesicles (*a*) and the olfactory placodes (*o*). The pigmented retina along the medial edge of each optic vesicle (*op*) is also visible. *Bar* 250 μm. **B** Frontal section of the left side of a stage 33/34 embryo (anterior is to the right; lateral is at the top). Continuous CII staining (*arrowheads*) lines the rhombencephalon (*r*) and mesencephalon (*m*). Staining of the dermis (*arrows*) is also visible. The auditory vesicle (*a*) is not well stained in this dorsal plane of section. *Bar* 50 μm. **C** Transverse section of a stage 33/34 embryo. Staining lines the notochord (*n*), the dermis (*arrow*), the ventrolateral surface of the rhombencephalon (*r*), and the auditory capsule (*a*), primarily on the ventral and medial walls. *Bar* 50 μm

By stage 25, CII lines the lateral surfaces of the posterior diencephalon and the ventrolateral surface of the spinal cord. All stage 26 specimens have diencephalic, mesencephalic, rhombencephalic, and spinal cord staining; this is continuous in the diencephalon and mesencephalon. By stage 29/30, specimens have continuous staining underlying the brain from diencephalon through rhomben-

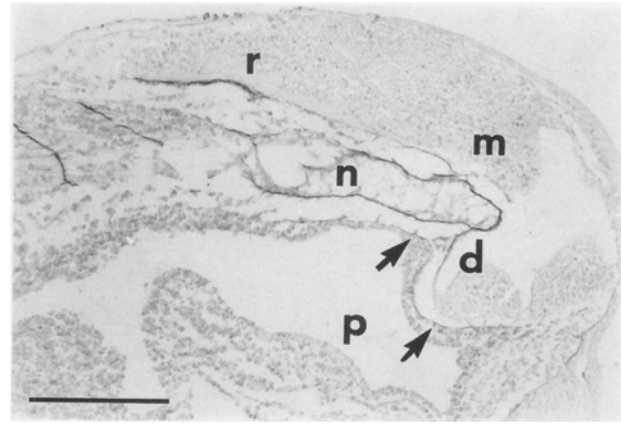

Fig. 4. Sagittal section through the head of a stage 35/36 embryo. Stain lines the ventrolateral surface of the brain (*d*, diencephalon; *m*, mesencephalon; *r*, rhombencephalon), notochord (*n*), and dorsal epithelium (*arrows*) of the pharynx (*p*). *Bar* 250 μm

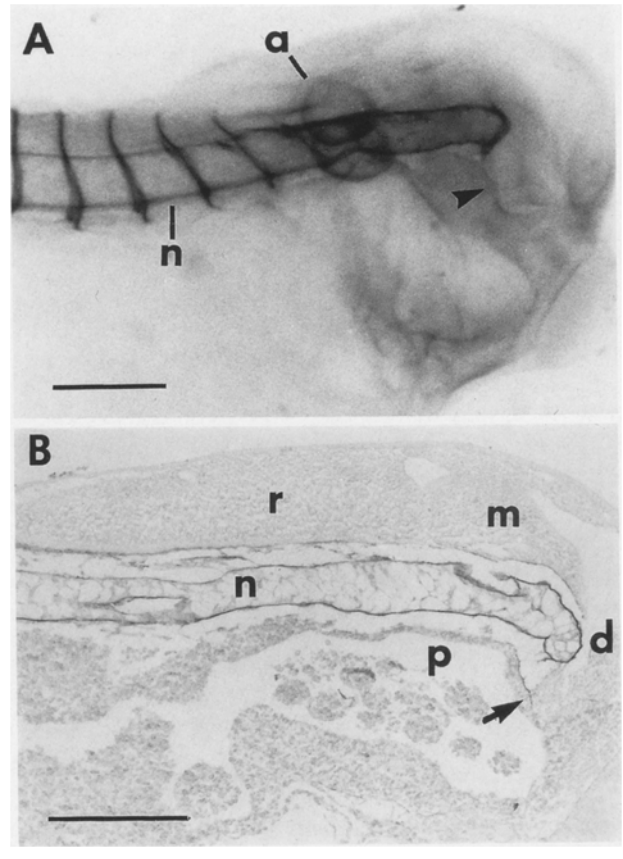

Fig. 5. A Lateral view of a stage 37/38 whole-mount embryo. Staining of the notochord (*n*) is strong at its rostral tip, but faint posteriorly. Although the auditory vesicles (*a*) remain strongly stained, staining of the brain is much fainter (*arrowhead*) than in earlier stages. *Bar* 250 μm. **B** Sagittal section through the head of a stage 37/38 embryo. The notochord (*n*) and the anterodorsal surface (*arrow*) of the pharynx (*p*) are stained, but staining of the ventrolateral surface of the brain is faint and patchy (*d*, diencephalon; *m*, mesencephalon; *r*, rhombencephalon) Pharyngeal stain extends more posteriorly in other sections of this specimen (not illustrated). *Bar* 250 μm

cephalon. At stage 31, faint staining is found on the ventral surface of the diencephalon, continuous with the stain on the lateral surfaces. The mesencephalic stain is stronger and extends further caudad, but it is not continuous with the rhombencephalic stain.

By stage 32, continuous staining extends from the posterior region of the forebrain onto the spinal cord (Fig. 2A). Staining is most intense on the lateral surfaces of the diencephalon and the mesencephalon rostral to the notochord. Stain is also present on the ventral surface of the diencephalon, and is continuous with that on the lateral surfaces caudal to the optic chiasma. Mesencephalic stain extends dorsally, lining the ventrolateral two-thirds of the midbrain. Rhombencephalic stain lines only the ventral surface.

At stage 33/34, CII staining is both more intense and more extensive, covering much of the ventrolateral surface of the brain (Fig. 3). CII is present on the lateral walls of the telencephalon, continuous with the diencephalic stain. The ventral and lateral diencephalic stain is continuous cauda to the optic nerve (n. II). There is strong staining of the ventrolateral surfaces of the mesencephalon and the rhombencephalon. However, staining is faint along the ventral midline immediately dorsal to the notochord; it is absent in places along the rhombencephalon caudal to the statoacoustic nerve (n. VIII). This staining pattern is retained through stage 35/36 (Fig. 4).

Staining weakens at stage 37/38. At this stage, telencephalic stain is reduced to a faint patch on the lateral surfaces surrounding the olfactory tract. There is faint staining on the lateral and ventral walls of the diencephalon at the level of the optic chiasma, but the posterior diencephalon, deflected ventral to the notochord, is unstained. The mesencephalic stain is still continuous with the lateral diencephalic stain, but it ends midway along the mesencephalon. The faint rhombencephalic stain is no longer continuous with that of the mesencephalon (Fig. 5B); it is strongest at the level of the fourth ventricle, where stain lines the ventrolateral surfaces. This pattern of faint, patchy staining of the brain is retained at stage 39 (Fig. 6).

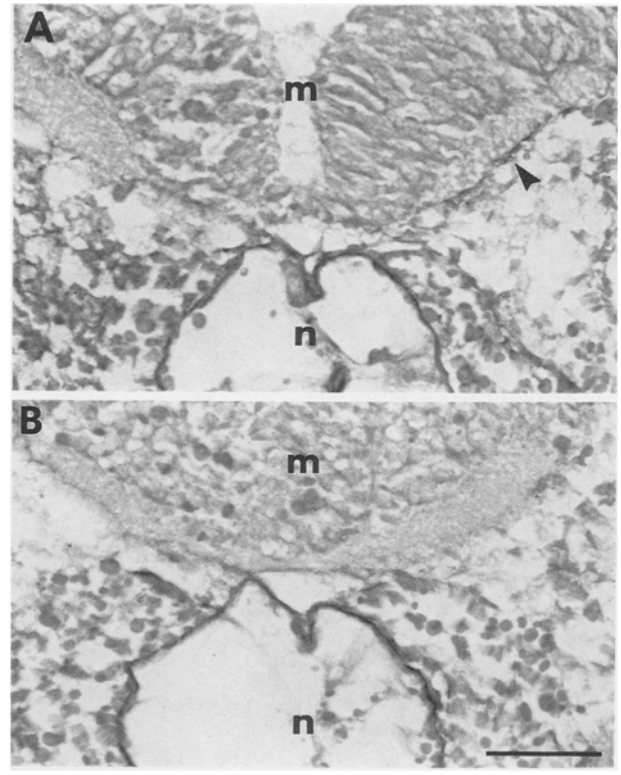

Fig. 6A,B. Transverse sections of a stage 39 embryo at two different positions along the mesencephalon (*m*). While the notochord (*n*) is stained at both levels, brain staining is patchy, being present rostrally (*arrowhead* in **A**), but not caudally (**B**). *Bar* 50 μm

Sense organs

Eye. CII associated with the developing special sense organs is first seen at stage 31, when faint stain is found along the posteromedial wall of the outer layer of the developing optic cup. This faint stain is retained in all subsequent stages examined.

Olfactory organ. CII is first detectable in the developing olfactory organ at stage 32, when it lines the ventromedial surface of the caudal two-thirds of the olfactory placode. Placodal staining is more extensive at stage 33/34 (Fig. 3A), and by stage 35/36 the entire placode is stained except for the surface of the invaginating nasal pit. This staining pattern remains in subsequent stages, although it is faint at stage 39.

Auditory organ. CII is first seen in the auditory vesicle at stage 32 (Fig. 2), when strong stain lines all but the lateral wall of the vesicle. By stage 33/34, stain surrounds the entire vesicle, except for the lateral surface adjacent to the dermis and patches on the dorsal surface (Fig. 3). This staining pattern is maintained through stage 39.

Oropharynx

Pharyngeal staining begins at stage 32, with faint stain limited to the basal surface of the dorsal epithelium caudal to the stomodeal-hypophyseal anlage. By stage 33/34, CII lines the dorsal pharyngeal epithelium between the first (mandibular) and third visceral pouches. Stain also separates the ventrolateral wall of the pharynx and the adjacent mesenchyme.

At stage 35/36, stain lines the dorsolateral epithelium of the oral evagination from its rostral end to the first visceral pouch, where the lateral stain is broken by the evagination of the pouch. Stain on the dorsal epithelium extends caudally. At the level of the first pouch it covers the dorsal half of the pharyngeal epithelium (Fig. 4); by the third pouch, it is restricted to the dorsal one-third of the pharynx (and ends at this level in one specimen). Caudal to the third pouch, the dorsal stain becomes restricted to a narrow strip roughly twice the diameter of the notochord. It is absent caudal to the fifth visceral pouch. There is stain on the ventral pharyngeal epithelium between the first and second pouches. Additionally, very faint stain lines the surface (incipient perichondrium) of

the pre-cartilaginous condensations of the hyoid and two subsequent branchial arches.

Staining is less intense at stage 37/38, present on only the dorsal and ventral surfaces of the pharyngeal epithelium (Fig. 5B). The dorsal stain is restricted to a narrow medial band at the level of the second arch; the ventral stain disappears caudal to the first arch. Faint, diffuse stain is seen in the ventral and ventrolateral mesenchyme of the first arch – the condensations of the infrarostral and Meckel's cartilages. Stain lines the perimeter of the ceratohyal and first ceratobranchial condensations in the second and third arches, respectively. Stage 39 is similar to stage 37/38, although the dorsal pharyngeal stain is absent caudal to the first arch.

Integument

The early tadpole skin is bilayered, with an outer, epithelial layer and an inner composite layer comprising the stratum germinativum of the epidermis plus investing cutis cells of the dermis (Nieuwkoop and Faber 1967). CII is localized to the basal surface of this inner layer. Stain is first visible at stage 32, lining the lateral integument beginning rostral to the optic vesicles and continuing caudad (Fig. 2B). Midway through the level of the optic vesicles, additional staining is found dorsolaterally. Caudal to the optic vesicles the stain is continuous along the entire inner dorsolateral surface of the integument. At stage 33/34, stain extends rostrad to the level of the olfactory vesicles. Separate staining of the ventral integument is found extending from the level of the optic vesicle to the level of the developing heart. Stage 35/36 specimens have stain on the dorsolateral and ventral integument from the rostral end of the head. At the level of the rhombencephalon, stain rings the entire dorsolateral integument (including that dorsal to the brain). At stages 37/38 and 39, stain is found both ventrally and lining the entire dorsolateral integument from the level of the olfactory vesicles caudad; it is interrupted only by contact with the optic vesicles.

Discussion

Epithelially expressed CII, alone or in combination with other factors, has been proposed as a template that mediates the patterning and differentiation of the cartilaginous neurocranium (Croucher and Tickle 1989; Heath and Thorogood 1989; Thorogood 1987; 1988; Thorogood et al. 1986; Wood et al. 1991). According to this "Flypaper Model," CII lining the neural epithelium and sensory vesicles entraps neurocranial skeletogenic mesenchyme derived from both paraxial mesoderm and neural crest (Hall and Hörstadius 1988; Sadaghiani and Thiébaud 1987; Seufert and Hall 1990), and supports its chondrogenic differentiation (Thorogood 1988; Thorogood et al. 1986). Support for this model of cranial development includes (1) the epithelial derivation of this CII (Bieker and Yazdani-Buicky 1992; Cheah et al. 1991; Su et al. 1991); (2) the transient expression of CII coincident with extracellular matrix-mediated interactions that affect chondrogenesis; (3) the localization of CII mainly in regions where neurocranial cartilages will later form; (4) the presence of a collagen receptor, Anchorin CII (von der Mark et al. 1986), on a subpopulation of cranial neural crest cells (Thorogood 1988) and (5) the demonstrated role of CII in supporting chondrogenesis in postcranial somites both in vitro (Kosher and Church 1975; Lash and Vasan 1978) and in vivo (Hall 1983; Vasan 1987). Originally described in chicken and quail, and subsequently in mice, this CII prepattern has been advanced as a general model for neurocranial development in all vertebrates (Thorogood 1988).

Reports of epithelially expressed CII in cranial regions of *Xenopus* (Bieker and Yazdani-Buicky 1992; Su et al. 1991; present study), extend the phylogenetic distribution of this ECM component to another vertebrate class, and support the view that this expression is an evolutionarily conservative and phylogenetically widespread feature of vertebrate cranial development. Moreover, the spatial distribution of CII in *Xenopus* is consistent with its purported role in mediating neurocranial differentiation, despite some CII expression in non-chondrogenic regions. However, we find that much of the CII expression occurs after cranial neural crest migration, seemingly too late to pattern these cells via entrapment in neurocranial regions.

Expression of CII in Xenopus

The spatiotemporal distribution of epithelially expressed type II collagen in the embryonic head of *Xenopus laevis* is summarized in Fig. 7. In Fig. 8, CII distribution along the brain and sensory vesicles is correlated with cranial neural crest migration and neurocranial cartilages. Consistent with its purported role in mediating chondrogenic differentiation (Thorogood 1988; Thorogood et al. 1986), the spatial distribution of much of the CII closely matches that of the larval neurocranium, which begins chondrogenesis at stage 40 (Nieuwkoop and Faber 1967). Staining of the anterior notochord, the intersegmental boundaries of the cranial somites, the midbrain, hindbrain, and auditory vesicles lies adjacent to sites where the posterior neurocranium will form (cranial trabeculae, basal plate, parachordals, and auditory capsules; Trueb and Hanken 1992; Fig. 8). Staining of the forebrain and oropharynx is adjacent to the future location of the anterior neurocranium, particularly the suprarostral and trabecular plates (Figs. 8, 9). As much of the posterior neurocranium is formed from paraxial mesoderm, this pattern of expression may indicate interactions between CII and skeletogenic mesenchyme that parallel those involved in somitic chondrogenesis (Hall 1983; Vasan 1987). Although the anterior neurocranium is largely derived from neural crest (Sadaghiani and Thiébaud 1987, Seufert and Hall 1990), interactions with CII may also be involved in its chondrogenic differentiation.

Unlike the situation in the neurocranium, there is little epithelially expressed CII that corresponds to the visceral skeleton. Pharyngeal CII is limited to the dorsal epitheli-

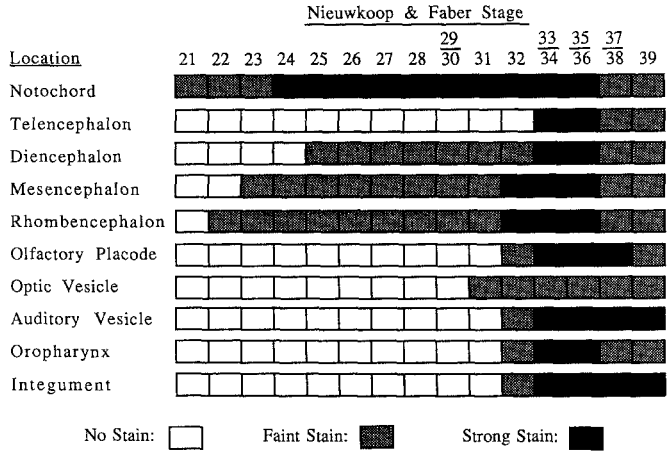

Fig. 7. Summary of the cranial distribution of type II collagen in embryonic *Xenopus laevis* showing two distinct phases of expression. Initially, CII is expressed on the notochord and, faintly, on the ventrolateral surfaces of the diencephalon, mesencephalon, and rhombencephalon. A second phase begins at stage 31, when additional CII is expressed in the telencephalon, sensory placodes, oropharynx, and integument. The distribution and intensity of CII expression reach a maximum between stages 33/34 and 35/36. The intensity of epithelial staining then declines in all regions except the auditory vesicles and integument

um, whereas visceral-arch cartilages differentiate primarily in ventral and lateral regions. The lone exception is faint staining of the ventral pharyngeal epithelium of the hyoid arch in which the ceratohyal cartilages will form.

Although the spatial distribution of much of the CII is consistent with its proposed role in neurocranial differentiation, our data do not corroborate a role for CII in patterning neurocranial cartilages by entrapping migrating skeletogenic mesenchyme, at least that derived from the neural crest. Much of the CII expression occurs after neural crest has migrated into the prospective neurocranial region. In *Xenopus*, cranial neural crest cell migration begins at stage 19 (Nieuwkoop and Faber 1967; Sadaghiani and Thiébaud 1987). Cells that contribute to the anterior neurocranium have arrived in this region by stage 25, yet expression of CII in the telencephalon and pharynx does not occur until six stages later. However, this observation does not preclude a role in cranial patterning by other mechanisms (Thorogood 1988; Thorogood et al. 1986). For example, by its possible role in mediating chondrogenic differentiation of skeletogenic mesenchyme, CII may influence where, as well as when, cranial cartilages form.

Expression of CII at non-chondrogenic sites (e.g., integument, olfactory and optic vesicles) represents an additional problem for the Flypaper Model. Several recent studies have reported embryonic expression of CII at non-chondrogenic sites within the head of other vertebrates, similar to our findings in *Xenopus* (Cheah et al. 1991; Fitch et al. 1989; Thorogood et al. 1986; Wood et al. 1991), as well as in the trunk (Kosher and Solursh 1989). However, the distribution of CII need not precisely match the subsequent location of cranial cartilages (Heath and Thorogood 1989; Wood et al. 1991). CII may affect cranial development only when mesenchymal cells with the ability to recognize CII are present, e.g., a subpopulation of cranial neural crest with the Anchorin CII receptor (Thorogood 1988; Wood et al. 1991), and/or when codistributed with other matrix components. For example, keratan sulfate-containing proteoglycans are codistributed with CII at the sites of the future olfactory and otic capsules in birds (Croucher and Tickle 1989; Heath and Thorogood 1989) and they are also involved in somite chondrogenesis in vitro (Lash and Vasan 1978). The embryonic distribution of keratan sulfate-containing proteoglycans in the head of *Xenopus* is not known. Studies of the distribution of this and other molecules, e.g., the Anchorin CII receptor, may be necessary before the role of CII in cranial development in amphibians can be fully evaluated.

Phylogenetic comparisons

A consistent pattern of CII expression, documented in a wide variety of taxa, is necessary although not sufficient to support a general role for CII in vertebrate cranial development (Thorogood 1988). All species examined so far – mouse, chicken, quail, and frog – show a similar distribution of CII, localized primarily on the ventrolateral surface of the developing brain, as well as in the notochord, special sensory vesicles, and integument. Assuming that the expression patterns in each species are characteristic of their respective higher taxa, this distribution of CII is a widespread and presumably evolutionarily conservative feature of vertebrate cranial development. The major difference among these species concerns pharyngeal staining, detected only in laboratory mice (Wood et al. 1991) and *Xenopus*. This additional staining need not suggest an additional function, such as patterning the visceral skeleton. For example, in *Xenopus*, restriction of CII primarily to the dorsal pharyngeal epithelium is consistent with a role in mediating the development of the anterior neurocranium.

Despite generally similar patterns of CII expression in the species studied, there are major interspecific differences in gross neurocranial morphology. For example, the three sensory vesicles are stained in all species studied, yet only in the birds do cartilaginous capsules form around all three sense organs. *Xenopus* and the mouse lack optic cartilages and, in *Xenopus*, nasal cartilages do not form until metamorphosis. (The very faint CII expression at the optic vesicle in *Xenopus* may reflect its lack of function in chondrogenesis.) It is tempting to speculate that the shared pattern of CII expression reflects the form of the primitive vertebrate skull, which included cartilaginous olfactory and optic, as well as otic, capsules (Maisey 1986). Thus, to the extent that CII mediates cranial development, it may affect only general features of the neurocranium, and not specify features that distinguish individual taxa.

This study has addressed the proposed role of a single ECM component, type II collagen, in mediating skull development. Other recent studies have emphasized the possible patterning role of factors intrinsic to the skeleto-

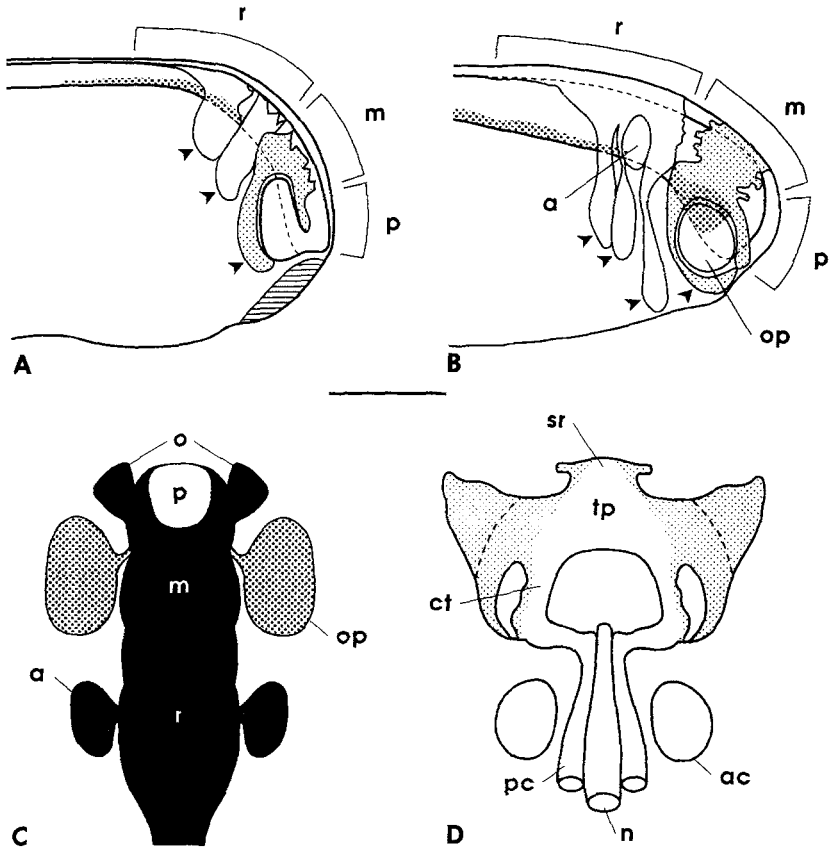

Fig. 8A–D. Schematic diagrams of three embryonic stages depicting CII distribution along the brain and sensory vesicles, cranial neural crest migration, and neurocranial cartilages. **A** Stage 22, lateral view. Cranial neural crest migration is under way (*arrowheads*), including the mandibular arch crest (*light stipple*) which contributes to the anterior neurocranium. CII is present only as faint patches (*bold stipple*) along the ventrolateral rhombencephalon (*r*). It is not detected along the mesencephalon (*m*) or prosencephalon (*p*). **B** Stage 26, lateral view. Streams of cranial crest (*arrowheads*) are penetrating the visceral arches. Mandibular arch crest (*light stipple*) has migrated into the region of the future anterior neurocranium. CII (*bold stipple*) covers the ventrolateral surfaces of the rhombencephalon, mesencephalon and diencephalic portion of the prosencephalon. It is absent from the pharynx and sensory vesicles (*a*, auditory vesicle; *op*, optic vesicle). Ventral view of the brain and sensory vesicles (**C**), and dorsal view of the neurocranium (**D**), after neural crest migration and expression of epithelial collagen, ca. stage 40. The maximum distribution of CII, present at stages 33/34 to 35/36, is shown on the brain and sensory vesicles (*bold stipple*, faint stain; *dark*, strong stain). Prosencephalic (*p*) and olfactory placode (*o*) staining correspond to the level of the suprarostral plate (*sr*) and the trabecular plate (*tp*), which are derived from both neural crest (*light stipple*) and paraxial mesoderm (*unshaded*). Mesencephalic (*m*) and rhombencephalic (*r*) staining correspond to the level of the cranial trabeculae (*ct*), basal plate (not shown, it lies ventral to the rostral tip of the notochord), and parachordal cartilages (*pc*), which are derived primarily from paraxial mesoderm. Auditory vesicle (*a*) staining corresponds to the mesodermally derived auditory capsule (*ac*). *n*, Notochord; *op*, optic vesicle. *Bar* 100 μm. Illustrations are modified from Sadaghiani and Thiébaud (1987; Figs. 14, 15), Trueb and Hanken (1992; Fig. 2), and personal observations

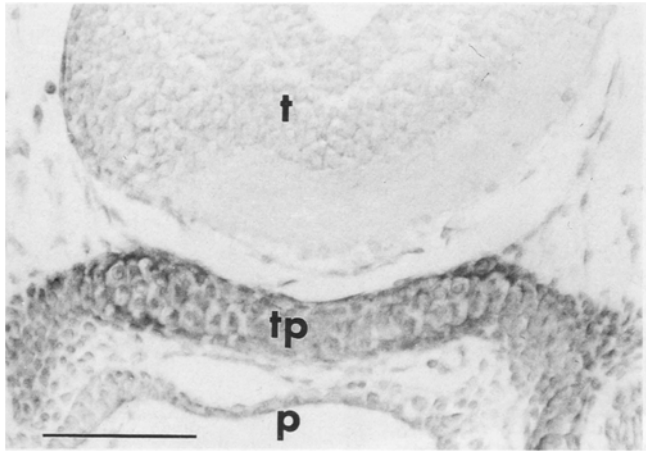

Fig. 9. Transverse section through the rostral region of the head of a stage 42 embryo. The cartilaginous trabecular plate (*tp*) is in close proximity to the epithelia of the pharynx (*p*) ventrally and the telencephalon (*t*) dorsally, both of which earlier express CII. *Bar* 100 μm

genic cells, as observed, for example, in neural crest grafting experiments (Noden 1983, 1988, 1991) and expression patterns of *Hox*-2 genes (Hunt et al. 1991a,b; Nieto et al. 1992). As cranial development is affected by intrinsic and extrinsic factors, a prominent challenge to future studies is to assess the relative contributions of both these influences on cranial form, and the extent to which they vary according to skeletal cell lineage, developmental stage, regional cranial components, and taxon.

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