MECHANICAL DEVELOPMENT OF THE NOTOCHORD IN *XENOPUS* EARLY TAIL-BUD EMBRYOS

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INTRODUCTION

How is a developing embryo shaped by the mechanical behavior of groups of cells? We address this question by focusing on a particular morphogenetic event: the elongation and straightening of the frog embryo during the late neurula and early tailbud stages (Fig. 1A).

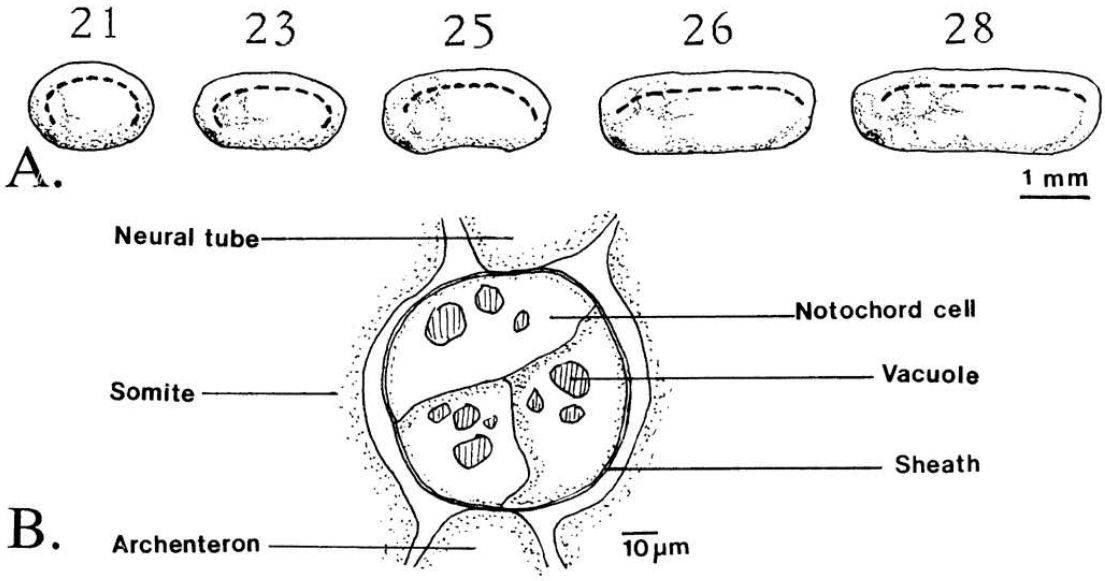

Fig. 1. A. Profiles of *Xenopus* embryos at various stages of development (indicated by numbers above diagrams). The position of the notochord within each embryo is indicated by the dashed line. Anterior is to the left. B. Diagram of a cross-section of the notochord and neighboring tissues of a stage 25 embryo.

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Our first step in unraveling the mechanisms responsible for this shape change in the embryo is to analyze the biomechanics of those components of the system that are likely to play important roles in the process. The component on which we focus in this paper is the notochord (Fig. 1). Although a number of studies have reported that removal of the notochord results in shorter embryos (Kitchen 1938; 1949; Lehman and Ris 1938; Horstadius 1944; Niewkoop 1946; Mookerjee 1953; Bijtel 1958), the mechanical role of the notochord in embryo elongation is controversial (e.g. Bijtel 1958; Malacinski and Youn 1982).

We have used two approaches to discover what the notochord does during the elongation and straightening of stage 20 - 28 (Niewkoop and Faber 1967) embryos of the frog *Xenopus laevis*. 1) We quantified how the morphology and the mechanical properties of the notochord change during these stages. 2) We conducted a series of manipulative experiments to sort out the roles of various components of the notochord, and to test hypotheses about particular mechanisms that might produce the changes we observe during these stages of notochord development. Most of the data on which the present analysis is based (summarized in Tables 1 and 2), as well as details of experimental protocol, are presented in Adams, Keller and Koehl (in prep.).

At the stages of development on which we are focusing, the notochord is composed of a sheath surrounding a stack of cells (Fig. 1B) that are flattened along their anterior-posterior axes and that are shaped like slices of pizza (Keller *et al.* 1989). Vacuoles, probably containing secreted glycosaminoglycans (GAG's, see Waddington and Perry 1962) within these cells begin enlarging at stage 23 (Niewkoop and Faber 1967).

HOW DOES THE MORPHOLOGY OF THE NOTOCHORD CHANGE?

The profile of the notochord at stages 21 - 28 is indicated in Fig. 1A, and its gross morphology is quantified in Table 1. The volume of the notochord increases about three-fold during this period of development. Significant lengthening of the notochord begins after stage 23, the stage

TABLE 1. *Xenopus laevis* Notochord Development

	stage 21	stage 23	stage 25	stage 26	stage 28
			[mean (S.D., n)]		
volume (m^3x10^{-11})	1.5 (0.6,10)	* 2.1 (0.6,10)	* 2.7 (1.1,10)	* 4.0 (1.8,10)	* 4.9 (1.3,10)
length ($mx10^{-3}$)	2.06 (0.24,10)	2.06 (0.18,10)	* 2.36 (0.27,10)	* 2.80 (0.22,10)	* 3.23 (0.33,10)
SI+	1.4 (0.2,10)	1.6 (0.2,10)	* 2.4 (0.5,10)	* 3.6 (1.4,10)	* 6.6 (3.2,10)
EI (Nm^2x10^{-14})	0.72 (0.35,5)	-	* 4.49 (1.89,5)	12.62 (8.29,5)	14.78 (16.51,5)
sheath fiber density (#/20μm)	15 (2,5)	* 26 (8,5)	* 35 (13,5)	-	23 (7,5)
L/D	21.8 (4.2,10)	* 18.4 (2.6,10)	* 20.5 (2.7,10)	21.8 (3.0,10)	23.6 (3.8,10)
sheathless in MNT, length ($mx10^{-3}$)	4.44 (0.41,5) @	4.31 (0.14,3) @	6.13 (0.78,2) @	* {2.42} {(0.34,3)} {stage 18}	
sheathless in MNT, diameter ($mx10^{-3}$)	0.06 (0.01,4)	0.06 (0.02,3)	0.07 (0.01,2)	-	-

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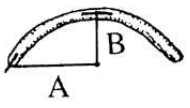
* Significant difference between stages to the right and left of the *, Kruskal-Wallis, Student Newman- Keuls, $p < 0.05$

@ Significant difference between sheathless notochord in MNT vs. in MNT + 1.0 M sorbitol, Mann-Whitney U, $p < 0.05$

\+ SI = straightness index = A/B



at which vacuolization starts (Niewkoop and Faber 1967). Hence, our data are consistent with the suggestion by Mookerjee *et al.* (1953) that vacuole enlargement may cause the notochord to elongate. The notochord also begins to straighten significantly after stage 23 and there is a significant direct association between the straightness of the notochord and that of the embryo (Kendall Tau, $n = 50$, $p < 0.01$).

HOW DO THE MECHANICAL PROPERTIES OF THE NOTOCHORD CHANGE?

Are the morphological changes in the notochord that occur between stages 21 and 28 accompanied by mechanical changes? Before attempting to answer this question, we must consider which mechanical property of the notochord is biologically important. As illustrated in Fig. 2A, if the notochord is to elongate or straighten, it must push on the surrounding tissues of the embryo. That push will be resisted by an equal and opposite force on the ends of the notochord. We view the notochord as a column bearing a compressive load along its long axis. If the force on such a slender column exceeds a critical value (F_E), the column bends or bows out elastically (i.e. undergoes Euler buckling, Fig. 2B). The higher the F_E of the notochord, the greater the force it can resist or exert on the surrounding tissues without buckling. Expressions for the F_E of columns of various geometries are given in many engineering texts (e.g. Roark and Young 1975; Gere and Timoshenko 1984); F_E is proportional to EI/L^2, where L is the length of the column and EI is its flexural stiffness (i.e. the resistance of a unit length of the column to being bent). Therefore, as the notochord lengthens, its likelihood of buckling should increase a great deal (F_E is inversely proportional to length *squared*) unless its EI is raised substantially to compensate. A column being pushed at its ends might also develop a local kink (i.e. undergo local buckling, Fig. 2B), as will be discussed below.

We used critical buckling force (F_E) as the measure of mechanical performance of the notochord. The flexural stiffness (EI) of notochords excised from embryos at various stages was measured by subjecting the notochords to three-point bending tests, as illustrated in Fig. 2C. Notochord EI increases by an order of magnitude between stages 21

and 28 (Table 1), the significant rise occurring sometime between stages 21 and 25. This drastic rise in EI leads to an increase in the F_E of the notochord, hence the notochord's resistance to Euler buckling increases as it elongates (Fig. 3).

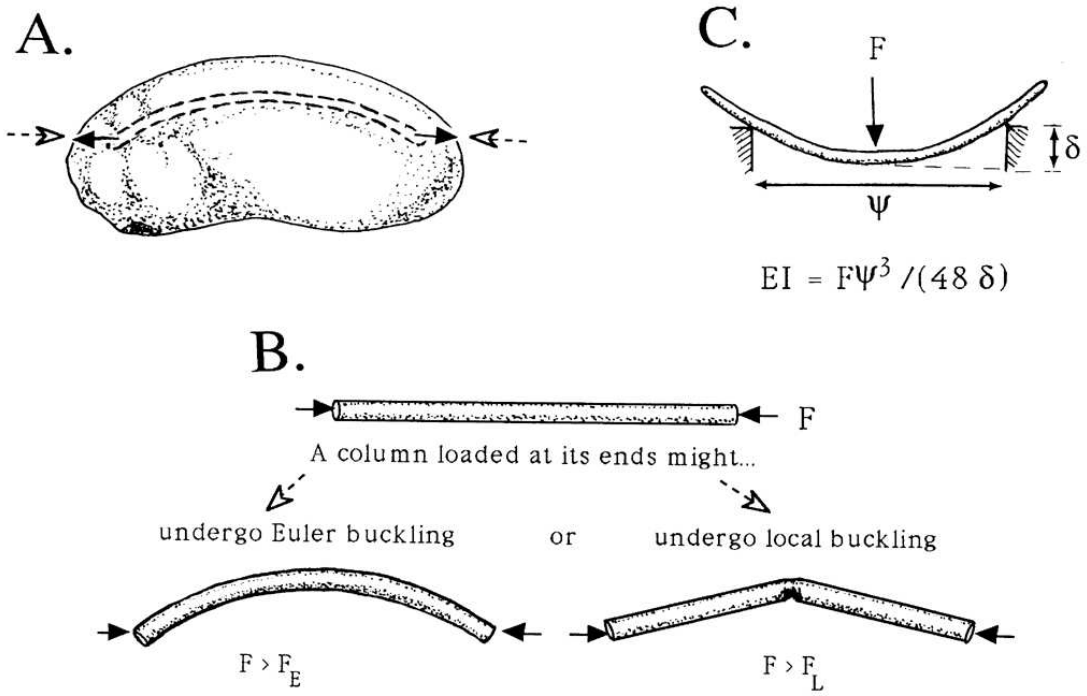


Fig. 2. A. Profile of an embryo, with dashed lines indicating the position within it of the notochord. Solid arrows indicate the force exerted on the surrounding tissues by the elongating or straightening notochord. Open arrows indicate the force imposed on the notochord by the surrounding tissues (unless the surrounding tissues are actively elongating at a rate equal to or greater than that of the notochord). B. Deformation of a column, as explained in the text. C. Three-point bending test of a notochord: F = applied force, ψ = distance between supports, δ = deflection of midpoint of notochord ($< 0.1\ \psi$), EI = flexural stiffness.

IS OSMOTIC SWELLING RESPONSIBLE FOR THE MORPHOLOGICAL AND MECHANICAL CHANGES THE NOTOCHORD UNDERGOES?

The notochord begins to lengthen, straighten, and stiffen significantly at the stage in development when the GAG-filled vacuoles of the notochord cells begin to expand. The high affinity of GAG's for

water (Grodzinsky 1983) leads us to speculate that osmotic swelling of the notochord might be responsible for the morphological and mechanical changes this structure undergoes. One way to test whether this is a reasonable idea is to osmotically swell excised notochords and to ascertain if such a treatment produces similar morphological and mechanical changes as those observed during normal development.

Excised notochords placed in doubly-distilled water (DDW) lengthen, straighten, and stiffen significantly (Table 2). When subsequently placed in modified Niu-Twitty solution (MNT, which is similar to Holtfreter's (1943) standard solution, and thus is isosmotic with most early amphibian cells), the notochords go back to their original configuration and stiffness. If returned to DDW, the notochords straighten again, and so on. Hence, we find that the morphological and mechanical changes that occur in the notochord during the early tailbud stages of development can be mimicked by osmotic inflation.

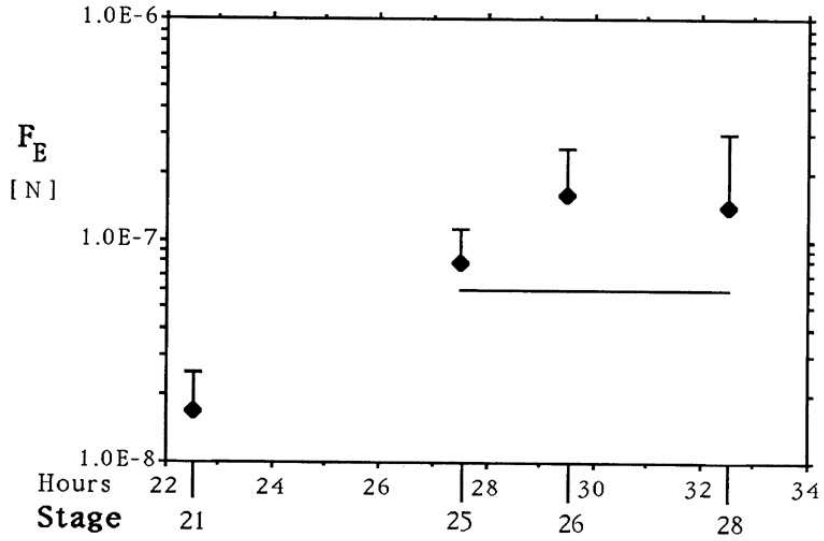


Fig. 3. Critical force to cause Euler buckling (F_E in Newtons, log scale) at various stages of development (hours after fertilization at 22°C); $F_E = (\alpha\ \pi^2\ EI)/L^2$, where $\alpha = 1$ (constant that depends on the how the ends of the column are constrained), EI = flexural stiffness, L = mean notochord length for a particular stage of development. Each point represents the mean for five notochords. Bars indicate one standard deviation. Line below points for stages 25, 26, and 28 indicates no significant difference between them; stage 21 is significantly different from stage 25 (Kruskal-Wallis, Student Newman-Keuls, $p < 0.05$).

THE NOTOCHORD AS A HYDROSTATIC SKELETON

The notochord is a sheath filled with vacuolated cells; if the osmotic pressure of those cells is altered, the shape, size, and stiffness of the notochord can be changed. Hence, it appears that the basic design of a notochord is similar to that of man-made pressure vessels (e.g. water tanks, pneumatic beams) and of biolological hydrostatic skeletons (e.g. plant cells, worms). All these structures (hereafter called "hydrostats") are composed of a tension-resisting sheath surrounding compression-resisting, but easily-sheared, material (usually fluid) under pressure. The mechanical behavior of such hydrostats has been extensively analyzed (e.g. Otto 1962; Faupel 1964; Sherrer 1967; Seymore 1970;

TABLE 2. Excised Stage 25 Notochords in Different Media

	MNT (deflated) [mean (SD, n)]	DDW (inflated)
length ($mx10^{-3}$)	2.40 (0,31,5#)	* 2.73 (0,42,5)
diameter ($mx10^{-3}$)	0.11 (0.01,7)	* 0.13 (0.02,7)
SI^{+}	2.1 (1.1,5)	@ 4.9 (3.3,5)
EI (Nm^2x10^{-14})	0.85 (0.68,7)	* 3.99 (1.86,7)

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* Parameter in MNT significantly different from in DDW, Wilcoxon signed rank, $p < 0.05$)

\# Cases where n = 5, notochords were placed in DDW first; cases where n = 7, two of the notochords were placed in MNT first, (no significant difference between parameter values for notochords placed in MNT before DDW vs. those placed in MNT after DDW, Wilcoxon signed rank, $p < 0.05$)

@ Wilcoxon signed rank, $p = 0.0796$

Swanson 1974; Roark and Young 1975; Wainwright *et al.* 1976; Koehl 1977; Hettiaratchi and O'Callaghan 1978; Wadepuhl and Beyn 1989). Here we shall briefly list some of the important properties of cylindrical hydrostats without formulating them quantitatively:

1. Inflation of limp hydrostats can straighten them unless the sheath is reinforced assymetrically.
2. For a given internal pressure, the tensile stress (force per cross sectional area of material bearing the load) in the sheath in the circumferential direction is twice as big as the stress in the longitudinal direction. Thus, if the walls of a cylindrical hydrostat are isotropic, the cylinder when inflated increases in girth at a greater rate than in length (or it develops a local increase in diameter, i.e. an anurism).
3. The walls of both man-made pressure vessels and biological hydrostatic skeletons are often reinforced with nearly inextensible fibers. The orientation of such fibers determines the shape changes that the cylinder undergoes when inflated as discussed by e.g. Clark and Cowey (1958), Green (1980), Alexander (1987), Cosgrove (1987) and Wainwright (1988).

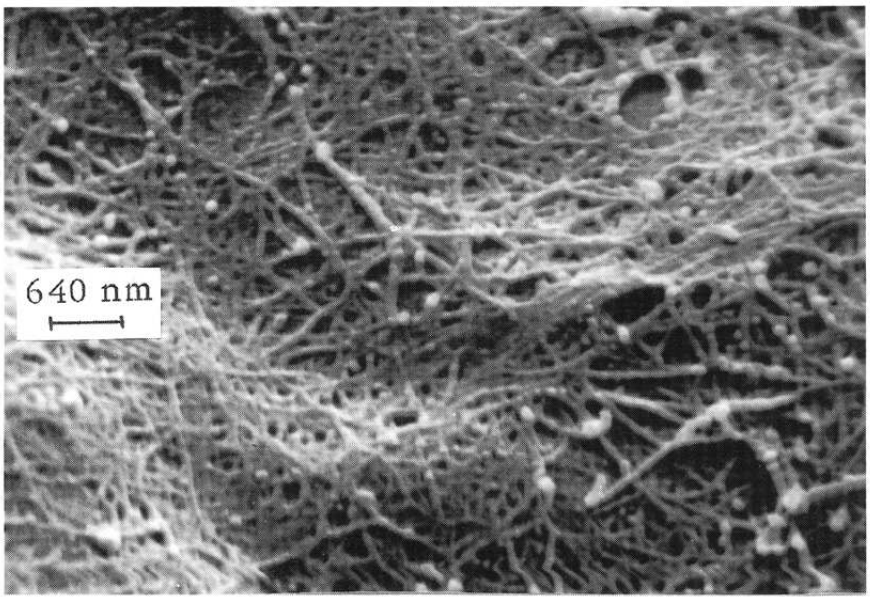

Fig. 4. Scanning electron micrograph of the surface of the notochord sheath of a stage 25 embryo. Fiber density, diameter and orientation do not differ significantly between anterior, middle and posterior regions of the notochord (Adams, *et al.*, in prep.).

4. The orientation of the sheath fibers also affects the mechanical response of a hydrostat to external forces. For example, at a given internal pressure, a cylinder reinforced with longitudinal and circumferential fibers kinks more easily (i.e. the force, F_L, required to produce local buckling is lower, Fig. 2B) than one reinforced with the same density of diagonal fibers.
5. The more resistant the walls of a pressure vessel are to being stretched, the higher the internal pressure that can be sustained.
6. The higher the internal pressure, the larger the load (F_L) it takes to kink (locally buckle) a hydrostatically-supported column.
7. The higher the internal pressure, the greater the flexural stiffness (EI) of a hydrostatic cylinder, hence the greater the load (F_E) it can bear without bending elastically (undergoing Euler buckling, Fig. 2B).

These features of hydrostatic cylinders suggest that we ought to have a closer look at the ontogeny of both the sheath and the internal pressure of the notochord.

DOES THE MORPHOLOGY OF THE NOTOCHORD SHEATH CHANGE?

The notochord sheath is indeed fibrous (Fig. 4) and contains collagen and GAG's (Weber 1961; Bruns and Gross 1970; Hay 1984). Hence, the notochord sheath, like other collagenous connective tissues, can be considered as a composite material composed of nearly inextensible fibers in a deformable matrix (e.g. Wainwright *et al.* 1976; Vincent 1982). Aspects of the microarchitecture of such tissues that are likely to affect their resistance to being stretched include density (number per transect length) and orientation of the fibers, and degree of cross-linking of fibers and matrix.

The notochord sheath changes between stages 20 and 28. A morphometric analysis of the notochord sheath revealed that most of the fibers are ≤ 1 μm wide, and that their density increases significantly by stage 25 (Table 1). Although we have not yet analyzed how the chemical composition of the sheath changes during development, we have observed that the sheath can be removed by collagenase digestion until stage 25; thereafter, trypsin in addition to collagenase is required for sheath removal (Adams *et al.* in prep.). Hence, at the same time

that the fiber density increases, the sheath becomes more difficult to digest away. These changes should result in a less stretchable sheath that can sustain higher internal pressures, although this remains to be tested.

Fiber orientation does not change with stage of development. The mean fiber angle (angle between a fiber and the long axis of the notochord) is 54° (99% confidence interval = 52° - 57°, n = 1500). Hence, the notochord is reinforced with diagonal fibers, an arrangement that makes it less likely to kink than if it had longitudinal and circumferential fibers. Furthermore, if the fibers in the wall of a cylindrical hydrostat are at 54°, their resistance to hoop and longitudinal stresses are balanced (e.g. Faupel 1964; Wainwright *et al.* 1976). Therefore, if the notochord behaves as a simple hydrostatic skeleton, we predict that it should inflate isometrically, i.e. that its length-to-diameter ratio (L/D) should remain constant as its volume increases.

Contrary to this prediction, the notochord L/D increases significantly after stage 23 (i.e. the notochord lengthens at a greater rate than it widens) (Table 1). Hence, the notochord does not behave as a simple hydrostatic skeleton whose shape changes upon inflation are constrained soley by the sheath. What then is the role of the notochord sheath?

WHAT IS THE MECHANICAL ROLE OF THE NOTOCHORD SHEATH?

An obvious way to determine the role of the notochord sheath is to remove it and observe the consequences. As mentioned above, the sheath can be removed by digestion with collagenase and trypsin. If the embryo is cut as indicated by the dashed line in Fig. 5A, the notochord can be exposed to a bath of enzymes while maintaining its normal relationship to the neighboring somitic mesoderm and neural anlagen. If such a digestion is conducted in MNT solution, the notochord elongates relative to the surrounding tissues. The elongating sheathless notochord does not appear to be stiff enough to stretch the surrounding tissues, but rather buckles and folds as it extends (Fig. 5B). The cells in such sheathless notochords are rounded, whereas they are flat in intact notochords (as described above). Does osmotic swelling of the cells produce these changes in sheathless notochords?

If the notochord sheath is digested away as described above, but in a bath of MNT to which sufficient sorbitol (a metabolically inert osmotic agent) has been added, the notochord does not lengthen or buckle, and its cells remain flat, as in the intact notochord. That the elongation of a sheathless notochord can be prevented by increasing the osmolality of the medium above that of MNT solution suggests that the notochord cells are more osmotically active than the surrounding tissues of the embryo.

Similar experiments can be conducted with notochords excised from their neighboring tissues. Such isolated notochords also lengthen (while their cells round up) if their sheaths are digested away in MNT solution, but do not elongate (while their cells remain flat) if the appropriate concentration of sorbitol is added to the bath. Measurement of such isolated notochords (Table 1) indicates that no significant osmotic lengthening of sheathless notochords occurs until stage 21. By stage 25, however, sheathless notochords in MNT solution roughly double in length. Surprisingly, sheathless notochords in MNT do not increase in

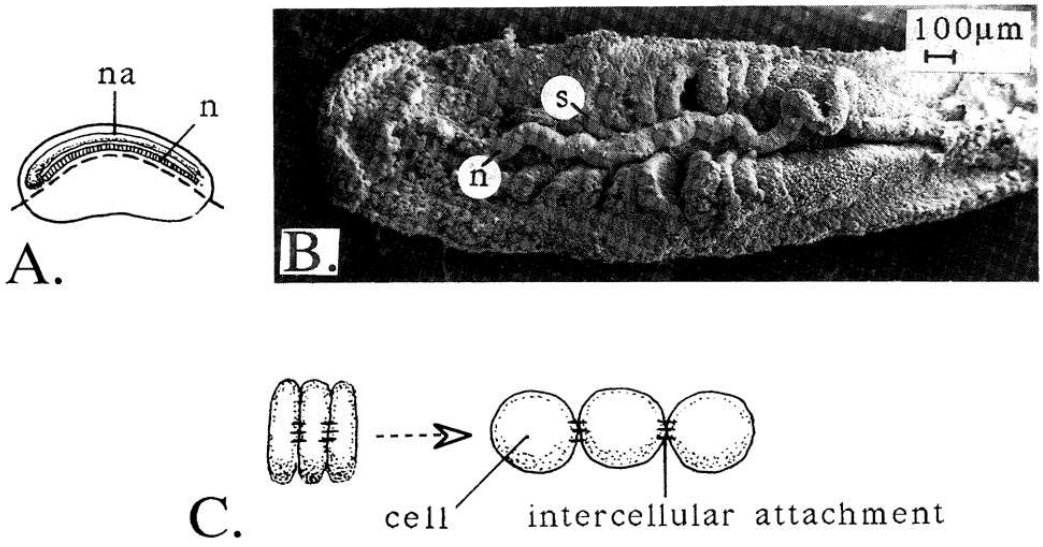

Fig. 5. A. Profile of an embryo with the positions within it of the notochord (n) and neural anlagen (na) indicated. The dashed line shows where embryos were cut (see text). B. SEM of a ventral view of a stage 21 embryo cut as shown in A and placed for 30 minutes at 22 °C in collagenase (1 mg/ml) and trypsin (1 mg/ml) in MNT; n = notochord, s = somite. C. Diagram of shape changes of osmotically inflated cells of a sheathless notochord (see text).

diameter at any of the stages we studied. Closer examination using SEM reveals many filiform connections between neighboring notochord cells. This connection of neighboring cells appears to constrain the direction in which a sheathless notochord can swell: cells that resembled a stack of pizza slices swell into a configuration reminiscent of a string of pearls (Fig. 5C). The role of the sheath, therefore, appears to be to prevent the osmotic swelling of notochord cells, thereby permitting the internal pressure (and hence the flexural stiffness) of the notochord to rise.

DOES THE INTERNAL PRESSURE OF THE NOTOCHORD CHANGE?

As mentioned above, the higher the internal pressure of a hydrostatic column, the greater its flexural stiffness, and the less likely it is to kink when loaded. We can use video recordings to determine whether notochords shrink or swell when their sheaths are removed in solutions of different osmolalities. By comparing the osmolality of a solution in which the notochord does not change dimensions with that of MNT solution (which is osmotically balanced with the surrounding embryonic tissues), we can estimate the hydrostatic pressure (technique described in Nobel 1970) in an intact notochord within an embryo. This work is now in progress, but our preliminary data indicates that the hydrostatic pressure of a stage 21 notochord is between 0.5 and 1.2 MN/m^2. This pressure is comparable to that of plant cells (e.g Nobel 1970) and is about an order of magnitude greater than that reported for *Xenopus* epidermal cells in culture (Strohmeier and Bereiter-Hahn 1987). By stage 25, the internal pressure of the notochord has increased to about 2.4 MN/m^2. This two- to three-fold increase in internal pressure occurs at the same time in the development of the notochord as the sharp rise in flexural stiffness.

SUMMARY AND PROPOSED MECHANISM

Between stages 21 and 25 the notochord of a *Xenopus* embryo begins to lengthen, straighten, and stiffen. At this time the fiber density in the notochord sheath increases and the sheath becomes more

difficult to digest away. Concurrent with this reinforcement of the sheath, the the osmotic activity of the notochord cells increases, GAG-filled vacuoles within these cells swell, and the internal pressure of the notochord increases 2- to 3-fold.

These observations lead us to propose the following mechanism for the shape, size, and stiffness changes that occur in the notochord during the early tail bud stages in *Xenopus* embryos. The osmotic acitvity of the notochord cells increases, probably due to the secretion of GAG's into their vacuoles. The flat cells in the notochord, therefore, tend to swell osmotically. Because of the connections between adjacent cells in the notochord, such swelling tends only to lengthen the notochord. However, this lengthening is limited by the fibrous sheath surrounding the notochord. The resultant rise in the internal pressure of the notochord not only increases the force required to kink the structure, but also raises the flexural stiffness, and hence increases the load that the notochrd can bear without bending elastically (Euler buckling). These changes in mechanical properties permit the notochord to elongate and straighten without being thrown into folds by the resistance of the surrounding tissues.

We see in the developing amphibian notochord an osmotic mechanism of force generation and shape-change production. In this regard the system is reminiscent of the hydrostatic mechanisms of morphogenesis in plants (e.g. Green 1980; Cosgrove 1987). The role of this elongation and straightening of the notochord in the morphogenesis of the surrounding embryonic tissues remains to be analyzed.

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REFERENCES

Adams DS, Keller RE, Koehl MAR. The mechanics of notochord elongation, straightening, and stiffening in the embryo of *Xenopus laevis*. (in prep.)

Alexander RMcN (1987) Bending of cylindrical animals with helical fibers in their skin or cuticle. J. Theor. Biol. 124: 97-110

Bijtel JH (1958) The mode of growth of the tail in urodele larvae. J. Embryol. Exp. Morph. 6: 466-478

Bruns RD, Gross J (1984) Studies on the tadpole tail: 1. Structure and organization of the notochord and its covering layers in *Rana catesbiana*. Am. J. Anat. 128: 193-224

Clark RB, Cowey JB (1958) Factors controlling the change of shape of some worms. J. Exp. Biol. 35: 731-748

Cosgrove DJ (1987) Mechanical and hydraulic aspects of plant cell growth. In: Bereiter-Hahn J, Anderson OR, Reif W-E (eds) Cytomechanics. Springer, Berlin Heidelberg New York, p. 215

Faupel JH (1964) Engineering design. John Wiley, New York

Gere JM, Timoshenko SP (1984) Mechanics of materials, 2nd edn. PWS Engineering, Boston

Green, PW (1980) Organogenesis - a biophysical view. Ann. Rev. Plant Physiol. 31: 51-82

Grodzinsky A (1983) Electromechanical and physiochemical properties of connective tissues. CRC Critical Rev. Biomed. Engin. 9: 133-199

Hay ED (1984) Collagen and embryonic development. In: Trelstad R (ed) The role of extracellular matrix in development. Alan R. Liss, New York, p 379

Holtfreter J (1943) Properties and function of the surface coat in amphibian embryos. J. Exp. Zool. 93: 251-323

Horstadius S (1944) Uber die Folge von Chordaextirpation an spaten Gastrulae und Neurulae von *Ambystoma punctatum*. Zool. Stolkh. 25: 75-88

Hettiaratchi DRP, O'Callaghan JR (1978) Structural mechanics of plant cells. J. Theor. Biol. 74: 235-257

Keller RE, Cooper M, Danilchik M, Tibbetts P, Wilson P (1989) Cell intercalation during notochord development in *Xenopus laevis*. J. Exp. Zool. 251: 134-154

Kitchen IC (1938) The effects of extirpation of the notochord undertaken at the medullary plate stage in *Ambystoma mexicanum*. Anat. Rec. 72: 34a

Kitchen, IC (1949) The effects of notochordectomy in *Ambystoma mexicanum*. J. Exp. Zool. 112: 393-415

Koehl MAR (1977) Mechanical diversity of the connective tissue of the body wall of sea anemones. J. Exp. Biol. 69: 107-125

Lehman FE, Ris H (1938) Weitere Untersuchungen uber die Entwicklung der Achsenorgene bei partiell chordalosen Tritonlarven. Rev. Suisse Zool. 45: 419-424

Malacinski GM, Youn BW (1982) The structure of the anuran amphibian notochord and a re-evaluation of its presumed role in early embryogenesis. Differentiation 21: 13-21

Mookerjee S (1953) An experimental study of the development of the notochordal sheath. J. Embryol. Exp. Morph. 1: 411-416

Mookerjee S, Deuchar GM, Waddington CH (1953) The morphogenesis of the notochord in amphibia. J. Embryol. Exp. Morph. 1: 399-409

Nieuwkoop PD (1946) Experimental investigations on the origin and determination of the germ cells and on the development of the lateral plates and germ ridges in urodeles. Arch. Neerl. Zool. 8: 1-205

Niewkoop PD, Faber J (1967) Normal table of *Xenopus laevis* (Daudin). North Holland, Amsterdam

Nobel PS (1970) Introduction to biophysical plant physiology. W. H. Freeman, San Francisco

Otto F (1962) Tensile structures. MIT Press, Boston

Roark RJ, Young WC (1975) Formulas for stress and strain, 5th edn. McGraw-Hill, New York

Seymore MK (1970) Skeletons of *Lumbricus terrestris* L. and *Arenicola marina* (L.). Nature 228: 383-385

Sherrer RE (1967) Filament-wound cylinders with axial-symmetric loads. J. Compos. Mat. 1: 344-355

Swanson CJ (1974) Application of thin shell theory to helically-wound fibrous cuticles. J. Theor. Biol. 43: 293-304

Strohmeier R, Bereiter-Hahn J (1987) Hydrostatic pressure in epidermal cells is dependent on Ca-mediated contractions. J. Cell Sci. 88: 631-640

Vincent JFV (1982) Structural biomaterials. Macmillan, London

Waddington CH and Perry MM (1962) The ultrastructure of the developing urodele notochord. Proc. Roy. Soc. B. 156: 459-483

Wadepuhl M, Beyn W-J (1989) Computer simulation of the hydrostatic skeleton. The physical equivalent, mathematics and application to worm-like forms. J.Theor. Biol. 136: 379-402

Wainwright SA (1988) Axis and circumference. The cylindrical shape of plants and animals. Harvard University Press, Cambridge

Wainwright SA, Biggs WD, Currey JD, Gosline JM (1976) Mechanical design in organisms. Edward Arnold, London

Weber R (1961) Similar pattern of fine structure in the basement lamella of the skin and the external sheath of the notochord in *Xenopus* larvae. Experientia 17: 365-366