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A Common Pattern of Somite Cell Rotation in Three Species of Pipidae

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During amphibian somitogenesis, presumptive myotomal cells change shape from round or polygonal to elongated and aligned parallel to the notochord (for reviews see Radice, et al., 1989; Keller, 2000). Although the final orientation of myotomal cells is always axial, the movements that achieve this final arrangement can differ greatly between species. The simplest movement is that seen in Bombina variegata, Pelobates fuscus, and Bufo bufo (Brustis et al., 1976; Brustis, 1979; Kielbowna, 1981). In these anurans, after segmentation myotomal cells simply elongate along the embryo's anteroposterior axis. The urodeles Ambystoma mexicanum and Pleurodeles waltli have a very different pattern; cells of the unsegmented mesoderm first elongate dorsoventrally then at segmentation become wedge-shaped and form a rosette surrounding a central myocoele. Subsequently, these cells reorient again and elongate, becoming parallel to the long axis of the embryo (Youn and Malacinski, 1981b).

A third distinctive pattern has been reported for Xenopus laevis. In this species, cells of the unsegmented paraxial mesoderm first elongate mediolaterally and perpendicular to the notochord. As segmentation begins, the cells rotate 90° to lie parallel to the notochord (Hamilton, 1969; Youn and Malacinski, 1981a). In Rana sphenoecephala, a similar but less extreme rotation occurs. Unsegmented cells elongate at an angle of about 45° to the notochord and then rotate the remaining 45° following segmentation (Youn and Malacinski, 1981b).

Why are there so many different mechanisms to achieve apparently identical results? Perhaps each pattern provides different functional advantages, but this explanation is difficult to test. Alternatively, the different patterns could represent historical constraints within specific lineages, but to date only single species within a family have been studied—comparative studies within lineages have not been done. To begin such an effort, we compared the pattern previously seen in Xenopus laevis with two additional members of the Pipidae: Xenopus tropicalis and Hymenochirus boettgeri. These taxa represent the three main lineages within Pipidae. Hymenochirus boettgeri represents the Hymenochirus-Pipa clade. X. tropicalis has an unduplicated genome that has been suggested to be ancestral to Xenopus (see review by Cannatella and de Sá, 1993), and X. laevis is a member of the clade of polyploid species (Biube et al., 1977; Kobel et al., 1998).

A total of 14 specimens of Hymenochirus boettgeri, seven specimens of Xenopus laevis were examined. Embryos of H. boettgeri were collected from naturally spawned (non-
hormone injected) clutches obtained from laboratory-reared adults. *Xenopus tropicalis* and *X. laevis* embryos were obtained from laboratory-reared adults injected with 50–100 I.U. (*X. tropicalis*) or 500–800 I.U. (*X. laevis*) of human chorionic gonadotropin (Sigma Chemical Co.) to induce spawning. The eggs were transferred to disposable culture dishes containing 20% Marc's Modified Ringer's solution (100% MMR is 100 mM NaCl, 2.0 mM KCl, 2.0 mM CaCl₂, 1 mM MgCl₂, 5.0 mM HEPES buffer, pH 7.4) and incubated at room temperature. Animals were staged according to Nieuwkoop and Faber's (1975) normal table for *Xenopus laevis* (NF). Embryos at NF stages 16–19 were preserved in Smith's fixative (12.5 ml glacial acetic acid, 2.5 g potassium dichromate, 50 ml 37% formaldehyde, 435 ml deionized H₂O), placed on a rotator for 24 h to enhance fixation, and then transferred to neutral buffered 3.7% formaldehyde for storage. Experiments were conducted in accordance with approved Institutional Animal Care and Use guidelines (University of Richmond Permit 98-2).

Observation of somitic cellular rearrangement patterns in fixed embryos were made by first embedding the specimens in Paraplast Plus wax (Oxford Scientific) or in glycol methacrylate (JB-4 Plus, Electron Microscopy Sciences). Embryos were oriented frontally. Wax-embedded specimens were sectioned at 10 µm, and plastic-embedded specimens were sectioned at 1–2 µm. Wax sections were transferred to glass slides and stained with Mallory-Heidenhain trichrome stain and hematoxylin (Presnell and Schreiber, 1997). Plastic sections were transferred to slides and stained with 0.1% toluidine blue in 1% sodium tetraborate for 1 min (Dawes, 1979). Sections were photographed using Kodak Technical Pan film.

We confirmed the pattern of somitic cellular rearrangement seen in *X. laevis* by Hamilton (1969) and Youn and Malacinski (1981a). Because somite development proceeds from anterior to posterior, favorable frontal sections of embryos during late neurula stages reveal the entire sequence of somite cell rearrangement. In posterior regions, paraxial mesoderm remains unsegmented. Here the mesoderm cells lie perpendicular to the notochord, elongated mediolaterally (Fig. 1A). Immediately anterior lies the most recently segmented somite. Here the cells have begun rotation and so lie at an angle to the notochord. Rotation is nearly complete in the next anterior somite and is completed in the third most recently segmented somite (Fig. 1A).

**Fig. 1.** Somite cell rotation in three species of pipid frogs: (A) *Xenopus laevis*; (B) *Xenopus tropicalis*; (C) *Hynenochirus boettgeri*. All specimens were examined at Nieuwkoop and Faber stage 19, oriented frontally, and sectioned at approximately the level indicated on the inset drawings (after Nieuwkoop and Faber 1975). Anterior of the embryo is toward the right. The notochord is indicated (n). Double arrows in each panel indicate the orientation of cells in unsegmented dorsal mesoderm or in somites. In the posterior region, cells in the unsegmented mesoderm are oriented mediolaterally. As segmentation proceeds, cells rotate and become oriented anteroposteriorly. All micrographs were printed at the same magnification. Note that cells in *X. laevis* are much larger than in *X. tropicalis* and *H. boettgeri*. Bar = 100 µm.

Observation of *X. tropicalis* and *H. boettgeri* specimens at NF stage 16–19 revealed the same cellular movement as those seen in *X. laevis* (Fig. 1B–C). Posterior unsegmented mesoderm cells lie perpendicular to the notochord, begin rotation at segmentation, and complete rotation by the time that two more somites have segmented. In all three species, the entire rotation occurs in about an hour at room temperature (Nieuwkoop and Faber, 1975; this study).

Different patterns of somite rearrangement among anurans might be required for mechanical reasons. Perhaps, for example, different patterns are more efficient for cells of different sizes. We found that myotome cell length is greater in *X. laevis* than in both *X. tropicalis* and *H. boettgeri*. The difference is readily apparent in Figure 1A–C which are shown at the same magnification. Measurement of 30 cells of each species in the rotated and aligned myotome revealed a mean cell length in *X. laevis* of 47.5 ± 2.7 µm, whereas in *H. boettgeri*, it is 29.4 ± 2, and in *X. tropicalis*, it is 29.9 ± 2.1 µm. *Xenopus laevis* cells are significantly longer (Student's t-test for both comparisons, P < 0.001). The difference in cell size may be related to ploidy level (or C-value) since *X. laevis*, although functionally dip-
loid, is thought to be derived from an allotetraploid ancestor, whereas *X. tropicalis* has retained the presumed ancestral diploid condition (Kobel and Du Pasquier, 1986). Whatever the reason for the dramatic difference in size, all three species showed the same pattern of somite cell rotation. Therefore, at least within the size range in these species, cell size does not seem to affect the mechanics of rotation.

Perhaps more surprising is that *X. laevis* and *H. boettgeri* have similar patterns of somitogenesis despite having distinct earlier differences in the mechanics of dorsal mesoderm formation (Minsuk and Keller, 1996) as well as differences later in myogenic timing (Smetanick et al., 1999, 2000). Of the myogenic events we have studied, somite cell rotation is the only one that occurs at the same time and in the same way in the two species. Thus, individual events of somitogenesis can vary independently in different species.

This unique process of myotome rearrangement may represent a synapomorphy for Pipidae. It has not been found in other species of anurans or urodèles studied thus far, including *Pelobates fuscus* (Kielbowna, 1981), a member of the Pelobatidae, a taxon that is relatively closely related to pipids (Ford and Cannatella, 1993). In *P. fuscus*, ventral somites do not elongate perpendicular to the body axis but rather fuse to form numerous myotubes, which become oriented parallel to the long axis directly, without rotation (Kielbowna, 1981). Similarly, rotation has not been seen in *Bufo bufo* (Brustis et al., 1976; Brustis, 1979) or *Bombina variegata* (Kielbowna, 1981), nor in the urodèles *Ambystoma mexicanum* and *Plethodon writh* (Youn and Malacinski, 1981b).

If this pattern of rotation is indeed synapomorphic for the Pipidae, then it should also occur in the genus *Pipa*, a prediction that can be confirmed when embryos of the appropriate stages are obtained. The genus *Pipa* will be particularly interesting to examine because there are both free swimming larvae (P. carinata) and direct developing (P. pipa) members of the genus. This could allow one to determine whether somitogenesis patterns are related to life history rather than historical events.

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LITERATURE CITED


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