

## Myotome Myogenesis in the Mexican Axolotl

Anton W. Neff

Medical Sciences Program  
Indiana University  
School of Medicine  
Bloomington IN 47460

The first muscles to differentiate during amphibian development are the myotomes of somites. The historical and current literature on early muscle induction and muscle differentiation in amphibia is extensive due to several factors: Myotomes make up the largest subdivision of the somites (others are: sclerotome-ventromedial and dermatome-dorsolateral) (Malacinski et al., 1989); myotome muscles are morphologically easily distinguishable from other axial tissues; many molecular markers for both structural (Radice and Malacinski, 1989) and muscle-specific regulatory transcription factors are available (Hopwood et al., 1989); and myotomal muscles can be induced in amphibian blastula animal caps by various purified growth factors (Smith, 1989). Because of the large body of data available on amphibian myotome myogenesis, one should be able to formulate generalizations about key features. However, this has proven to be difficult; comparative morphological as well as limited molecular analysis of myotome myogenesis among different species of amphibians suggest a similar general pattern with substantial differences in detail (reviews—Malacinski et al., 1989 and Radice et al., 1989).

Initially, inductive influences from growth factors in the vegetal hemisphere of blastulae induce mesoderm in the marginal zone, which differentiates into presomitic mesoderm, which gives rise to myotomes, among other mesoderm components. The presomitic mesoderm presegments into somitomeres (Jacobson, 1988), which then rearrange and segment in an anterior-to-posterior wave (Keynes and Stern, 1988). Primary myotomes are formed which typically contain myocytes (which may or may not be mononucleated, depending on species). These then give rise to secondary myotomes containing secondary myotubes, which in all species so far studied are multinucleated. Primary to secondary myocyte (tube) transition also occurs among other vertebrates, such as avian and mam-

malian species (e.g., Harris et al., 1989). The details of when in development muscle-specific genes get activated (e.g., before or after somitogenesis), of the morphology/morphogenesis of the somites, of the morphology/morphogenesis of the primary myotomes, and of the fates of individual myotomes (e.g., Chung et al., 1989) during development vary considerably among different amphibian species. Considering that only a small number of the total number of amphibian species have been studied, the variation could be even greater. For example, myogenesis has not been studied in direct developing amphibians (which bypass functional larval stages) (e.g., Del Pino, 1989).

Currently, because of scientific momentum and ease of rearing adult animals, most research on early myogenesis in amphibia is being done on the anuran *Xenopus laevis*. We are learning much about the details in this species. But can we make generalizations from the *Xenopus* data to all amphibians? This author's answer is *no*. To fully understand the causative forces of myotome myogenesis, especially in the absence of an extensive developmental genetics approach, we need comparative data from a wide range of amphibians. To set the foundation for this proposed comparative investigation this author believes that myotome myogenesis should be investigated in detail at the molecular level in at least one other laboratory-reared amphibian that shows considerable variance from myotome myogenesis in *Xenopus laevis*. This author believes that a second species should be the Mexican axolotl (*Ambystoma mexicanum*). In this report myotomogenesis in the axolotl is compared to myotomogenesis in *Xenopus laevis* (see Table).

This minireview does not permit extensive discussion of each one of those features. The transient polar pattern of myotome differentiation in the axolotl will be discussed in some detail. In contrast to *Xenopus laevis*, where primary myotomes form by the 90 degree rotation of already committed (sarcomeric actin- and myosin-positive) individual presomitic myocytes such that they lie parallel to the notochord resulting in single mononucleated myocytes spanning individual myotomes, *the pattern in the axolotl is more complex*. Presomitic mesoderm cells undergo orderly craniocaudal shape changes such that they form rosettes about an internal cavity (myocoel). Then, individual mononucleated cells on the anterior and medial side of the

**Several comparative features of myotome myogenesis in the anuran *Xenopus laevis* and the urodele *Ambystoma mexicanum*.**

Feature	<i>Xenopus laevis</i>	<i>Ambystoma mexicanum</i>
Origin of the mesoderm	Internal (Smith and Malacinski, 1983) Muscle-specific transcription factor (e.g., MyoD) expressed during gastrula stage (Hopwood et al. 1989)	External (Smith and Malacinski, 1983)
Expression of sarcomeric actin and myosin mRNAs	Late gastrula (Sturgess et al., 1980) Presomitic mesoderm (Neff et al., 1989).	Late tailbud stage (Forman and Slack, 1980) Segmented somites (Neff et al., 1989)
Segmentation of somites	90° rotation of aligned cells (Youn and Malacinski, 1981a) Cranial/caudal heat sensitive wave (Elsdale et al., 1976) Begins at the neural groove stage (Youn and Malacinski, 1981a)	Rosette formation with myocoele (Youn and Malacinski, 1981b) Cranial/caudal heat sensitive wave (Armstrong and Graveson, 1988) Begins at the late tailbud stage (Youn and Malacinski, 1981b)
Primary myotomes	Mononucleated myocytes (Hamilton, 1969) Polyploid nuclei (Kielbowna, 1966) Electrically coupled (Blackshaw and Warner, 1976)	Multinucleated myocytes (Youn and Malacinski, 1981b) Transient polar pattern of myotome differentiation (Neff et al., 1989) Electrically insulated (Blackshaw and Warner, 1976)
Regional differences in myotomes	Transient differentiation and degeneration of 6 cranial myotomes (Chung et al., 1989)	No equivalent myotomes
Secondary myotomes	Multinucleated with diploid nuclei (Kielbowna, 1966)	Multinucleated with diploid nuclei

myotomes begin to express sarcomeric actin and myosin (as seen by immunocytology with heterologous monoclonal antibodies, Neff et al., 1989). Fusion of what appear to be already actin- and myosin-positive myocytes begins on the medial side of the developing myotomes (Youn and Malacinski, 1981b) eventually creating multinucleated myotubes. Subsequently, more cells in the anterior and medial side of the myotome and, eventually, in the posterior and lateral side of the myotome fuse such that by the late tailbud stage multinucleated myotubes span individual myotomes. Transplantation experiments have

shown that polar myotome differentiation is an autonomous property of individual cells (Neff, et al., 1989). When presomitic mesoderm is inverted with respect to the anterior/posterior axis, the original differentiation pattern is retained. Which axolotl myocytes are equivalent to the *Xenopus* primary mononucleated myocytes—the pre-fusion myosin/actin-positive mononucleated myocytes on the anterior and medial side of the early differentiating myotome or the multinucleated myotubes? How and when is the transient polar differentiation pattern regulated? Is the control at the level of transcription or translation? Is the transient

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differentiation pattern prepatterned in the somitomeres, or even earlier? Do cell interactions within a myotome determine the polar pattern? How universal is this polar myotome differentiation pattern among other urodeles and other anurans? Transitory polarity within myotomes has been reported in other organisms such as the Japanese fire belly newt (Neff et al., 1989) and the chick (Kaehn et al., 1988).

The variation in the key features of myotomogenesis shown in the table no doubt reflects variation at the molecular level (e.g., regulation of transcription by muscle differentiation factors such as MyoD) and at the cellular level (e.g., cell movements and rearrangements during segmentation of the presomitic mesoderm). However, there are gaps in our knowledge concerning myotomogenesis in the axolotl, especially at the molecular level. In contrast to *Xenopus*, where many muscle markers (e.g., actin, Mohun et al., 1989; myosin, Radice and Malacinski, 1989) and putative regulatory factors localized to muscle have been cloned (e.g., MyoD, Harvey, 1990), none have been cloned from the axolotl. This author believes that understanding axolotl myotome myogenesis requires this gap to be filled. Perhaps with the utilization of *Xenopus laevis* probes and probes from other urodeles, such as the newt (e.g., Casmir et al., 1988) homologous genes can be cloned from axolotl cDNA libraries.

## Literature Cited

- Armstrong, J. B., and A. C. Graveson. 1988. Progressive patterning precedes somite segmentation in the Mexican axolotl (*Ambystoma mexicanum*). *Dev. Biol.* 126:1-6.
- Blackshaw, S. E., and A. E. Warner. 1976. Low resistance junctions between mesoderm cells during development of trunk muscles. *J. Physiol.* 255:209-230.
- Casmir, C. M., P. B. Gates, P. B. Ross-McDonald, J. F. Jackson, R. K. Patient, and J. P. Brockes. 1988. Structure and expression of a newt cardio-skeletal myosin gene. Implications for the C value paradox. *J. Mol. Biol.* 202:287-296.
- Chung, H.-M., A. W. Neff, and G. M. Malacinski. 1989. Autonomous death of amphibian (*Xenopus laevis*) cranial myotomes. *J. of Exp. Zool.* 251:290-299.
- Del Pino, E. M. 1989. Modifications of oogenesis and development in marsupial frogs. *Development* 107:169-187.
- Elsdale, T., M. Pearson, and M. Whitehead. 1976. Abnormalities in somite segmentation following heat shock to *Xenopus* embryos. *J. Embryol. exp. Morph.* 35:625-535)
- Forman, D., and J. M. W. Slack. 1980. Determination and cellular commitment in the embryonic amphibian mesoderm. *Nature* 286:482-484.
- Hamilton, L. 1969. The formation of somites in *Xenopus*. *J. Embryol. exp. Morph.* 22:253-264.
- Harris, A. J., M. J. Duxson, R. B. Fitzsimons, and F. Rieger, F. 1989. Myonuclear birthdates distinguish the origins of primary and secondary myotubes in embryonic mammalian skeletal muscles. *Development* 107:771-784.
- Harvey, R. P. 1990. The *Xenopus* MyoD gene: an unlocalized maternal mRNA predates lineage restricted expression in the early embryo. *Development* 108:669-680.
- Hopwood, N. D., A. Pluck, and J. B. Gurdon. 1989. MyoD expression in the forming somites is an early response to mesoderm induction in *Xenopus* embryos. *EMBO* 11:3409-3417.
- Jacobson, A. G. 1988. Somitomeres: mesodermal segments of vertebrate embryos. *Development* 104:209-220.
- Kaehn, K., H. J. Jacob, B. Christ, K. Mimrichsen, and R. E. Poelmann. 1988. The onset of myotome formation in the chick. *Anat. Embryol.* 177:191-201.
- Keynes, R. J., C. D. and Stern. 1988. Mechanisms of vertebrate segmentation. *Development* 103:413-429.
- Kielbowna, L. 1966. Cytological and cytophotometrical studies on myogenesis in *Xenopus laevis* (Daudin) *Zool. Pol.* 17: 247-255.
- Malacinski, G. M., A. W. Neff., G. M. Radice, and H.-M. Chung. 1989. Amphibian somite development: Contrasts of morphogenetic and molecular differentiation patterns between the laboratory archetype species *Xenopus* (anuran) and Axolotl (urodele). *Zoological Science* 6:1-14.
- Mohun, T. J., M. V. Taylor, N. Garrett, and Gurdon, J. B. 1989. The CARG promoter sequence is necessary for muscle-specific transcription of the cardiac actin gene in *Xenopus* embryos. *EMBO J.* 8:1153-1161.
- Neff, A. W., G. M. Malacinski, and H.-M. Chung. 1989. Amphibian (urodele) myotomes display transitory anterior/posterior and medial/lateral

- differentiation patterns. *Dev. Biol.* 132:529-543.
- Radice, G. P., and G. M. Malacinski. 1989. Expression of myosin heavy chain transcripts during *Xenopus laevis* development. *Dev. Biol.* 53:562-568.
- Radice, G. P., A. W. Neff, Y. H. Shim, J.-J. Brustis, and G. M. Malacinski. 1989. Developmental histories in amphibian myogenesis. *Int. J. Dev. Biol.* 33:325-343.
- Smith, J. C. 1989. Mesoderm induction and mesoderm-inducing factors in early amphibian development. *Development* 105:665-677.
- Smith, J. C., and G. M. Malacinski. 1983. The origin of the mesoderm in an anuran *Xenopus laevis* and a urodele *Ambystoma mexicanum*. *Dev. Biol.* 98:250-254.
- Sturgess, E. A., J. E. M. Ballantine, H. R. Woodland, P. R. Mohun, C. D. Lane, and G. J. Dimitriadis. 1980. Actin synthesis during the early development of *Xenopus laevis*. *J. Embryol. exp. Morphol.* 58:303-320.
- Youn, B. W., and G. M. Malacinski. 1981a. Somitogenesis in the amphibian *Xenopus laevis*: scanning electron microscopic analysis of intrasomitic cellular arrangements during somite rotation. *J. Embryol. exp. Morph.* 64:23-43.
- Youn, B. W., and G. M. Malacinski. 1981b. Comparative analysis of amphibian somite morphogenesis: cell rearrangement patterns during rosette formation and myoblast fusion. *J. Embryol. exp. Morph.* 66:1-26.