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Original Article

Morphological and biochemical analysis of regeneration after cardiotoxin injury of *Xenopus laevis* fast muscles

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ABSTRACT The anterior brachial muscle of *Xenopus laevis* forelimb was characterized as a fast-type muscle composed of type II fibers exclusively. Larval and adult muscles showed three distinct isomyosins composed by two different heavy chains, HCl and HCf, respectively, associated with the same fast light chains. Muscle regeneration was examined after degeneration of the myofibers by injection of cardiotoxin, a snake toxin. 24 h after the injury no myofibers and no myosin were detected. New myosins of larval and adult fast types started to be synthesized two weeks after the injury, during a stage of proliferation of mononucleated cells. 1 month after the injury, the regenerated muscles which showed structural differences with the normal muscle contained only fast isomyosins. The precocious larval to fast heavy chain transition observed in regenerating muscles of the adult *X. laevis* without any thyroid hormone influence shows that the myogenic program in adult muscle regeneration is regulated by factors that are different from those regulating normal development.

KEY WORDS: *myosin isoforms, fast muscle, regeneration, Xenopus*

Introduction

Degeneration and then regeneration of the muscle fibers take place following muscle injury (Allbrook, 1981; Plaghki, 1985). The mononucleated satellite cells, which in normal muscle are located between the plasma membrane and the basal lamina, proliferate and fuse to form new fibers (Mauro, 1961; Snow, 1977; Campion, 1984). In mammals, the same myosin isoforms, i.e., embryonic, neonatal and adult, are synthesized during regeneration as observed during normal development, therefore suggesting that regeneration recapitulates the developmental process (Carraro *et al.*, 1983; Marechal *et al.*, 1984; Plaghki, 1985; d'Albis *et al.*, 1989; Whalen *et al.*, 1990). Nevertheless, d'Albis *et al.* (1987b, 1988) have shown that the appearance of adult isomyosins was more precocious in regenerating as compared to developing muscles in adult rat and mouse.

It is known that thyroid hormone regulates the neonatal to adult myosin isoform transition during normal development (d'Albis *et al.*, 1987a; d'Albis and Butler-Browne, 1993). The acceleration of synthesis of fast myosin in regenerating muscles of adult mammals has been attributed to differences in thyroid hormonal status between newborn and adult animals (d'Albis *et al.*, 1987b).

In amphibians as observed in mammals, there is a myosin isoform transition during the course of muscle development. This transition is characterized by the progressive replacement of larval isomyosins by the adult isoforms during the time of anatomical metamorphosis, is correlated with transitory increase of thyroid hormone levels (Chanoine *et al.*, 1987, 1990a). An essential difference between thyroidal status of amphibians and that of mammals is that thyroid hormone is not detected in serum of adult amphibians (Leloup and Buscaglia, 1977; Chanoine *et al.*, 1987). So, the amphibian model makes it possible to study the regeneration process without any thyroid hormone influence.

In this work, we study the regeneration of fast type muscles following cardiotoxin injury in the adult *Xenopus laevis*. We carry out our analysis at the histological and biochemical levels. The myosin isoforms and their heavy chain subunits synthesized during the new myogenesis were characterized in relation to the histological stage of the regenerating muscle.

Results

Normal muscle features

Analysis of the myosin ATPase profile of adult muscle revealed a uniform strong staining of the myofibers after preincubation at pH 9.4 (Fig. 1A). In contrast, we showed a complete reversion of the staining following acidic preincubation (pH 4.35); all myofibers displayed only a weak staining reaction (Fig. 1B) and were classified as type II fibers, which are characteristic of the fast-type muscles.

Larval and adult fast muscles were characterized by three myosin isoforms, the three adult isoforms migrating slightly slower than the three larval ones (Fig. 2). Comigration of myosin extracted from larval and adult muscles showed that the two faster migrating

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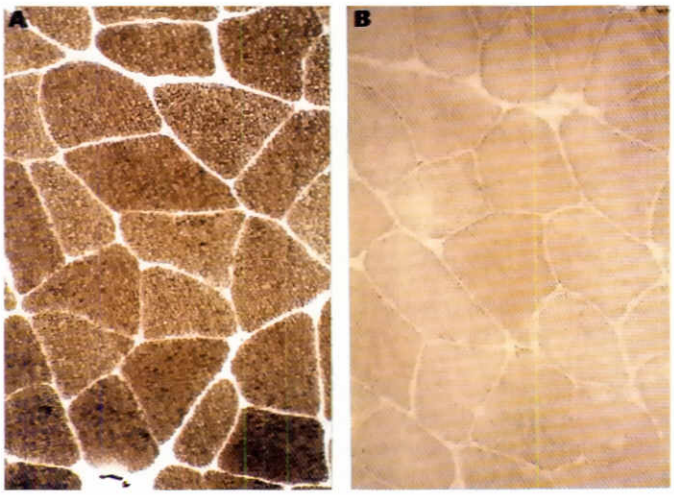

Fig. 1. Transversal sections in the *anterior brachial* muscle of adult animal stained for myosin ATPase. (A) *Preincubation at pH 9.4.* **(B)** *Preincubation at pH 4.35.*

adult isoforms comigrated with the two larval isoforms migrating slower.

Analysis of the myosin heavy chains using SDS-PAGE in presence of 37.5% glycerol showed that larval and adult fast myosins were composed of two different heavy chains, namely HCl and HCf, respectively (Fig. 3).

Two-dimensional gel analysis revealed the same three fast light chains, LC1f, LC2f and LC3f in larval and adult muscles (Fig. 4).

Regenerating muscles

Histological analysis

By 24 h post-injection of cardiotoxin, all the myofibers were degenerated (Fig. 5B). The degenerated muscle retained the appearance of continuous tubes due to the persistence of the basal lamina sheaths of the myofibers (Couteaux *et al.*, 1988). The fifteenth day post-injection was characterized by an important proliferation of mononucleated cells (Fig. 5C). New myotubes with central nuclei were observed as early as the nineteenth day post-injection (Fig. 5D-G) and the progressive transformation of myotubes into myofibers took place, characterized by the migration of the

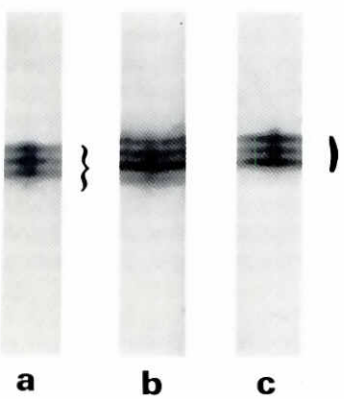

Fig. 2. Electrophoretic analysis under non-dissociating conditions of native myosin extracted from *anterior brachial* muscles. *Larval* **(a)** *and adult* **(c)** *muscles.* **(b)** *Comigration of* **(a)+(c)** *[)] Larval isomyosins; [)] adult fast isomyosins.*

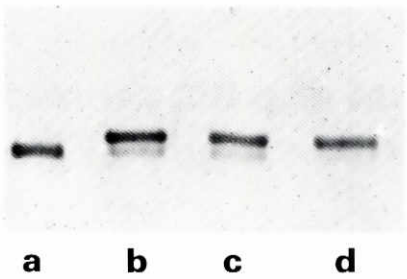

Fig. 3. SDS-PAGE in presence of 37.5% glycerol of myosin heavy chains extracted from *anterior brachial* muscles. *Larval* **(a)** *and adult* **(d)** *muscles.* **(b,c)** *Comigration of* **(a)+(d).**

central nuclei to the periphery. One and 2 months following cardiotoxin injection, the regenerated muscle presented morphologicl features distinguishing it from normal muscle (Fig. 5H-J). Myofibers were less closely juxtaposed than in normal muscle, which was clearly observed by immunohistochemistry using antibodies against laminin (Fig. 5K-M). A large scatter of the diameter of the new myofibers was also visible. Moreover, many myonuclei are abnormally located in the center of the fibers.

Analysis of myosin isoforms

No myosin isoforms were detected before the fifteenth day post-injection where four isoforms were observed in pyrophosphate gel electrophoresis (Fig. 6a). As early as 1 month post-injection, only the three isomyosins with the slower electrophoretic mobility were detected (Fig. 6c). These observations suggested that larval and adult fast isomyosins were expressed as early as the beginning of the regeneration process. That was confirmed by the analysis of myosin heavy chains in SDS-PAGE in the presence of 37.5% of

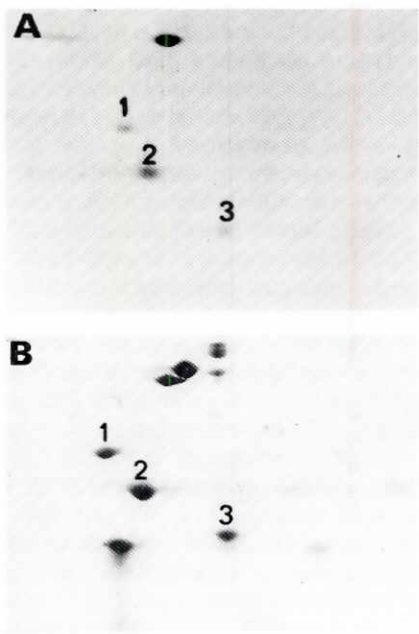

Fig. 4. Two-dimensional electrophoresis of myosin light chains extracted from *anterior brachial* muscles. *Larval* **(A)** *and adult* **(B)** *muscles. 1,2,3, LC1f, LC2f and LC3f, respectively.*

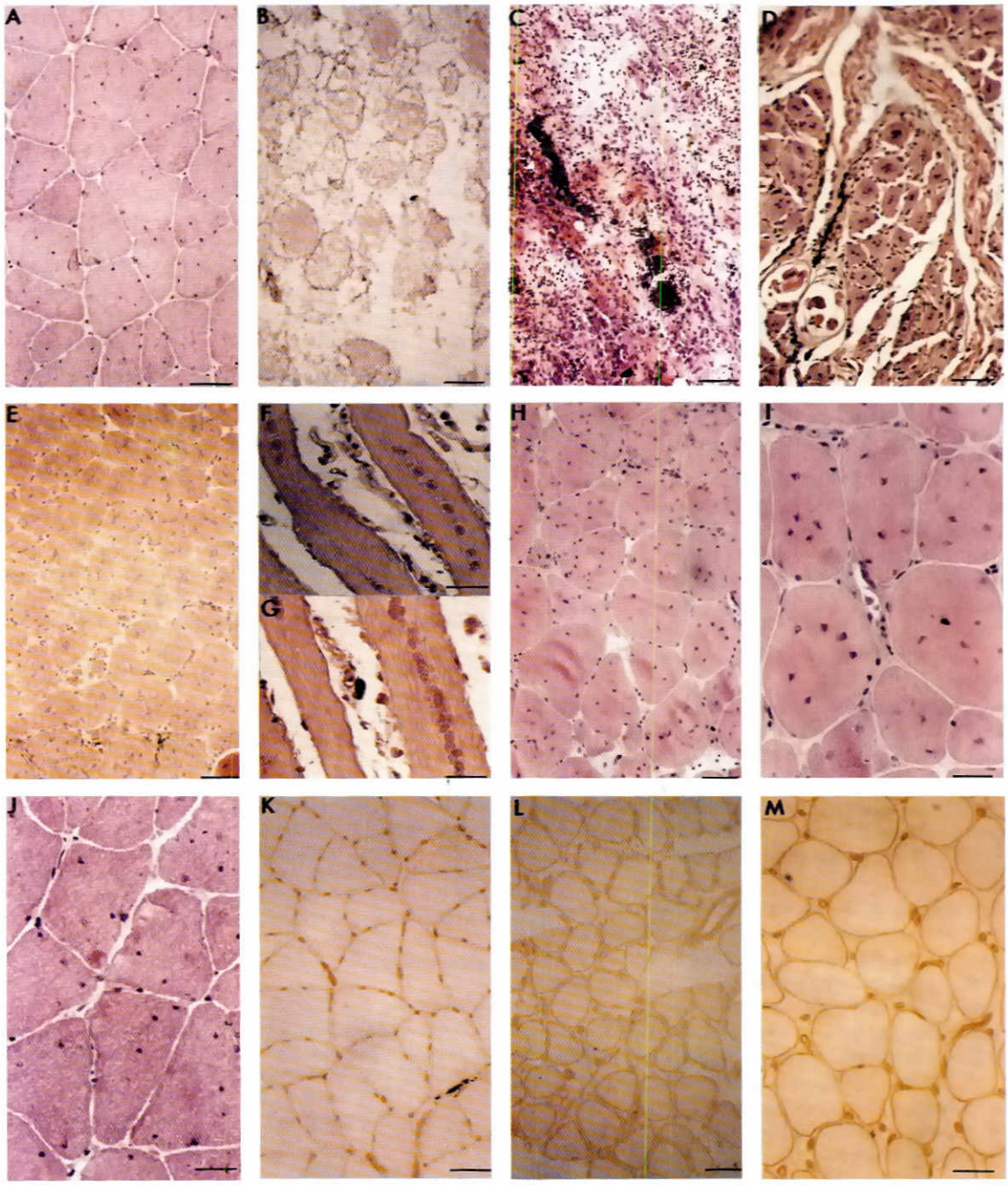

Fig. 5. Hematoxylin-eosin histological stains of degenerated (B), regenerating (C-I) and control adult (A,J) muscles. *The degenerated and the regenerating muscles were taken at 24 h* **(B)**, *15 days* **(C)**, *19 days* **(D)**, *21 days* **(E,F,G)**, *1 month (H) and 2 months* **(I)** *after cardiotoxin treatment. Immunohistochemical staining with a laminin antibody of control* **(K)** *and regenerating muscles taken at 21 days* **(L)** *and 2 months* **(M)**. *All the sections of muscle were transverse sections except for* **(F)** *and* **(G)** *which were longitudinal sections. Bars: A-E and K-M, 20 μm, F-G and I-J, 10 μm.*

glycerol (Fig. 7). Larval and fast heavy chains were expressed during the first stage of regeneration, at 15 days post-injection, when the mononucleated cells proliferated. As early as 1 month post-injection, when the new fibers were formed, only the fast heavy chain was detected. It should be noted that the fast myosin heavy chain was mostly expressed as early as the beginning of muscle regeneration.

Discussion

Only a few reports have studied the biochemical features of muscles from anuran amphibians (Pliszka *et al.*, 1981; Lannergren, 1987). In this work, we show that the *anterior brachial* muscle of *X. laevis* is a fast-type muscle composed exclusively of type II fibers. Kordylewski *et al.* (1989) demonstrated that larval and fast muscles

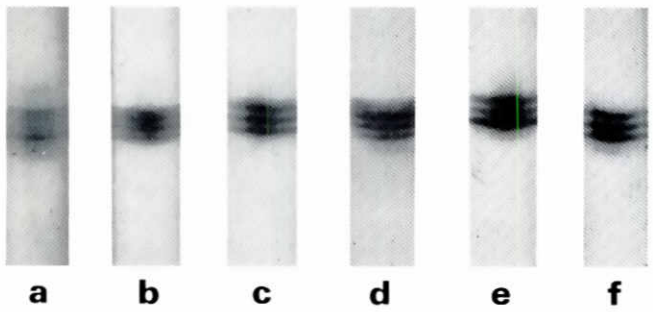

Fig. 6. Electrophoretic analysis under non-dissociating conditions of native myosin extracted from regenerating muscles. *15 days* **(a)**, *21 days* **(b)** *and 1 month* **(c)** *after cardiotoxin treatment. Adult* **(e)** *and larval* **(f)** *myosin isoforms used as controls.* **(d)** *Comigration of* **(c)+(f)**.

of *X. laevis* are composed of three distinct isomyosins. We have shown that the three larval and the three fast myosin isoforms are characterized by two different heavy chains, HCl and HCf, respectively, associated with the same three fast light chains. These isomyosins probably represent different combinations of the three light chains either with HCl or with HCf as previously observed in mammals (d'Albis *et al.*, 1985), urodelan amphibians (Chanoine and Gallien, 1989; Chanoine *et al.*, 1991) or fishes (Chanoine *et al.*, 1990b; Martinez *et al.*, 1990).

Cardiotoxin purified from the venom of *Naja mossambica* offered the advantage of selective action. It induced degeneration of the myofibers but did not affect the satellite cells, blood vessels and muscle innervation (Couteaux and Mira, 1985).

Whereas satellite cells have been demonstrated to be present in *X. laevis* muscles (Franquinet *et al.*, 1988), these cells have not yet been clearly identified in urodelan amphibian muscles (Lo *et al.*, 1993).

During the fifteenth day post-injection, the mononucleated cells, which proliferate, were probably composed of satellite cells mixed with leukocytes and macrophages, as a result of the cardiotoxin treatment. New myotubes formed during the following days and the progressive transformation into myofibers took place.

The structural differences observed between the architecture of the regenerated and normal muscle could be attributed to different explanations. On the one hand, in avian muscles, it has been shown that the connective tissue plays an organizing role in muscle development (Chevallier and Kieny, 1982). Couteaux *et al.* (1988) suggested that the presence of an altered connective tissue at the beginning of muscle regeneration might explain in part some of the deviations from normal myogenesis observed during this process. On the other hand, the structural features of the regenerated muscle could be imputed to properties of satellite cells which might be different from that of embryonic myoblasts.

Our analysis of muscle regeneration following cardiotoxin injury showed a myosin isoform expression pattern which clearly differs from that observed during normal development. Fast myosin heavy chain was expressed as early as the first stage of muscle regeneration. This contrasted with what happened during normal muscle development where fast heavy chain began to appear at the time of anatomical metamorphosis during the terminal differentiation of the developing muscle (Kordylewski *et al.*, 1989; Radice and Malacinski, 1989).

It has been clearly demonstrated that the increase of thyroid hormone levels during anatomical metamorphosis regulates the larval to fast myosin isoform transition, T3 playing an essential role in initiating the disappearance of the larval isoforms in amphibian muscles (Chanoine *et al.*, 1987, 1989). In the adult rat, the precocious expression of fast myosin heavy chain following cardiotoxin injury has been attributed to the high, constant plasma concentration of thyroid hormone in comparison to the low concentration observed in the newborn, hyperthyroidism promoting the synthesis of fast isomyosins and hypothyroidism promoting that of neonatal isomyosins (d'Albis *et al.*, 1987b). The precocious larval to fast heavy chain transition observed in regenerating muscles of the adult *X. laevis* without any thyroid hormone influence shows that the myogenic program in adult muscle regeneration is regulated by other factors than those regulating normal development. This suggests that satellite cells and embryonic myoblasts might be endowed with specific developmental programs and would agree with the proposal of Chevallier *et al.* (1987) that satellite cells and embryonic myoblasts represent non-equivalent myogenic cells.

The precocious larval to fast heavy chain transition during muscle regeneration without any thyroid hormone influence seems to be a common feature of amphibians since similar results had been previously observed during limb regeneration of urodelan amphibians following amputation (Saadi *et al.*, 1993).

Materials and Methods

Muscle injury and sampling

Injury was performed on adult *Xenopus laevis*. Animals were anesthetized with tricaine methane sulfate (MS222) and pure cardiotoxin from *Naja mossambica nigricollis* venom (Latoxan, France) (10^{-5} M in 0.9% NaCl) was injected into the right anterior brachial muscle of the forelimb. We checked that intramuscular injection of the solvent did not induce any deterioration of the muscles. Samples for histological, histochemical and immunohistochemical studies were plunged in isopentane and frozen in liquid nitrogen. For biochemical studies, samples were placed in cryotubes and immediately frozen in liquid nitrogen.

Biochemistry

Myosin extraction

Preparation of crude myosin was performed in ice according to Pliszka *et al.* (1981). The muscles were cut in small pieces and washed with 4 vol. of 600 mM KCl, 40 mM $NaHCO_3$, 10 mM Na_2CO_3, 1 mM $MgCl_2$, 10 mM $Na_4P_2O_7$ pH 8.8 (buffer A). Myosin was then extracted with 4 vols. of buffer A; after 90 min of gentle agitation, the mixtures were centrifuged at 10000xg

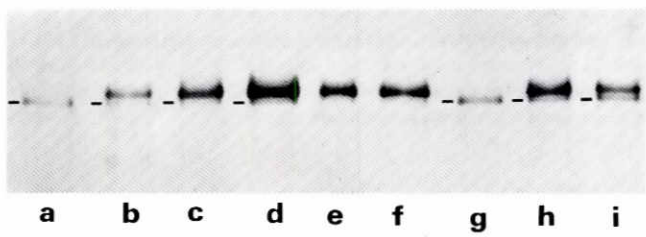

Fig. 7. SDS-PAGE in presence of 37.5% glycerol of myosin heavy chains extracted from regenerating muscles. *15 days* **(b,i)**, *19 days* **(c,h)**, *21 days* **(d)** *and 1 month* **(e)** *after cardiotoxin treatment. Larval* **(a,g)** *and adult* **(f)** *heavy chains used as controls. (-) Larval heavy chain.*

and the myosin containing supernatants were diluted twice with glycerol for conservation and study of native myosin.

Electrophoretic analysis of native myosin

Non-dissociating conditions were used (d'Albis *et al.*, 1985) but for different modifications (Chanoine *et al.*, 1990b). Running buffer was 20 mM $Na_4P_2O_7$ (pH 8.5), 10% glycerol, 0.01% 2-mercaptoethanol, 2 mM $MgCl_2$ and 2 mM ATP. Cylindrical gels (6x0.5 cm) were 4% in polyacrylamide; about 1 μg myosin per band was loaded. Electrophoresis was carried out at 80 V for 16h30 at -2°C/-3°C.

Two-dimensional electrophoretic analysis of myosin light chains

Crude myosin extracts were dissolved in 9 M urea, 2% nonidet P-40, 2.2% ampholines (pH 4-6.5) and 5% 2-mercaptoethanol. Analysis of myosin light chains was performed by the technique of O'Farrell (1975) using 2.2% ampholines in the first dimension and 14% polyacrylamide, 0.1% SDS in the second dimension (Laemmli, 1970).

Electrophoretic analysis of myosin heavy chains in the presence of SDS

This was performed according to Carraro and Catani (1983) in 5% polyacrylamide slab gels, in the presence of 0.1% sodium dodecyl sulfate (SDS) and 37.5% glycerol. The extracts were diluted in 20% glycerol, 5% SDS, 5% 2-mercaptoethanol, 1 mM EDTA and boiled for 2 min. Electrophoresis was carried out at 55 V for 18 h. Gels were stained with silver nitrate according to Ansorge (1983).

Histology, histochemistry, immunohistochemistry

For the histological, histochemical and immunohistochemical results presented, transverse frozen sections, 8 μm thick, were used. They were stained with hematoxylin and eosin. The histochemical reaction for myosin ATPase activity was carried out according to the method described by Padykula and Herman (1955) and Brooke and Kaiser (1970) for mammals using the experimental conditions defined by Johnston *et al.* (1974) for fishes. Staining of myosin ATPase was performed for 2 min in alkaline pH (10.4) or for 10 min in acidic pH (4.35). Fiber types were described following the nomenclature proposed by Brooke and Kaiser (1970) for mammals. Immunohistochemical staining for detection of laminin using indirect immunoperoxidase was carried out using vectastain kit. The laminin antibody was a rabbit antiserum, and was a gift of Dr. Daniel Louvard (Institut Pasteur, Paris, France).

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References

ALLBROOK, D.B. (1981). Striated muscle regeneration. *Muscle Nerve 4:* 234-245.

ANSORGE, W. (1983). Fast visualization of protein bands by impregnation in potassium permanganate and silver nitrate. In *Electrophoresis 82* (Ed. D. Stathakos). De Gruyter, Berlin, pp. 235-242.

BROOKE, M.H. and KAISER, K.K. (1970). Muscle fiber types. How many and what kind. *Arch. Neurol. 25:* 369-379.

CAMPION, D.R. (1984). The muscle satellite cell: a review. *Int. Rev. Cytol. 87:* 225-251.

CARRARO, U. and CATANI, C. (1983). A sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochem. Biophys. Res. Commun. 116:* 793-802.

CARRARO, U., DALLA LIBERA, L. and CATANI, C. (1983). Myosin light and heavy chains in muscle regenerating in absence of the nerve: transient appearance of the embryonic light chain. *Exp. Neurol. 79:* 106-117.

CHANOINE, C. and GALLIEN, C.L. (1989). Myosin isoenzymes and their subunits in urodelan amphibian fast skeletal muscle — coexistence of larval and adult heavy chains in neotenic individuals. *Eur. J. Biochem. 181:* 125-128.

CHANOINE, C., D'ALBIS, A., GUYOT-LENFANT, M., JANMOT, C. and GALLIEN, C.L. (1987). Regulation by thyroid hormones of terminal differentiation in the skeletal dorsal muscle. II. Urodelan amphibians. *Dev. Biol. 123:* 33-42.

CHANOINE, C., GUYOT-LENFANT, M., D'ALBIS, A., DURAND, J.P., PERASSO, F., SALLES-MOURLAN, A.M., JANMOT, C. and GALLIEN, C.L. (1989). Thyroidal status and isoenzymic pattern in the skeletal dorsal muscle of urodelan amphibians — the perennibranchiate *Proteus anguinus*. *Cell Differ. 28:* 135-144.

CHANOINE, C., GUYOT-LENFANT, M., SAADI, A., PERASSO, F., SALLES-MOURLAN, A.M. and GALLIEN, C.L. (1990a). Myosin structure and thyroidian control of myosin synthesis in urodelan amphibian skeletal muscle. *Int. J. Dev. Biol. 34:* 163-170.

CHANOINE, C., SAADI, A., GUYOT-LENFANT, M. and GALLIEN, C.L. (1991). Myosin polymorphism in urodelan amphibians dorsal skeletal muscle. *Comp. Biochem. Physiol. 100B:* 153-159.

CHANOINE, C., SAADI, A., GUYOT-LENFANT, M., HEBBRECHT, C. and GALLIEN, C.L. (1990b). Myosin structure in the eel (*Anguilla anguilla* L.). Demonstration of three heavy chains in adult lateral muscle. *FEBS Lett. 277:* 200-204.

CHEVALLIER, A. and KIENY, M. (1982). On the role of the connective tissue in the patterning of the chick limb musculature. *Roux Arch. Entw.Mech. Org. 191:* 277-280.

CHEVALLIER, A., PAUTOU, M.P., HARIS, A.J. and KIENY, M. (1987). On the non-equivalence of skeletal muscle satellite cells and embryonic myoblasts. *Arch. Anat. Microsc. Morphol. Exp. 75:* 161-166.

COUTEAUX, R. and MIRA, J.C. (1985). Remodelling of neuromuscular junctions during repair of muscle fibres. In *Molecular Basis of Nerve Activity* (Eds. J.P. Changeux, F. Hucho, A. Maelicke and E. Neumann). De Gruyter, Berlin, pp. 35-45.

COUTEAUX, R., MIRA, J.C. and D'ALBIS, A. (1988). Regeneration of muscles after cardiotoxin injury. I. Cytological aspects. *Biol. Cell 62:* 171-182.

D'ALBIS, A. and BUTLER-BROWNE, G. (1993). The hormonal control of myosin isoform expression in skeletal muscle of mammals: a review. *Basic Appl. Myol. 3:* 7-16.

D'ALBIS, A., COUTEAUX, R., JANMOT, C. and MIRA, J.C. (1989). Myosin isoform transitions in regeneration of fast and slow muscles during postnatal development of the rat. *Dev. Biol. 135:* 320-325.

D'ALBIS, A., COUTEAUX, R., JANMOT, C., ROULET, A. and MIRA, J.C. (1988). Regeneration after cardiotoxin injury of innervated and denervated slow and fast muscles of mammals. Myosin isoform analysis. *Eur. J. Biochem. 174:* 103-110.

D'ALBIS, A., GUYOT-LENFANT, M., JANMOT, C., CHANOINE, C., WEINMAN, J. and GALLIEN, C.L. (1987a). Regulation by thyroid hormones of terminal differentiation in the skeletal dorsal muscle. I. Neonate mouse. *Dev. Biol. 123:* 25-32.

D'ALBIS, A., JANMOT, C. and BECHET, J.J. (1985). Myosin switches in skeletal muscle development of an urodelan amphibian *Pleurodeles waltlii*. Comparison with a mammalian, *Mus musculus*. *Biochem. Biophys. Res. Commun. 128:* 94-100.

D'ALBIS, A., WEINMAN, J., MIRA, J.C., JANMOT, C. and COUTEAUX, R. (1987b). Regulatory role of thyroid hormones in myogenesis. Analysis of myosin isoforms during muscle regeneration. *C.R. Acad. Sci. (III) 305:* 697-702.

FRANQUINET, R., AAMIRI, A., GAUTRON, J. and LE MOIGNE, A. (1988). *In vitro* proliferation and differentiation of myogenic cells from adult *Xenopus*. *Biol. Struct. Morphol. 1:* 84-88.

JOHNSTON, I.A., PATTERSON, S., WARD, P. and GOLDSPINK, G. (1974). The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. *Can. J. Zool 52:* 871-877.

KORDYLEWSKI, L., FABER, J., GORLICH, A. and KILARSKI, W. (1989). Histochemistry and isomyosins of tail musculature in *Xenopus*. *J. Muscle Res. Cell Motil. 10:* 290-296.

LAEMMLI, U. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:* 680-684.

LANNERGREN, J. (1987). Contractile properties and myosin isoenzymes of various kinds of *Xenopus* twitch muscle fibers. *J. Muscle Res. Cell Motil. 8:* 260-273.

LELOUP, J. and BUSCAGLIA, M. (1977). La triiodothyronine, hormone de la métamorphose des amphibiens. *C.R. Acad. Sci. Paris 284D:* 2261-2263.

LO, D.C., ALLEN, F. and BROCKES, J.P. (1993). Reversal of muscle differentiation during urodele limb regeneration. *Proc. Natl. Acad. Sci. USA 90:* 7230-7234.

MARECHAL, G., SCHWARTZ, K., BECKERS-BLEUKX, G. and GHINS, E. (1984). Isozymes of myosin in growing and regenerating rat muscles. *Eur. J. Biochem. 138:* 421-428.

MARTINEZ, I., OFSTAD, R. and OLSEN, R.L. (1990). Myosin isoforms in red and white muscles of some marine teleost fishes. *J. Muscle Res. Cell Motil. 11:* 489-495.

MAURO, A. (1961). Satellite cell of skeletal muscle fibers. *J. Biophys. Biochem. Cytol. 9:* 493-498.

O'FARRELL, P.H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem. 250:* 4007-4021.

PADYKULA, H.A. and HERMAN, E. (1955). The specificity of the histochemical method of adenosine triphosphatase. *J. Histochem. Cytochem. 17:* 170-195.

PLAGHKI, L. (1985). Régénération et myogénèse du muscle strié. *J. Physiol. (Paris) 80:* 51-110.

PLISZKA, B., STRZELECKA-GOLASZEWSKA, H., PANTALONI, C. and D'ALBIS, A. (1981). Comparison of myosin isoenzymes from slow-tonic and fast-twitch fibers of frog muscle. *Eur. J. Cell Biol. 25:* 144-149.

RADICE, G.P. and MALACINSKI, G.M. (1989). Expression of myosin heavy chain transcripts during *Xenopus laevis* development. *Dev. Biol. 133:* 562-568.

SAADI, A., GALLIEN, C.L., GUYOT-LENFANT, M. and CHANOINE, C. (1993). A new approach of urodele amphibian limb regeneration: study of myosin isoforms and their control by thyroid hormone. *Mech. Dev. 43:* 49-56.

SNOW, M.H. (1977). Myogenic cell formation in regenerating rat muscle injured by mincing. II. An autoradiographic study. *Anat. Rec. 188:* 200-218.

WHALEN, R.G., HARRIS, J.B., BUTLER-BROWNE, G.S. and SESODIA, S. (1990). Expression of myosin isoforms during notexin-induced regeneration of rat soleus muscles. *Dev. Biol. 141:* 24-40.

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