The Journal of Experimental Biology 209, 372-379
Published by The Company of Biologists 2006
doi:10.1242/jeb.02006

In situ hybridisation of a large repertoire of muscle-specific transcripts in fish larvae: the new superficial slow-twitch fibres exhibit characteristics of fast-twitch differentiation

F. Chauvigné, C. Ralliere, C. Cauty and P. Y. Rescan*

National Institute for Agricultural Research, the Joint Unit Research for Fish Physiology, Biodiversity and the Environment, INRA Scribe, IFR140, Campus de Beaulieu, 35042 Rennes, France

*Author for correspondence (e-mail: pierre-yves.rescan@rennes.inra.fr)

Accepted 17 November 2005

Summary

Much of the present information on muscle differentiation in fish concerns the early embryonic stages. To learn more about the maturation and the diversification of the fish myotomal fibres in later stages of ontogeny, we investigated, by means of *in situ* hybridisation, the developmental expression of a large repertoire of muscle-specific genes in trout larvae from hatching to yolk resorption. At hatching, transcripts for fast and slow muscle protein isoforms, namely myosins, tropomyosins, troponins and myosin binding protein C were present in the deep fast and the superficial slow areas of the myotome, respectively. During myotome expansion that follows hatching, the expression of fast isoforms became progressively confined to the borders of the fast muscle mass, whereas, in contrast, slow muscle isoform transcripts were uniformly expressed in all the slow fibres. Transcripts for several enzymes involved in oxidative metabolism such as citrate synthase, cytochrome oxidase component IV and succinate dehydrogenase, were present throughout the whole myotome of hatching embryos but in later stages became concentrated in slow fibre as well as in lateral fast fibres. Surprisingly, the slow fibres that are added externally to the single superficial layer of the embryonic (original) slow muscle fibres expressed not only slow twitch muscle isoforms but also, transiently, a subset of fast twitch muscle isoforms including MyLC1, MyLC3, MyHC and myosin binding protein C. Taken together these observations show that the growth of the myotome of the fish larvae is associated with complex patterns of muscular gene expression and demonstrate the unexpected presence of fast muscle isoform-expressing fibres in the most superficial part of the slow muscle.

Key words: fish, larva, myogenesis, contractile protein, *in situ* hybridisation, expressed sequence tags.

Introduction

Adult fish have two main skeletal muscle types anatomically separated in the myotome: a superficial layer of slow oxidative fibres (the red muscle) covering a deeper, larger mass of fast glycolytic fibres (the white muscle). Very often, a layer of fibres called intermediate fibres, identified on the basis of their histochemical properties, separates the slow and the fast muscle fibres. It is now well established that the embryonic formation of the superficial slow muscle fibres involves the lateral migration of adaxial cells, which flank the notochord, from the deep part of the myotome toward the lateral surface (Devoto et al., 1996; Stoiber, 1998). The fast muscle fibres of the myotomal muscle arise from non-migrating lateral presomitic cells that do not contact the notochord. The specification of the different muscle cell identities depends upon distinct levels of activity of the morphogens of the hedgehog family (Du et al., 1997; Blagden et al., 1997; Wolff et al., 2003). The early differentiation of embryonic muscle fibres in fish embryos involves a complex temporal sequence of gene activations (Xu et al., 2000; Hall et al., 2003) including transient expression of fast (Chauvigné et al., 2005; Bryson-Richardson et al., 2005) as well as slow (Chauvigné et al., 2005) muscle isoforms in superficial slow fibres and deep fast fibres, respectively. Following embryonic myogenesis, an expansion of the myotome occurs in fish larvae involving the recruitment of new muscle fibres in germinal zones (Veggetti et al., 1990; Johnston et al., 1998; Galloway et al., 1999; Rowlerson and Veggetti, 2001). It has been shown that the addition of new slow fibres in the borders of the myotome does occur even in zebrafish mutants that lack hedgehog signalling (Barresi et al., 2001). This indicates that the mechanisms regulating the identity of the new slow fibres produced in larvae are distinct from those specifying the slow fate of adaxial cells in early embryos. In spite of the developmental importance of the muscle expansion

occurring in the larvae, very little has been reported regarding the gene activations that lead to the differentiation of new muscle fibres and the maturation of original (embryonic) muscle fibres in post-hatching embryos. In this study, we took advantage of the identification of a large repertoire of muscle ESTs for examining the developmental expression of muscle genes in the expanding myotome of fish larvae. Our major finding is that the slow fibres that are added externally to the single superficial layer of embryonic slow muscle transiently express several fast muscle isoforms. This challenges the simple view that the superficial part of the developing fish myotome is composed of a population of muscle fibres with only slow characteristics.

Materials and methods

Fertilisation, incubation conditions and fish rearing

Eggs from rainbow trout *Oncorhynchus mykiss* Walbaum were collected in the experimental facilities of the INRA Drennec fish farm (Finistère, France). After artificial insemination, eggs were incubated at 10°C in recirculating dechlorinated water. Chemical water parameters were regularly monitored. Oxygen levels were always above 98% saturation. Before fixation, larvae were rapidly anaesthetised with phenoxyethanol (Sigma, Poole, UK).

Simple and double in situ hybridisation of sections.

Fast myosin heavy chain, slow myosin heavy chain, fast myosin light chain 1 and fast myosin light chain 3 cDNAs have been characterised previously (Gauvry and Fauconneau, 1996; Rescan et al., 2001; Thiebaud et al., 2001). Other muscle-specific cDNAs have been identified from a large-scale, rainbow trout 3′ and 5′ sequencing project (AGENAE research program). Digoxigenin-labelled antisense RNA probes were synthesised from a PCR-amplified template using T3 RNA polymerase. Single and double *in situ* hybridisations were performed on transverse sections of rainbow trout embryos according to the method of Gabillard et al. (2003) with minor modifications. The double in situ hybridisations were performed with fluorescent markers. The green fluorescence was obtained, once the hybridisation was performed, by incubation of sections with mouse anti-digoxigenin antibody (Roche) followed by an incubation with Alexa Fluor 488-conjugated rabbit-derived anti-mouse IgG antibodies (Molecular Probes, Leiden, The Netherlands). The red fluorescence was obtained by incubation of sections with goat anti-fluorescein antibodies (Vector, Peterborough, UK) followed by incubation with Alexa Fluor 594-conjugated donkey-derived anti-goat IgG antibodies (Molecular Probes). FITC (revealing Alexa Fluor 488) and Texas Red (revealing Alexa Fluor 594) filters were then used for confocal microscopy. The confocal microscope system used in this study was a Leica TCS NT (Milton Keynes, UK) equipped with Kr/Ar laser and mounted on a Leica DMB microscope

Results

Fast muscle isoform transcripts accumulate in differing proportions in the borders of the fast muscle mass of the developing fish larvae

At prehatching stages, around 27 d.p.f. (days post-fertilisation) the genes for the fast muscle-specific proteins

Table 1. *Summary and accession numbers of the muscle-specific cDNA clones used for the in situ hybridisation experiments*

Gene name	Accession no.	Most homologous cDNA	Accession no.	aa sequence identity (%)
(A)				
Fast myosin heavy chain	Z48794	*Onchorhynchus mykiss* fast skeletal myosin heavy chain	CAA88724	–
Fast myosin light chain 1	AF330140	*Onchorhynchus mykiss* fast myosin light chain 1	AAK60614	–
Fast myosin light chain 3	AF330141	*Onchorhynchus mykiss* fast myosin light chain 3	AAK60615	–
Fast tropomyosin	BX078951	*Salmo salar* fast myotomal muscle tropomyosin	AAB36559	99
Fast troponin C	BX078175	*Danio rerio* fast skeletal muscle troponin C	AAF78473	92
Fast troponin I	BX077831	*Salmo salar* fast myotomal muscle troponin I	AAC23580	95
Fast protein C	BX079906	*Homo sapiens* fast myosin binding protein C	Q14324	52
Slow myosin heavy chain	AF211172	*Onchorhynchus mykiss* slow myosin heavy chain	AAF74412	–
Slow myosin light chain 1	BX076946	*Caranx delicatissimus* myosin light chain 1	BAB69817	83
Slow troponin T	BX075548	*Salmo trutta* slow myotomal muscle troponin T isoform 1S	AAB58912	90
Slow protein C	BX309915	*Homo sapiens* slow myosin binding protein C	NP_002456	59
(B)				
Aldolase A	BX081163	*Danio rerio* aldolase A	AAG94593	88
Enolase β	BX085209	*Danio rerio* enolase 3 (β, muscle)	NP_999888	94
Lactate dehydrogenase A	BX077037	*Chromis xanthochira* lactate dehydrogenase A	AAP44528	88
Succinate dehydogenase	BX083576	*Danio rerio* succinate dehydrogenase complex, subunit A	AAH45885	97
Cytochrome oxydase	BX307833	*Danio rerio* cytochrome *c* oxidase subunit IV isoform 1	AAQ91229	78
Citrate synthase	BX865536	*Danio rerio* citrate synthase	AAH45362	91

(A) Genes encoding components of striated muscle cytoarchitecture; (B) genes encoding metabolic enzymes.

[myosin heavy chain (MyHC), myosin light chain (MyLC1, MyLC3), troponin C, troponin I, myosin binding protein C and tropomyosin; Table 1) were uniformly expressed in the deep fast muscle fibres (Fig. 1A–C). From hatching (35 d.p.f.) onwards, while several myosepta separating adjacent W-shaped myotomes are visible in transverse sections, we observed a progressive rarefaction of the fast muscle transcripts within the fibres of the deep domain of the myotome while they were abundant in the fibres located in dorsal, ventral and lateral domains of the fast muscle mass. (Fig. 1D–I). Double *in situ* hybridisation with fluorescent antisense riboprobes revealed, in post-hatched embryos, that there was not a strict overlap of the labelling of the fast muscle isoform transcripts (Fig. 2A–C). This indicates that fast muscle isoform transcripts are present in differing proportions in fast muscle fibres.

The most lateral fast fibres of the fish larvae express enzymes involved in oxidative activity

To know more about the metabolic differentiation of the muscle fibres of the fish larvae, we investigated the developmental expression of several enzymes involved in muscle metabolism. At the eyed stage (20 d.p.f.), transcripts for citrate synthase, cytochrome oxidase component IV and succinate dehydrogenase, were present throughout the whole myotome. This indicated that the energy supply in the superficial slow and the deep fast muscle fibres depends mostly on aerobic metabolism. As the myotome matures, at around 45 d.p.f., these transcripts that encode enzymes of the aerobic metabolic pathway became confined to the superficial slow fibres and, to a lesser extent, to the lateral fast fibres (Fig. 3A–C). However, intense staining for enolase β, lactate dehydrogenase A and aldolase A transcripts, which encode enzymes regulating anaerobic glycogenolysis, were found throughout the whole fast muscle mass. Taken together, these observations demonstrate the emergence, during yolk resorption, of a distinct lateral subpopulation of fast aerobic fibres.

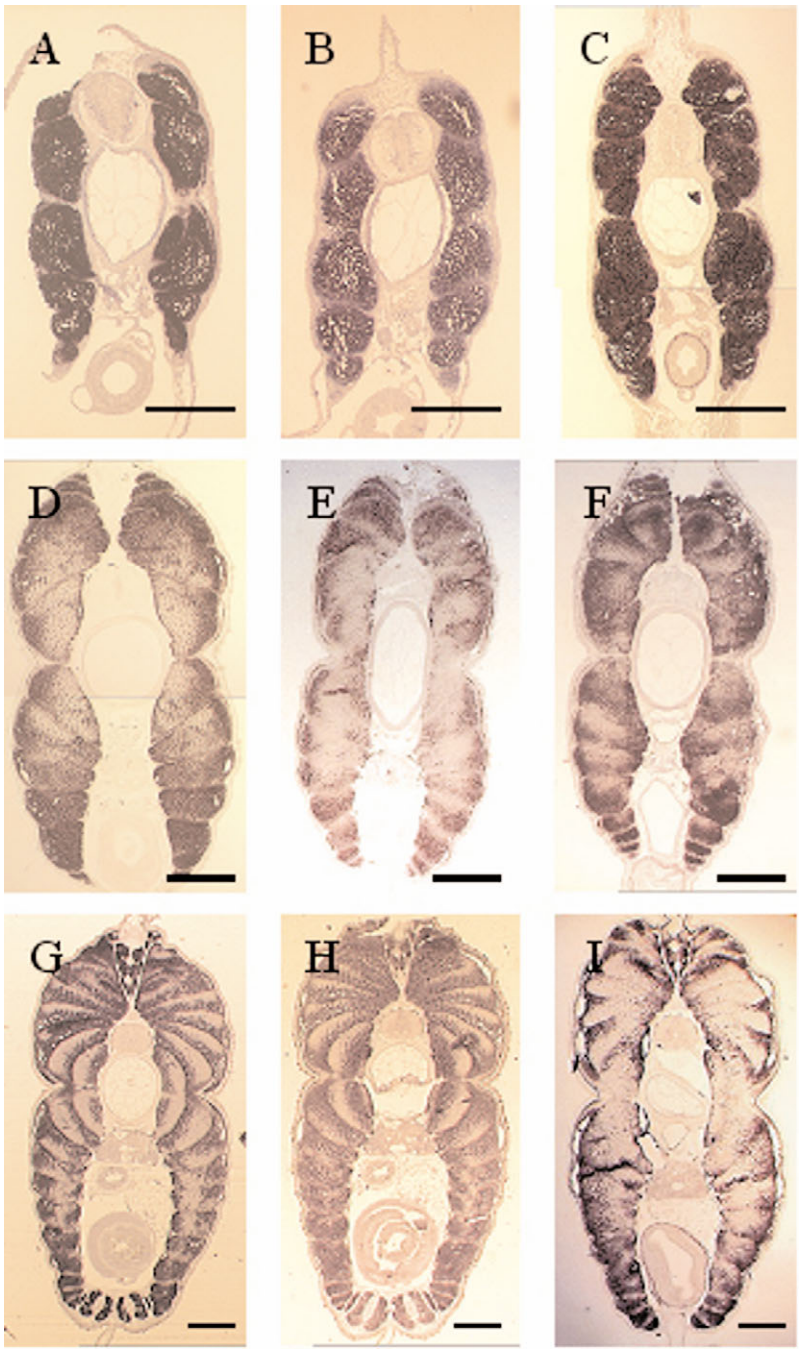

Fig. 1. Expression of fast myosin heavy chain (MyHC; A,D,G), fast myosin binding protein C (B,E,H), and fast tropomyosin (C,F,I) in trout larvae. Transverse sections through the myotome at 27 (A–C), 41 (D–F) and 50 (G–I) days post-fertilisation. Fast isoform transcripts initially present throughout the whole fast muscle mass (A–C) become confined to the edges of adjacent myotomes (D–I). Scale bars, 200 μm.

The most external slow fibres of the fish larvae express a subset of fast muscle isoforms

During the larval stages, the superficial slow muscle layer thickens by the addition of new layers of muscle fibres externally to the single superficial layer of original (embryonic) slow muscle fibres (Fig. 4). This recruitment is predominant in the regions closest to the lateral line. During this myotome expansion, slow myosins (light and heavy chains), slow troponin T and slow myosin binding protein C were expressed in both deep embryonic (original) and superficial neoformed slow fibres (Fig. 5A–D). However, unexpectedly, several fast isoforms RNAs, including MyLC1, MyLC3, MyHC, as well as fast myosin binding protein C, but not fast tropomyosin nor fast troponin I and fast troponin C, were found to selectively accumulate in the superficial neoformed slow muscle. This expression was first observed in

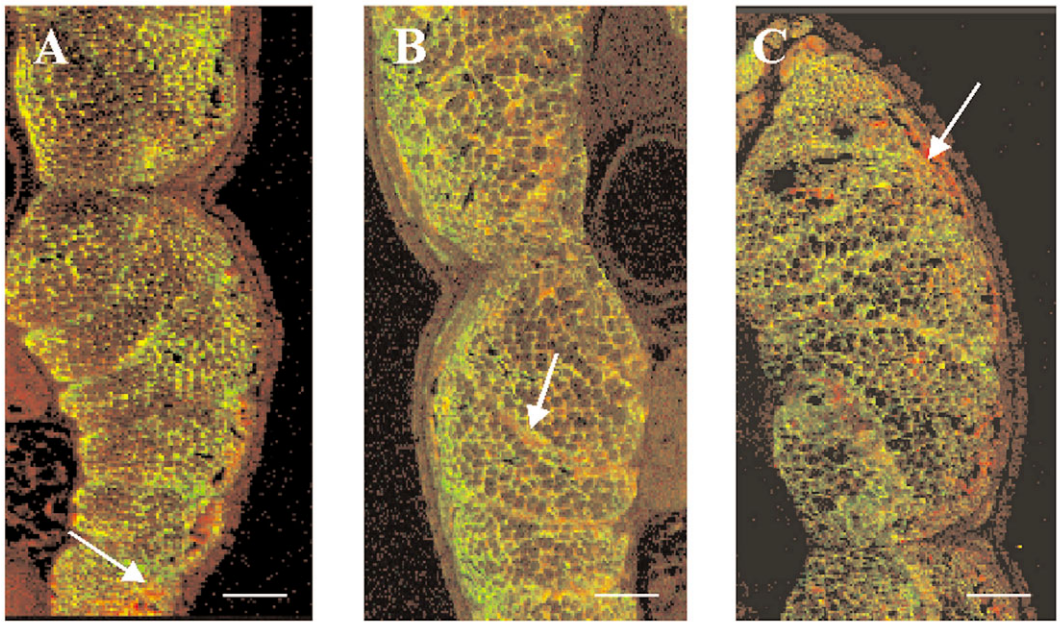

Fig. 2. Merged immunofluorescence images of transverse sections of fast muscle at 48 days post-fertilisation. Expression of (A) fast myosin heavy chain (MyHC; green) and fast troponin C (red), (B) fast MyHC (green) and fast tropomyosin (red), (C) fast MyHC (green) and fast myosin light chain 1 (MyLC1; red). Incomplete overlap of the fluorescent markers (arrows) show that fast muscle fibres contain differing proportions of fast-twitch muscle isoform transcripts. Scale bars, 100 μm.

40 d.p.f. embryos, in a monolayer of small slow fibres covering the original embryonic slow fibres (Fig. 6A–D). In later stages of myotome expansion, whereas the slow muscle had thickened by the addition of multilayers of neoformed fibres, the expression of fast isoforms was observable only within the most superficial neoformed slow fibres and was no longer detectable in the deeper layers of neoformed slow fibres (Fig. 6E–H). This shows that the expression of fast isoforms was only transient in the neoformed slow fibres. Double *in situ* hybridisation using a MyLC1 probe specific for the slow twitch fibres and probes corresponding to fast MyLC1, fast MyLC3, fast MyHC and fast myosin binding protein C (Fig. 6I–K) further confirmed that the external fibres of the slow muscle exhibited both slow and fast characteristics.

Discussion

Although the cellular mechanisms of post-embryonic muscle growth has been described in many fish species (for reviews, see Stoiber et al., 1999; Rowlerson and Veggetti, 2001) very little is known regarding the gene transcriptions that underlie the differentiation of newly recruited muscle fibres

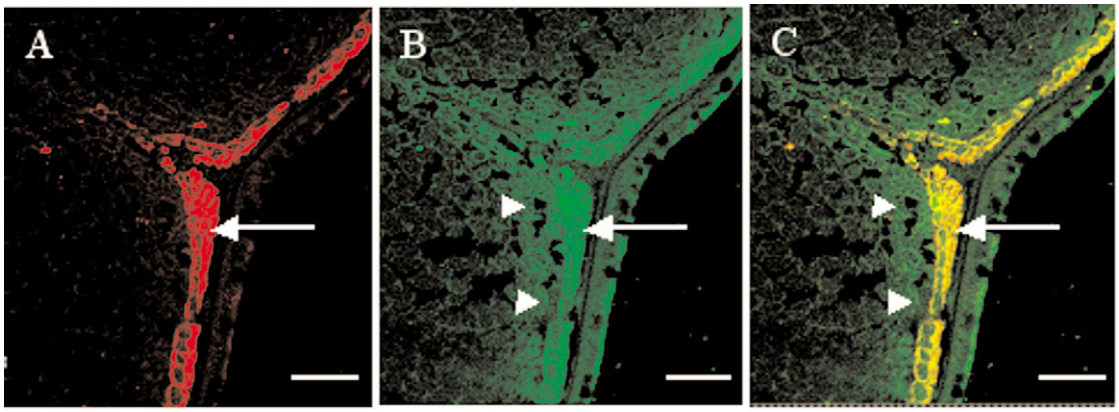

Fig. 3. Expression of slow myosin light chain 1 (MyLC1; red) and cytochrome C oxidase subunit IV (green) transcripts in 48 days post-fertilisation trout larvae. Separate (A,B) and merged (C) immunofluorescence images of transverse sections at the horizontal septum. Cytochrome *c* oxidase subunit IV transcripts accumulate in slow MyLC1-expressing superficial muscle fibres (arrows) and in lateral fast muscle fibres (arrowheads). Scale bars, 80 μm.

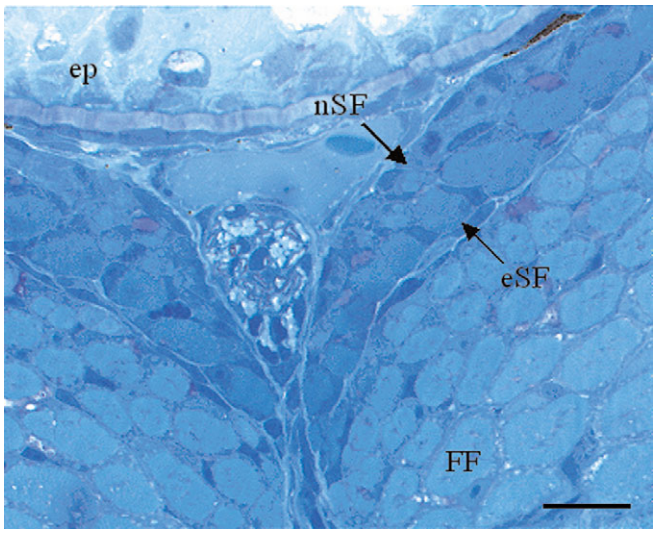

Fig. 4. Transverse section of lateral muscle at 44 days post-fertilisation. Semi-thin section showing region around the horizontal septum; Richardson stain. New small diameter slow fibres (nSF) are added laterally to the original embryonic monolayer of slow fibres (eSF). FF, fast fibres; ep, epidermis. Scale bars, 20 μm.

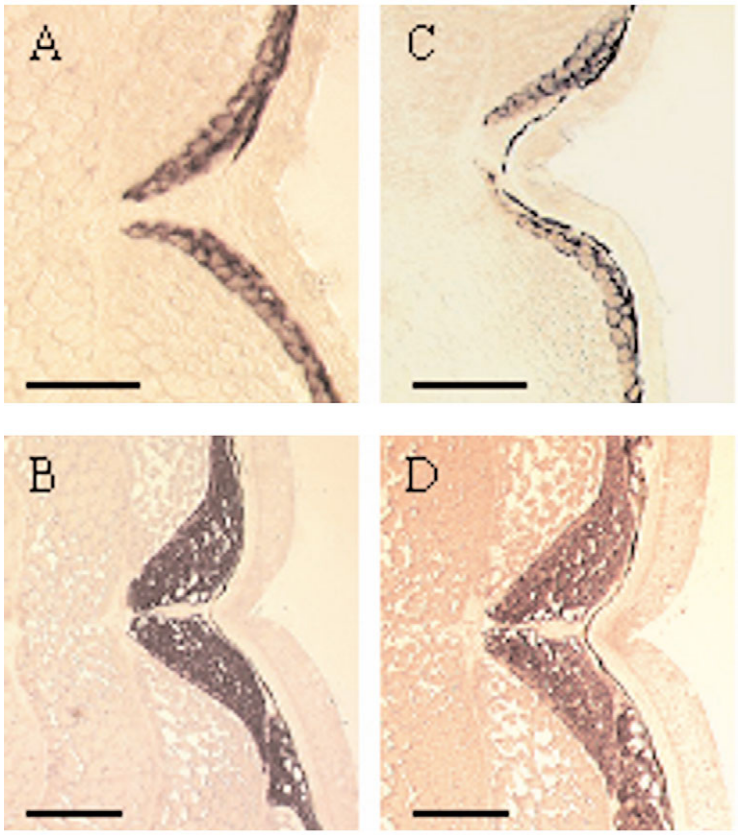

Fig. 5. Expression of slow myosin light chain 1 (MyLC1; A,B) and slow troponin T (C,D). Transverse sections at the horizontal septum of trout larvae 41 (A,C) and 76 (B,D) days post-fertilisation. Slow MyLC1 and slow troponin T isoform transcripts accumulate in all the slow muscle fibres. Scale bars, 40 μm.

and the maturation of embryonic muscle fibres in fish larvae. In this study, to complete our knowledge on fibre differentiation during muscle expansion in fish larvae, we carried out single and double in situ hybridisation with a large repertoire of muscle-specific riboprobes. Our results show that around the time of hatching, slow and fast muscle isoforms, such as myosin, tropomyosin and troponin, were distributed throughout the slow and fast muscle, respectively. This spatial distribution of fast and slow muscle isoform transcripts is consistent with the previous identification of two major fibre types in recently hatched zebrafish larvae as shown by means of immunohistochemistry using anti-myosin sera (Van Raamsdonk et al., 1982). However, in the course of post-hatching development, a redistribution of transcripts for fast muscle isoforms was observed in the growing myotome: these latter accumulated mostly in the borders of the fast muscle mass. Furthermore, double in situ hybridisation revealed that the fast isoform transcripts accumulated in differing proportions within individual fast fibres, revealing an unexpected fibre diversity in fast muscle. In the light of observations on the spatial distribution of different sized white fibres in fish larvae (Veggetti et al., 1990; Rowlerson and Vegetti, 2001; Galloway et al., 1999) and more especially in salmonid larvae (Stickland et al., 1988), it is likely that the fibres situated in the borders of the fast muscle and that accumulate high level of fast isoform transcripts correspond to muscle fibres added during myotome expansion of the trout larvae. In keeping with this growth pattern, it has been suggested that myogenic cells originating from the perimyotomal mesenchymal tissue are a possible source of new muscle fibres (Stoiber and Sänger, 1996).

Regarding the metabolic differentiation, we observed that transcripts for enzymes of the aerobic metabolic pathways were present throughout the whole myotome at the eyed stage. This observation is consistent with the general statement that energy metabolism in embryos and larvae of fish is almost entirely aerobic (Wieser, 1995). During subsequent maturation of the myotome, transcripts encoding enzymes of the aerobic metabolic pathway were found to selectively accumulate in the superficial slow oxidative fibres and, to a lesser extent, in the most lateral fast muscle fibres, which were also shown to express enzymes of glycolysis. Given their location and their metabolic properties, it is probable that these lateral fast fibres correspond to the presumptive intermediate or pink muscle previously identified in older fish on the basis of their histochemical properties, such as the pH sensitivity of the mATPase and intermediate SDH activity (Sanger and Stoiber, 2001). The appearance of fast aerobic muscle fibres at the end of yolk resorption in trout coincides with that observed in zebrafish and red sea bream (van Raamsdonk et al., 1982; Matsuoka and Iwai, 1984). Recently, Wolff et al. (2003) have identified a subpopulation of fast fibres

(MFFs) that express the Engrailed 1 and 2 homoproteins but not slow MyHC in 24 h post-fertilisation zebrafish embryos. Given the location of these Engrailed-expressing fast muscle fibres beneath the superficial slow fibres and in the vicinity of the horizontal myoseptum, it would be of interest to determine whether this subpopulation of fast fibres prefigures the aerobic fast muscle fibres. To our knowledge, so far, no intermediate muscle fibre-specific myosin isoforms have been reported, although peptide mapping has suggested they may exist (Scapolo and Rowlerson, 1987). The generalisation of *in situ* hybridisations of muscle-specific EST-derived riboprobes in various fish species will help, in the near future, to determine whether a specific contractile differentiation, if any, takes place in the intermediate muscle fibres.

Our major and unexpected finding was that a subset of superficial slow fibres exhibit fast characteristics. Indeed, simple and double *in situ* hybridisation showed that the small diameter slow fibres that are added laterally to the embryonic slow fibres co-expressed both slow and fast-twitch muscle isoforms, in particular fast myosin heavy and light chains. The expression of fast muscle isoforms in the superficial slow muscle is consistent with, and extends, the work by Johnston

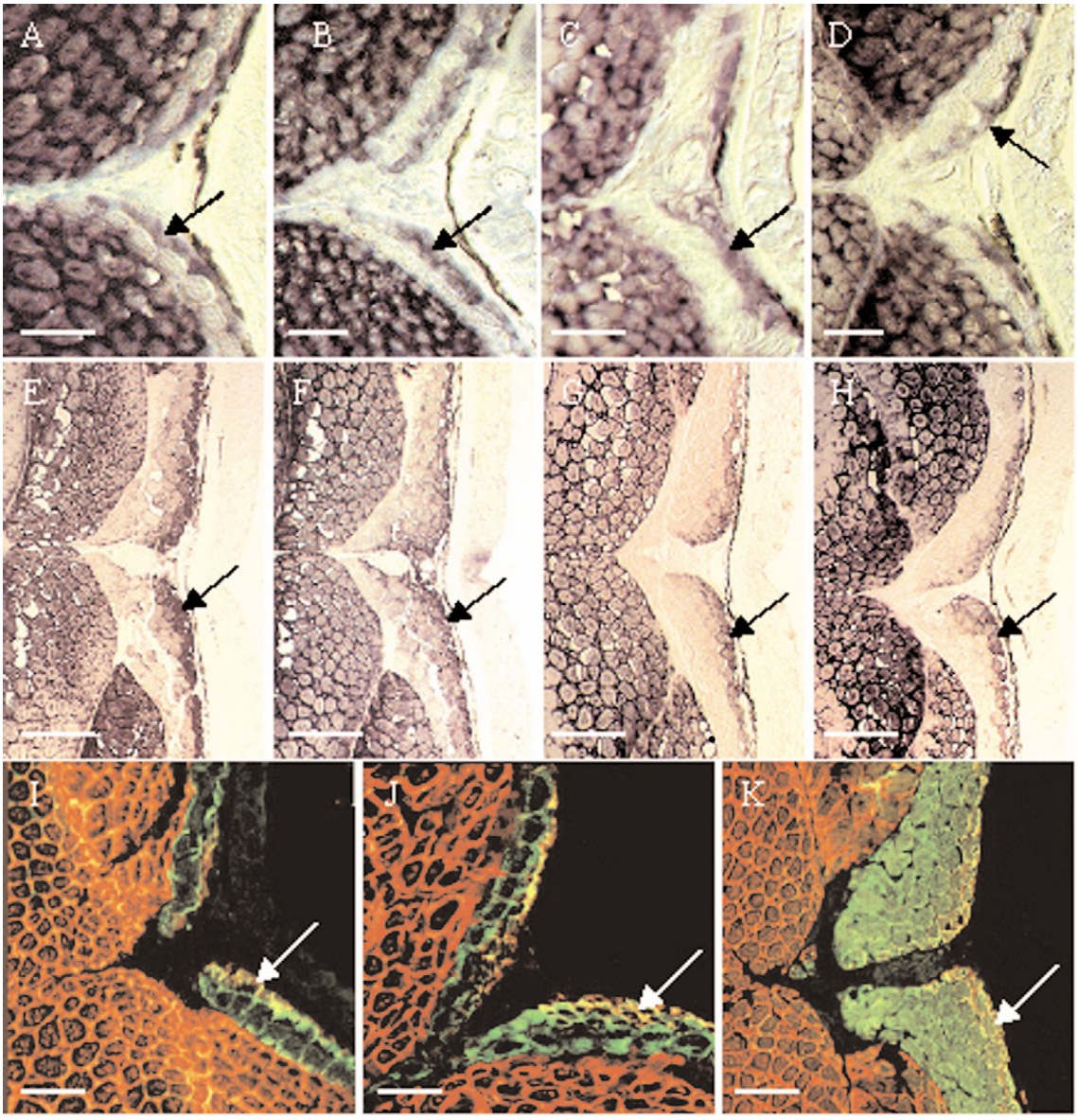

Fig. 6. Expression of fast myosin heavy chain (MyHC; A,E), fast myosin binding protein C (B,F), fast myosin light chain 1 (MyLC1; C,G), fast MyLC3 (D,H), and slow MyLC1 (green) and fast MyHC (red) (I–K) in trout larvae. Transverse sections of lateral myotomal muscle at 41 (A–D and I), 48 (J) and 76 (E–H,K) days post-fertilisation. Labelling for fast muscle isoforms is observed in fast muscle fibres and in the superficial layers of slow fibres (arrows, A–H). Merged immunofluorescence images (I–K) show that the most external layers of superficial fibres express both slow and fast muscle isoforms (see arrows showing the overlap of the two fluorescent markers). Scale bars, 40 μm (A–D); 80 μm (E–H); 40 μm (I–J) and 80 μm (K).

et al. (1998) who reported, using peptide mapping, the presence of adult fast myosin light chain isoforms in superficial slow muscle extracts of herring yolk-sac larvae. Our observations are also consistent with previous histochemical data reporting variable myosin ATPase (m-ATPase) activity between deep and peripheral slow muscle fibres of gilthead seabream larvae (Ramirez-Zarzoza, 1995), and support the pioneer work by Mascarello and colleagues (1995) showing heterogeneous myosin immunostaining in slow muscle fibres of developing sea bream larvae. It is important to note, however, that in contrast to fast growing large fish species such as trout or sea bream, small fish such as zebrafish, which are largely used for developmental studies, display limited recruitment of new slow myofibres from hatching onwards. Therefore, we can not exclude that some phenotypic variations may exist in post-embryonic muscle fibres, from one fish species to another, in relation to growth patterns.

The expression of fast twitch muscle isoforms in newly recruited slow fibres is only transient, showing that the final phenotype of the neoformed superficial fibres in larvae is ultimately slow. It is important to note that such a down-regulation of fast muscle genes has also been observed early in the adaxially derived embryonic slow fibres during trout (Chauvigné et al., 2005) and zebrafish (Bryson-Richardson et al., 2005) development. The expression of fast skeletal muscle isoform in embryonic muscle fibres destined to become slow has also been described previously in chick (Sweeney et al., 1989) showing that this property is largely shared among vertebrates. Thus the transient expression of fast muscle isoforms may be necessary to build the initial myofibrillar infrastructure in nascent slow muscle fibres before the complete replacement of fast muscle isoforms by slow ones. The gradual acquisition of the slow phenotype in newly recruited superficial fibres during larval life raises a question about the mechanisms controlling the developmental programme expression of muscle genes in these fibres. It is possible, as shown in differentiating primary and secondary myofibres in chick (Lefeuvre et al., 1996), that neuronal inputs resulting from the invasion of motoneurones in myotome participates to the regulation of muscle-specific gene transcription in fish larvae. In order to investigate this, it would be of interest to use surgical and pharmacological methods to interfere with innervation of the fish larvae myotome. Also, signalling molecules that are secreted by neighbouring tissues may influence the myogenic programme of newly recruited slow muscle fibres. In this regard, it is interesting to note that Wnt11, a member of a family secreted proteins implicated in vertebrate myotome patterning, is produced by the adaxially derived embryonic slow fibres (Makita et al., 1998). This inductive influence may regulate, through the stabilisation of cytosolic β catenin (Seidensticker and Behrens, 2000) the differentiation programme of myogenic cells that form new slow fibres laterally to the embryonic slow fibres. One of the best ways to test the hypothesis of such a regulation would be to examine the phenotypic properties of nascent myofibres during the larval period in the zebrafish mutant *silberblick* that lacks the Wnt11 signalling pathway (Heisenberg et al., 2000). Several other mutants such as *pipetail, you too* and *Acerebellar* that lack the Wnt5, sonic hedgehog and Fgf8 signalling pathways, respectively (Rauch et al., 1997; Ingham and Kim, 2005; Reifers et al., 1998) may also be useful for examining the regulation of the myogenic programme of nascent slow fibres by signalling molecules.

The cellular source of the newly recruited larval slow fibres is not yet clearly identified. In fish embryos some external cells separate the superficial slow fibres from the epidermis. These cells, which have been described in zebrafish (Waterman, 1969), herring (Johnston, 1993), sea bass and sea bream (Veggetti et al., 1990; Lopez-Albors et al., 1998), have been proposed to be, during the larval period, a source of myoblasts contributing to the addition of new superficial slow muscle fibres in the region close to the lateral line (Veggetti et al., 1990). This hypothesis is supported by the recent observation that external cells on the surface of the fish myotome express the myogenic determination genes *Pax3* and *Pax7* (Groves et al., 2005; Devoto et al., 2005). However, the fact that the external cells do not exhibit feature of muscle differentiation such as the presence of myofibrils and that they are positive for collagen I led to the suggestion that they form an epithelial cell layer sharing many characteristics with amniote dermatome (Rescan et al., 2005). These two interpretations are, however, not exclusive and it is quite possible that the external cell layer produces both myogenic and dermal precursors. If so, the fish external cell layer would form a structure homologous to the dermomyotome in the amniotes (Devoto et al., 2005). Nevertheless, it is clear that only a lineage tracing analysis can definitely demonstrate that the somitic external cell layer does produce myogenic precursors contributing to the recruitment of muscle fibres during fish larval stages.

This work was supported by grants from the Institut National de la Recherche Agronomique, OFIMER, IFOP and the CIPA. We thank François Piumi for providing the EST cDNA clones and Roselyne Primault for her help in the observation of sections by confocal microscopy (Service de Microscopie Electronique, Faculté de Médecine, Université de Rennes I, France).

References

Barresi, M. J. F., D'Angelo, J. A., Hernandez, L. P. and Devoto, S. H. (2001). Distinct mechanisms regulate slow-muscle development. *Curr. Biol.* **11**, 1432-1438.

Blagden, C. S., Currie, P. D., Ingham, P. W. and Hughes, S. M. (1997). Notochord induction of zebrafish slow muscle mediated by Sonic hedgehog. *Genes Dev.* **11**, 2163-2175.

Bryson-Richardson, R. J., Daggett, D. F., Cortes, F., Neyt, C., Keenan, D. G. and Currie, P. D. (2005). Myosin heavy chain expression in zebrafish and slow muscle composition. *Dev. Dyn.* **233**, 1018-1022.

Chauvigné, F., Cauty, C., Ralliere, C. and Rescan, P. Y. (2005). Muscle fiber differentiation in fish embryos as shown by in situ hybridization of a large repertoire of muscle-specific transcripts. *Dev. Dyn.* **233**, 659-666.

Devoto, S. H., Melancon, E., Eisen, J. S. and Westerfield, M. (1996).

Identification of separate slow and fast muscle precursor cells *in vivo*, prior to somite formation. *Development* **122**, 3371-3380.

Devoto, S. H., Stoiber, W., Hammond, C. L., Steinbacher, P., Haslett, J. R., Barresi, M. J. F., Patterson, S. E., Adiarte, E. and Hugues, S. M. (2005). Generality of vertebrate developmental patterns: evidence for a dermomyotome in fish. *Evol. Dev.* (in press).

Du, S. J., Devoto, S. H., Westerfield, M. and Moon, R. T. (1997). Positive and negative regulation of muscle cell identity by members of the hedgehog and TGF-beta gene families. *J. Cell Biol.* **139**, 145-156.

Gabillard, J. C., Duval, H., Cauty, C., Rescan, P. Y., Weil, C. and Le Bail, P. Y. (2003). Differential expression of the two GH genes during embryonic development of rainbow trout *Oncorhynchus mykiss* in relation with the IGFs system. *Mol. Reprod. Dev.* **64**, 32-40.

Galloway, T. F., Kjorsvik, E. and Kryvi, H. (1999). Muscle growth and development in Atlantic cod larvae (*Gadus morhua* L.), related to different somatic growth rates. *J. Exp. Biol.* **202**, 2111-2120.

Gauvry, L. and Fauconneau, B. (1996). Cloning of a trout fast skeletal myosin heavy chain expressed both in embryo and adult muscles and in myotubes neoformed in vitro. *Comp. Biochem. Physiol. Biol.* **115B**, 183-190.

Groves, J. A., Hammond, C. L. and Hugues, S. H. (2005). Fgf8 drives myogenic progression of a novel lateral fast muscle fibre population in zebrafish. *Development* **132**, 4211-4222.

Hall, T. E., Cole, N. J. and Johnston, I. A. (2003). Temperature and the expression of seven muscle-specific protein genes during embryogenesis in the Atlantic cod *Gadus morhua* L. *J. Exp. Biol.* **206**, 3187-3200.

Heisenberg, C. P., Tada, M., Rauch, G. J., Saude, L., Concha, M. L., Geisler, R., Stemple, D. L., Smith, J. C. and Wilson, S. W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76-81.

Ingham, P. W. and Kim, H. R. (2005). Hedgehog signalling and the specification of muscle cell identity in the zebrafish embryo. *Exp. Cell Res.* **306**, 336-342.

Johnston, I. A. (1993). Temperature influences muscle differentiation and the relative timing of organogenesis in herring (*Clupea harengus*) larvae. *Mar. Biol.* **116**, 363-379.

Johnston, I. A., Cole, N. J., Abercromby, M. and Viera, V. L. A. (1998). Embryonic temperature modulates muscle growth characteristics in larval and juvenile herring. *J. Exp. Biol.* **201**, 623-646.

Lefeuvre, B., Crossin, F., Fontaine-Perus, J., Bandman, E. and Gardahaut, M. F. (1996). Innervation regulates myosin heavy chain isoform expression in developing skeletal muscle fibers. *Mech. Dev.* **58**, 115-127.

Lopez-Albors, O., Gil, F., Ramirez-Zarzosa, G., Vazquez, J. M., Latorre, R., Garcia-Alcazar, A., Arencibia, A. and Moreno, F. (1998). Muscle development in gilthead sea bream (*Sparus aurata*, L.) and sea bass (*Dicentrarchus labrax*, L.): further histochemical and ultrastructural aspects. *Anat. Histol. Embryol.* **27**, 223-229.

Makita, R., Mizuno, T., Koshida, S., Kuroiwa, A. and Takeda, H. (1998). Zebrafish wnt11: pattern and regulation of the expression by the yolk cell and No tail activity. *Mech. Dev.* **71**, 165-176.

Mascarello, F., Rowlerson, A., Radaelli, G., Scapolo, P. A. and Veggetti, A. (1995). Differentiation and growth of muscle in the fish *Sparus aurata* (L): I. Myosin expression and organization of fibre types in lateral muscle from hatching to adult. *J. Muscle Res. Cell Motil.* **16**, 213-222.

Matsuoka, M. and Iwai, T. (1984). Development of the myotomal musculature in the red sea bream. *Bull. Jpn. Soc. Fish.* **50**, 29-35.

Ramirez-Zarzosa, G., Gil, F., Latorre, R., Ortega, A., Garcia-Alcaraz, A., Abellan, E., Vazquez, J. M., Lopez-Albors, O., Arencibia, A. and Moreno, F. (1995). The larval development of lateral musculature in gilthead sea bream sparus aurata and sea bass *Dicentrarchus labrax*. *Cell Tissue Res.* **280**, 217-224.

Rauch, G. J., Hammerschmidt, M., Blader, P., Schauerte, H. E., Strahle, U., Ingham, P. W., McMahon, A. P. and Haffter, P. (1997). Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harb. Symp. Quant. Biol.* **62**, 227-234.

Reifers, F., Bohli, H., Walsh, E. C., Crossley, P. H., Stainier, D. Y. and Brand, M. (1998). Fgf8 is mutated in zebrafish *acerebellar* (*ace*) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. *Development* **125**, 2381-2395.

Rescan, P. Y., Collet, B., Ralliere, C., Cauty, C., Delalande, J. M., Goldspink, G. and Fauconneau, B. (2001). Red and white muscle development in the trout (*Oncorhynchus mykiss*) as shown by *in situ* hybridisation of fast and slow myosin heavy chain transcripts. *J. Exp. Biol.* **204**, 2097-2101.

Rescan, P. Y., Ralliere, C., Chauvigné, F. and Cauty, C. (2005). Expression patterns of collagen I (α1) encoding gene and muscle-specific genes reveal that the lateral domain of the fish somite forms a connective tissue surrounding the myotome. *Dev. Dyn.* **233**, 605-611.

Rowlerson, A. and Veggetti, A. (2001). Cellular mechanisms of post-embryonic muscle growth in aquaculture species. In *Muscle Development and Growth. Fish Physiology Series,* vol. 18 (ed. I. A. Johnston), pp. 103-140. San Diego: Academic Press.

Sanger, A. M. and Stoiber, W. (2001). Muscle fiber diversity and plasticity. In *Muscle Development and Growth. Fish Physiology Series,* vol. 18 (ed. I. A. Johnston), pp. 187-250. San Diego: Academic Press.

Scapolo, P. A. and Rowlerson, A. (1987). Pink lateral muscle in the carp (*Cyprinus carpio* L.) histochemical properties and myosin composition. *Experientia* **43**, 384-386.

Seidensticker, M. J. and Behrens, J. (2000). Biochemical interactions in the Wnt pathway. *Biochim. Biophys. Acta* **1495**, 168-182.

Stickland, N. C., White, R. N., Mescall, P. E., Crook, A. R. and Thorpe, J. E. (1988). The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L.). *Anat. Embryol. (Berl.)* **178**, 253-257.

Stoiber, W. and Sanger, A. M. (1996). An electron microscopic investigation into the possible source of new muscle fibres in teleost fish. *Anat. Embryol. (Berl.)* **194**, 569-579.

Stoiber, W., Haslett, J. R., Goldschmid, A. and Sanger, A. M. (1998). Patterns of superficial fibre formation in the European pearlfish (*Rutilus frisii meidinger*i) provide a general template for slow muscle development in teleost fish. *Anat. Embryol.* **197**, 485-496.

Stoiber, W., Haslett, J. R., Goldschmid, A. and Sanger, A. M. (1999). Myogenic patterns in teleosts: what does the present evidence really suggest? *J. Fish Biol.* **55**, 84-89.

Sweeney, L. J., Kennedy, J. M., Zak, R., Kokjohn, K. and Kelley, S. W. (1989). Evidence for expression of a common myosin heavy chain phenotype in future fast and slow skeletal muscle during initial stages of avian embryogenesis. *Dev. Biol.* **133**, 361-374.

Thiebaud, P., Rescan, P. Y., Barillot, W., Ralliere, C. and Theze, N. (2001). Developmental program expression of myosin alkali light chain and skeletal actin genes in the rainbow trout *Oncorhynchus mykiss*. *Biochim. Biophys. Acta* **1519**, 139-142.

van Raamsdonk, W., van't Veer, L., Veeken, K., Heyting, C. and Pool, C. W. (1982). Differentiation of muscle fiber types in the teleost *Brachydanio rerio*, the zebrafish. *Anat. Embryol.* **164**, 51-62.

Veggetti, A., Mascarello, F., Scapolo, P. A. and Rowlerson, A. (1990). Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax* (L.). An ultrastructural and morphometric study. *Anat. Embryol.* **182**, 1-10.

Waterman, R. E. (1969). Development of the lateral musculature in the teleost, *Brachydanio rerio*: a fine structural study. *Am. J. Anat.* **125**, 457-494.

Wieser, W. (1995). Energetics of fish larvae, the smallest vertebrates. *Acta Physiol. Scand.* **154**, 279-290.

Wolff, C., Roy, S. and Ingham, P. W. (2003). Multiple muscle cell identities induced by distinct levels and timing of hedgehog activity in the zebrafish embryo. *Curr. Biol.* **13**, 1169-1181.

Xu, Y., He, J., Wang, X., Lim, T. M. and Gong, Z. (2000). Asynchronous activation of 10 muscle-specific protein (MSP) genes during zebrafish somitogenesis. *Dev. Dyn.* **219**, 201-215.