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Short Contribution

Restricted expression of the zebrafish *hsp90α* gene in slow and fast muscle fiber lineages

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ABSTRACT Members of the heat shock protein 90 (Hsp90) family of molecular chaperones play important roles in allowing a select group of intracellular signaling molecules reach and maintain functionally active conformations. We have previously shown that *hsp90α* gene expression in early zebrafish embryos is restricted to a subgroup of paraxial-mesoderm derived somitic cells prior to muscle formation and that the gene is downregulated in mature trunk and tail muscle fibers. Here we have compared the expression of the *hsp90α* gene to muscle regulatory genes during development of slow and fast muscle fibers in normal embryos and in embryos carrying mutations which affect somitic muscle formation. We show that *hsp90α* is first expressed early during the development of slow somitic muscle progenitors shortly following *myoD* activation and at a point prior to or co-incident with the expression of other known muscle regulatory genes. Expression of *hsp90α* is also activated in the midline of *flh* mutants when these cells switch from a notochord to a muscle fate. Conversely, expression is not detectable in cells of the paraxial mesoderm lineage which fail to converge in *spt* mutants and which do not activate expression of other muscle specific marker genes. Finally, expression of *hsp90α* is downregulated in slow muscle fibers by 24 h of age but becomes detectable in the later developing fast fibers at this time. Thus, *hsp90α* is expressed in developing muscle progenitors during short temporal and spatial windows of both slow and fast fiber lineages in the zebrafish somite.

KEY WORDS: *heat shock protein, Hsp90, myogenesis, MyoD, zebrafish*

We have previously shown that expression of the heat shock protein 90alpha (*hsp90α*) gene is restricted to a subgroup of developing muscle cells during normal development of the zebrafish somite at control temperatures (Sass *et al.*, 1996). These data were interesting in light of the fact that Hsp90 interacts with a number of molecules involved in signal transduction pathways and the regulation of gene expression in other vertebrates, including several which play important roles during striated muscle development. In the latter category, biochemical studies have shown that murine Hsp90 can stimulate the DNA binding activity of the myogenic regulatory protein MyoD *in vitro* (Shaknovich *et al.*, 1992; Shue and Kohtz, 1994). Hsp90 also interacts with casein kinase II, which has recently been implicated as a potential regulator of MyoD activity during mammalian myogenesis (Johnson *et al.*, 1996). Further, we have also reported that *hsp90α* mRNA is enriched in the developing somites of chicken embryos (Sass and Krone, 1997), a result which suggests that elevated levels of *hsp90* gene expression are required for aspects of muscle development in other vertebrates as well.

In order to more clearly define the expression pattern of the *hsp90α* gene in zebrafish somitic muscle, whole-mount *in situ* hybridization was carried out on early and mid-somitogenesis stage embryos. As shown in Figure 1, the *hsp90α* gene is strongly expressed in the cells known as adaxial cells, which lie on either side of the notochord. These cells will subsequently give rise to the two types of zebrafish slow muscle known as muscle pioneers and non-muscle pioneer slow fibers (Devoto *et al.*, 1996; see also Fig. 3). Expression is detectable prior to segmentation (open arrow in panel A) at a temporal point approximately 2-3 h following activation of the earliest known marker of adaxial cells, *myoD* (Weinberg *et al.*, 1996), but prior to *myogenin* (panel C) and prior to or coincident with a number of other muscle specific markers such as members of the *mef2* family (data not shown; Ticho *et al.*, 1996). Slightly later in development, expression of *hsp90α* remains strongest in the adaxial cells but is also detectable at low levels in the

Abbreviations used in this paper: hsp, heat shock protein; flh, floating head; spt, spadetail.

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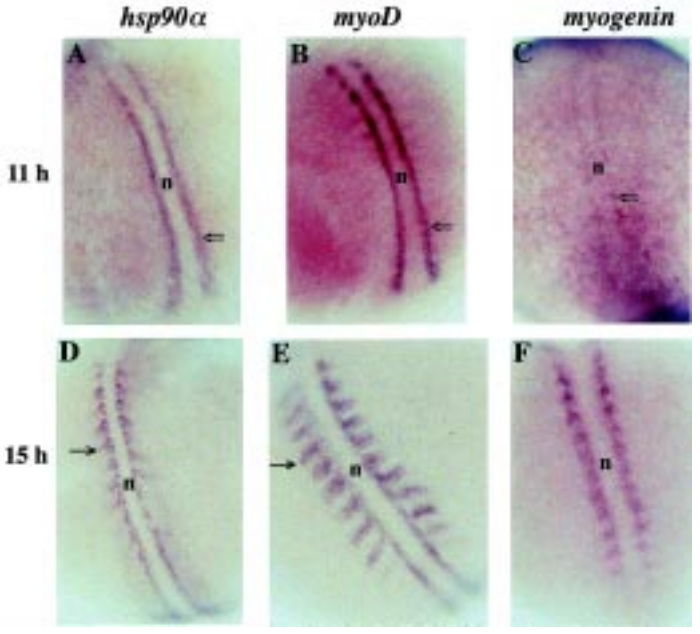

Fig. 1. Analysis of *hsp90α, myoD,* and *myogenin* expression in early and mid-somitogenesis zebrafish embryos using whole-mount *in situ* hybridization analysis. *All embryos are shown in dorsal view with anterior to the top of the panel. Both hsp90α and myoD are strongly expressed in the adaxial cell progenitors of somitic slow muscle fibers prior to segmentation (open arrows in* **A** *and* **B**). *Expression of myogenin is barely detectable at this stage (open arrow in* **C**). *Cells lateral to the adaxial cells express much lower levels of hsp90α mRNA by the 11-12 somite stage (solid arrow in* **D**); *many of these cells express higher levels of myoD at this point in development* **(E)**. *n, notochord.*

presomitic and somitic cells which lie lateral to the adaxial cells (solid arrow in panel D) and which will later give rise to fast muscle fibers (Devoto *et al.*, 1996). In all embryos, expression occurs in only a subset of *myoD*-positive lateral cells and is not detectable in the notochord (n) or the neural tube (see also Fig. 3).

We next turned to our attention to several zebrafish mutants which exhibit aberrations in somitic muscle formation. If *hsp90α* expression is activated as part of the muscle development pathway, we would expect expression of the gene to be altered in a predictable fashion when cells in these embryos switch either towards or away from a striated muscle fate. In embryos carrying the *floating head* mutation (*flh*^{n1}; a 2bp deletion in the zebrafish *Xnot* homolog; Halpern *et al.*, 1995; Talbot *et al.*, 1995), axial cells which would normally form notochord switch to a striated muscle fate and express characteristics typical of adaxial cells (e.g. *myoD*; Fig. 2, panel D; Halpern *et al.*, 1995; Melby *et al.*, 1996). If *hsp90α* is expressed as part of the muscle development program, we would predict that it should be activated in these cells as well. As shown in Figure 2, these transfated cells strongly express the *hsp90α* gene early during their formation (compare staining at arrows in panels A and D to the non-staining notochord (n) in panels A and B of Fig. 1). In *spadetail* (*spt*^{b106}) embryos, which represents a mutation in a zebrafish T-box gene (Kimmel *et al.*, 1989; Griffin *et al.*, 1998), cells of the paraxial mesoderm lineage fail to converge dorsally and fail to become incorporated into the trunk, resulting in an embryo with very little trunk muscle. These cells instead enter the tail bud to form the enlarged "spade" tail and do not express muscle specific markers (Weinberg *et al.*, 1996). Unlike the trunk, somitic muscle in the tail of these embryos develops relatively normally. As expected if *hsp90α* is expressed as part of the muscle development program, *hsp90α* mRNA levels are greatly reduced in 15 h old *spt* embryos (Fig. 2, panels B and E) and the gene is not expressed in mismigrated cells of the paraxial mesoderm lineage which form the "spade" tail (asterisk in panel C). Trunk expression is only detectable in isolated islands of cells which later form small sporadic clusters of trunk muscle (solid arrows in panels B,C,E, and F; Kimmel *et al.,* 1989). However, both *hsp90α* and *myoD* are strongly expressed in the relatively normal somitic muscle of the tail (open arrows). These data clearly show that *hsp90α* is expressed in a predictable fashion in cells which switch fates either towards or away from a muscle fate in embryos carrying these mutations.

In order to more clearly define the expression of *hsp90α* in different somitic muscle fiber lineages, we examined expression in sections of embryos at different points during formation of slow and fast fibers. As shown in Figure 3, panel B, the gene is strongly expressed in the three to five rows of adaxial cells which lie immediately adjacent to the notochord prior to segmentation.

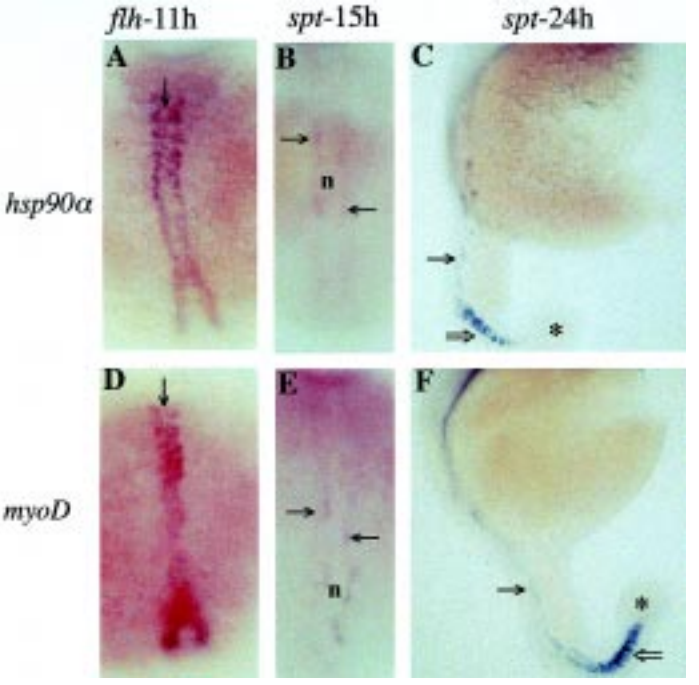

Fig. 2. Analysis of *hsp90α* expression in embryos carrying the *flh*^{n1} and *spt*^{b106} mutations. *Eleven and 15 h embryos are dorsal views whereas 24 h embryos are in lateral view. Anterior is to the top in all panels. Expression of hsp90α is activated in axial cells of flh mutants which would normally form notochord but instead switch to a striated muscle fate (arrowhead in* **A**). *Myotomes in these embryos eventually fuse below the neural tube (Halpern et al., 1995). These axial cells do not normally express hsp90α or other muscle markers in wild-type embryos (see Fig. 1). Expression of hsp90α and muscle markers is reduced in the trunk of spt embryos with only a few isolated patches of expressing cells visible (solid arrows in* **B,C,E** *and* **F***) whereas relatively normal expression of hsp90α and myoD is observed in the normal tail muscle (open arrows). The mismigrated cells which eventually end up in the expanded tailbud express neither hsp90α nor other muscle markers (asterisks in panels C and F). n, notochord*

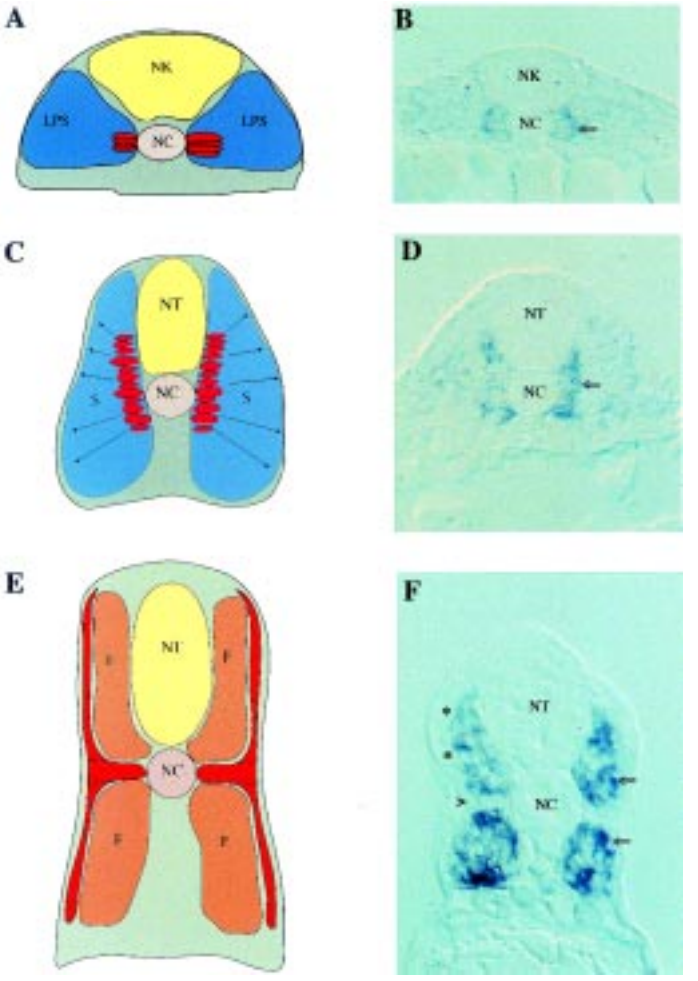

Fig. 3. The *hsp90α* gene is expressed during formation of both slow and fast muscle fibers. (A,C *and* **E)** *Schematic diagrams of slow and fast muscle development in the zebrafish somite as described by Devoto et al., 1996.* **(B,D** *and* **F)** *Cross sections through embryos stained for hsp90α mRNA at comparable stages of somite formation. Devoto et al. (1996) have previously demonstrated that slow and fast muscle fibers are identifiable as molecularly and anatomically distinct populations of cells within the early zebrafish somite. The progenitors of slow muscle fibers are the adaxial cells which lie laterally adjacent to the notochord and can be identified by their distinct morphology and their early expression of myoD (Weinberg et al., 1996; red cells in panel A). Following segmentation, these cells move dorsally and ventrally to form a column of elongated cells along the notochord and neural tube which spans the anterio-posterior length of the somite (red cells in panel C). Shortly thereafter, a subpopulation of these cells migrate through the somite and assume a final position at the periphery of the myotome as non-muscle pioneer slow muscle. The other subpopulation of these cells, which will develop into muscle pioneers, elongate laterally to the periphery of the somite but retain their contact with the notochord in 24 h embryos (panel E). Fast muscle fibers form later in development and do not begin to express their phenotype until migration of non-muscle pioneer slow muscle to the periphery of the myotome (orange in panel E). The hsp90α gene is strongly expressed in the developing adaxial cells prior to segmentation (open arrow in panel B) and during the time period when these cells form a single column which spans the anterior-posterior length of the somite (open arrow in panel D). At 24 h of development, hsp90α mRNA is detectable in neither the muscle pioneer (arrowhead in panel F) nor the non-muscle pioneer slow fibers (asterisk in panel F). However, strong expression of the gene now occurs in the developing fast muscle fibers which have recently initiated their differentiation (open arrow in panel F). LPS, lateral presomitic cells; NC, notochord; NK, neural keel; S, somite: NT, neural tube; F, developing fast muscle fibers. Slow muscle fibers and their progenitors, the adaxial cells, are shown in red.*

Following segmentation, the gene continues to be expressed within these slow muscle progenitors as they move dorsally and ventrally to form a single column of approximately 20 cells adjacent to the notochord and neural tube (panel D). However, expression of the gene is no longer detectable in the non-muscle pioneer slow fibers following their migration through the somite and subsequent differentiation in their final position at the periphery of the myotome (asterisks in panel F). The muscle pioneer slow muscle cells, which span the myotome at the dorsal-ventral midline, have also downregulated the *hsp90α* gene at this point (arrowhead in panel F). However, the gene is now strongly expressed in the fast muscle (open arrows), which begins to develop following the migration of the slow fibers to the periphery of the myotome. We have previously shown that the gene is subsequently downregulated in all somitic muscle by 40-48 h of age (Sass *et al.*, 1996). Thus, expression of *hsp90α* is limited to relatively short temporal windows during formation of both slow and fast somitic muscle fibers and expression is not detectable as part of the mature fiber phenotype. In summary, the data presented here indicate that the *hsp90α* gene is expressed as part of the muscle development program in both slow and fast fibers of zebrafish. This suggests that a specific requirement for *hsp90α* gene expression may exist during formation of both slow and fast striated muscle and that the previously identified biochemical interactions of Hsp90 with muscle regulatory proteins may be of biological significance. This is further supported by recent studies in our laboratory which have shown that inhibition of Hsp90 function early during zebrafish development disrupts muscle pioneer development at a temporal point which coincides with *hsp90α* expression (Lele *et al.*, 1999).

Experimental Procedures

Zebrafish

Adult zebrafish were maintained according to standard methods (Westerfield, 1995) and staged as described by Kimmel *et al.* (1995). In all experiments, embryos were maintained and staged at a normal incubation temperature of 28.5°C.

Whole-mount in situ hybridization

Digoxigenin-11-UTP (Boehringer-Mannheim) labeled sense and antisense RNA probes were synthesized by *in vitro* transcription. The *in situ* hybridization protocol of Puschel *et al.* (1992) was used with minor modifications (Akimenko *et al.*, 1994; Sass *et al.*, 1996; Lele and Krone, 1997). As templates, we used the previously described cDNAs encoding zebrafish *myoD* and *myogenin* (Weinberg *et al.*, 1996), and *hsp90α* (Krone and Sass, 1994).

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