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Dystrobrevin and dystrophin family gene expression in zebrafish

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Abstract

Dystrophin/dystrobrevin superfamily proteins play structural and signalling roles at the plasma membrane of many cell types. Defects in them or the associated multiprotein complex cause a range of neuromuscular disorders. Members of the dystrophin branch of the family form heterodimers with members of the dystrobrevin branch, mediated by their coiled-coil domains. To determine which combinations of these proteins might interact during embryonic development, we set out to characterise the gene expression pattern of dystrophin and dystrobrevin family members in zebrafish. γ -dystrobrevin (*dtng*), a novel dystrobrevin recently identified in fish, is the predominant form of dystrobrevin in embryonic development. *Dtng* and *dmd* (*dystrophin*) have similar spatial and temporal expression patterns in muscle, where transcripts are localized to the ends of differentiated fibres at the somite borders. *Dtng* is expressed in the notochord while *dmd* is expressed in the chordo-neural hinge and then in floor plate and hypochord. In addition, *dtng* is dynamically expressed in rhombomeres 2 and 4–6 of the hindbrain and in the ventral midbrain. α -dystrobrevin (*dtna*) is expressed widely in the brain with particularly strong expression in the hypothalamus and the telencephalon; *drp2* is also expressed widely in the brain. *Utrophin* expression is found in early pronephros and lateral line development and *utrophin* and *dystrophin* are both expressed later in the gut. β -dystrobrevin (*dtnb*) is expressed in the pronephric duct and widely at low levels. In summary, we find clear instances of co-expression of dystrophin and dystrobrevin family members in muscle, brain and pronephric duct development and many examples of strong and specific expression of members of one family but not the other, an intriguing finding given the presumed heterodimeric state of these molecules.

Keywords

muscle; zebrafish; notochord; midbrain; rhombomere; gene expression; utrophin; dystrophin; dystrobrevin; drp2; dystrotelin

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1.0 Results and Discussion

1.1 Relative expression of dystrophin and dystrobrevin family members

We recently described the structure and phylogenetic relationships of the metazoan members of the dystrophin/dystrobrevin/dystrotelin superfamily (Jin et al., 2007). These sub-plasma membrane cytoskeletal proteins and their associated membrane complexes are of significant cell biological and clinical interest due to their role in cell-matrix interactions and their dysfunction in many muscular dystrophies (Cohn and Campbell, 2000). Members of the dystrophin branch of the superfamily (one protein in invertebrates, three paralogous proteins in vertebrates) have been shown to interact with members of the dystrobrevin branch (one protein in invertebrates, two or three paralogous proteins in vertebrates) both genetically and physically, in both mice and nematodes (Gieseler et al., 1999a; Gieseler et al., 1999b; Grady et al., 1999; Sadoulet-Puccio et al., 1997). This heterodimerisation between dystrophins and dystrobrevins is mediated by protein domains which vary little (dystrophins) or not at all (dystrobrevins) between the paralogues, and the interactions appear to be correspondingly relatively promiscuous (Sadoulet-Puccio et al., 1997). Presumably, therefore, the composition of dystrophin/dystrobrevin complexes is largely determined by their overlapping patterns of expression at the cellular and subcellular level. We therefore set out to analyse the co-expression of the superfamily members during development of the zebrafish. Dystrophin and its plasma membrane anchor dystroglycan are expressed in the embryo and larva of the zebrafish, *Danio rerio* (Bolanos-Jimenez et al., 2001a; Bolanos-Jimenez et al., 2001b; Parsons et al., 2002; Fig. 1). *Dystrophin* expression has been reported in skeletal muscle and is required in muscle for plasma membrane integrity (Bassett et al., 2003) (Fig. 1I). *Dystrophin* paralogues *utrophin* and *dystrophin-related protein 2* (*drp2*) are also expressed in muscle and other tissues in amniotes (Dixon et al., 1997; Schofield et al., 1993) as well as in fish (Fig. 1J-O, U, V). Zebrafish have three *dystrobrevin* genes: α -, β - and γ -*dystrobrevin* (*dtna*, *dtnb*, *dtng*), whereas amniotes have only α and β (Jin et al., 2007). Analysis of EST frequencies revealed that *dtna* and *dtnb* transcripts are much less abundant than those of *dtng* (Fig. 1A; Jin et al., 2007), and this is confirmed by RT-PCR analysis of mRNA from several stages of zebrafish embryos (Fig. 1B). Whereas *dmd*, *utrn*, *dtna*, *dtng* and *dytn* (*dystrotelin*) show more-or-less constant levels of expression in the first 5 days (5d) of life, *drp2*, *dp116* (a short isoform of *dmd*), *G-utrn* (the utrophin paralogue of the *dp116* transcript), and *dtnb* levels increase between 16h and 5d of development.

1.2 Expression of dystrobrevins and dystrophins in axial structures

By in situ mRNA hybridisation, *dtng* mRNA is expressed strongly from at least 8 somites stage (8s) in the notochord (Fig. 1C, C' and data not shown). Notochord expression of *dtng* declines at 17s, so that by 22s it is detected only in the less matured posterior notochord (Fig. 1D), and by 24 hpf is no longer detected (Fig. 1E, E' and data not shown). *Dystrophin* (*dmd*) is expressed in the axial chorda mesoderm at tailbud (tb) stage (Fig. 1F and F') and remains strong in the posterior floor plate and the chordo-neural hinge in a similar way to *shhb* (Ekker et al., 1995) and in the hypochord (Fig. 1G,H,H'; www.zfin.org and data not shown). It is likely that the axial expression is mainly of Dp71 transcripts as shown by (Bolanos-Jimenez et al. (2001b). This short form of *dmd* can be detected by our 3' UTR probe. Another *dmd* transcript, Dp116, seems to be expressed only from 5d (Fig. 1B)-in amniotes Dp116 expression is confined to the central and peripheral nervous systems (Byers et al., 1993; Schofield et al., 1994). Like *dtng*, *dmd* mRNA expression in the axial structures declines by 24 hpf (Fig. 1I and I'). *Utrn*, *dtna*, *dtnb* and *drp2* transcripts are detected very weakly in the notochord at early stages of somitogenesis (Fig. 1J,Q, S, U) and none are significantly expressed in the notochord or other axis structures at 24 hpf (Fig. 1O, R, T, V). Dystrophin and Dp71, as well as utrophin and dystroglycan, have been reported to be expressed in the mouse floor plate (Wertz and Fuchtbauer, 1998; Schofield et al., 1995); we

found no reports of axial expression of dystrobrevins. A possible binding partner for dystrophin and γ -dystrobrevin in the plasma membrane of the axial structures could be dystroglycan, which is expressed at early somitogenesis in all midline structures and later in the hypochord (Parsons et al., 2002). Several laminins are also expressed in these structures (Pollard et al., 2006; Scott and Stemple, 2005). We conclude that, before and at early somitogenesis stages, two genes are expressed strongly in axial structures: *dtng* in the notochord and *dmd* dorsal to the notochord, in the floor plate and ventral in the hypochord. Both genes become down-regulated in the midline by the end of somitogenesis. The other genes in these families have little expression in the notochord.

1.3 Expression of dystrobrevins and dystrophins in kidney, gut and lateral line development

Utrophin is expressed in the pronephric duct, detected by both the *utr*n probe (detects all *utr*n transcripts) and the *R-utr*n probe (detects specifically the rod domain of full length utrophin and not the short G-utrophin) as early as 13-15s (Fig. 1J-M). This expression remains strong at 21-22s, 24 and 48 hpf (Fig. 1N and O, 2L and L'). *Dtnb* is expressed in the pronephric duct at 48 hpf (Fig. 2K and K'). No detectable levels of *dtng*, *dmd*, *dtna* or *drp2* mRNAs were found in the pronephric duct (Fig. 1C-I, Q-V and Fig. 2A-J and data not shown). In mice, several isoforms of utrophin, dystrophin, α - and β -dystrobrevin are expressed in the developing kidney and the urogenital system (Blake et al., 1998; Blake et al., 1995; Durbeek et al., 1997; Haenggi et al., 2005; Rees et al., 2007). Our data show that during early kidney development in zebrafish, *utrophin* from the dystrophin family and β -*dystrobrevin* from the dystrobrevin family are potential binding partners.

At later stages, we observed dystrophin, utrophin and β -sarcoglycan proteins in the hindgut (Fig. 2O, R, U, U'). By this stage the intestinal musculature is formed and it is likely that these proteins are part of the smooth muscle DGC complex. In mice, utrophin is also expressed in gut and intestinal epithelia (Driss et al., 2006) where it is mainly driven by its A-promoter (Takahashi et al., 2005). Murine α -dystrobrevin is strongly expressed in the developing gut and intestinal smooth muscle (Lien et al., 2004). At later stages, other proteins from the dystrophin family are also present in mouse intestinal smooth muscle in mice (Straub et al., 1999).

*Utr*n is also expressed from around 24 hpf in the lateral line primordia (Fig. 1O) and is detected at least until 48 hpf in the lateral line neuromasts (Fig. 2L and L'). We have not detected any other dystrobrevin/dystrophin family gene expression in the lateral line. *Dag1* (*dystroglycan*) and *cav1* (*Caveolin 1*) are expressed in the lateral line neuromasts (www.zfin.org; Nixon et al., 2007). Both were recently shown to form a complex with utrophin in human umbilical vein endothelial cells (Ramirez-Sanchez et al., 2007), and may therefore form a complex with utrophin in the lateral line system.

1.4 Expression of dystrophins and dystrobrevins in muscle

At the early stages of somitogenesis, *dmd* is detected weakly in muscle cells (Fig. 1E; www.zfin.org; Bolanos-Jimenez et al., 2001b; Bassett et al., 2003). As the slow cells migrate and fast fibres differentiate around 18-21s (Blagden et al., 1997; Devoto et al., 1996), *dmd* mRNA is up-regulated and becomes localised to the somite borders with a location and timing that parallels elongation and intersomitic attachment of terminally differentiated slow and fast fibres, starting in the rostralmost somites (Fig. 2A; www.zfin.org; Bassett et al., 2003). *Dmd* mRNA remains localised at somite borders at 48hpf, and 5d of development and probably beyond (Fig. 2B,C,E and data not shown). It becomes detectable between 1-3d in several head muscles (compare Fig. 2D and 3O). *Dtng* mRNA is also localized at the anterior and posterior termini of differentiated muscle fibres

around 18–21s (Fig. 2F), and later at 24hpf, 48 hpf, and until at least 5d (Fig. 2G, H and data not shown). Thus, *dtng* and *dmd* mRNAs appear to co-localize in muscle of developing zebrafish embryos. No localized somite expression of *dtna* and *dtnc* mRNA is detected within the myotome prior to 24 hpf (Fig. 1J–M), but after this time a weak signal appears to be excluded from the somite borders (Fig. 2I and K). In mice *dystrophin* and α -*dystrobrevin* are localised mainly to the sarcolemma of muscle fibres (Wertz and Fuchtbauer, 1998; Lien et al., 2004), whereas β -*dystrobrevin* is not expressed in mouse skeletal muscle (Blake et al., 1998). Zebrafish *utrophin* mRNA is very weakly detected in medial somites at 13s, especially when the pan-*utrn* probe was used (Fig. 1J and K). At 22s, *utrn* mRNA is localized to the somite borders, as are *dmd* and *dtng* mRNAs (Fig. 1N). This localization is still evident at 24 hpf in the chevron-shaped somites of wild type (wt) embryos and in the U-shaped somites of *smo* mutant embryos (Fig. 1O, P). As *smo* embryos lack embryonic slow muscle, we conclude that *utrn* mRNA is localized at the ends of fast muscle fibres, and is not dependent on the presence of slow muscle fibres. By 48 hpf, *utrn* mRNA has become diffuse (Fig. 2L and data not shown). In mice, *utrophin* mRNA is localise primarily to the neuromuscular junctions (NMJs) but can also be found in other parts of the muscle (Vater et al., 1998). Our EST and RT-PCR data show that *dmd*, *dtng* and *utrn* are the main genes expressed at most stages of development (Fig. 1A and B). This agrees with the in situ hybridization results, and may reflect the large quantity of muscle in the embryo. *Drp2* and *dystrotelin* mRNA are not detected in somites by in situ hybridization at 13s, 24 hpf and 48 hpf (Fig. 1R and S, 2J and data not shown). Similarly, *Drp2* is absent from murine muscle (Dixon et al., 1997).

Overall, mRNAs for dystrophin/dystrobrevin family genes show precise sub-cellular localization in maturing muscle fibres. That prompted us to analyse the localisation of proteins of the dystrophin family. As reported previously, dystrophin protein co-localizes with its mRNA and with β -dystroglycan protein at the somite border at 24hpf, whereas *dystroglycan* (*dag1*) mRNA is diffuse in the fibres (Fig. 2P; Parsons et al., 2002). Subsequently, low levels of *dystrophin* become more broadly distributed on the muscle fibre surface, although still concentrated near its mRNA at the somite borders (Fig. 2A–E, Q, R). β -sarcoglycan protein remains highly concentrated at the somite borders until 5d (Fig. 2S–U). In contrast, utrophin protein is more evenly distributed from 48 hpf and onwards, matching the dispersed location of its mRNA at these stages (Fig. 2L–O). Interestingly, utrophin appear to be concentrated at costamere-like structures in older fibres (Fig. 2O''). We conclude that the localisation of dystrophin family proteins and their mRNAs is under complex and precise regulation during the early maturation of muscle fibres.

1.5 Expression of dystrobrevins and dystrophins in the CNS

In mice, genes from the dystrobrevin and dystrophin families are expressed in many parts of the CNS (Blake et al., 1995; Lien et al., 2004; Wertz and Fuchtbauer, 1998). *Dtng* mRNA is strongly expressed in four stripes in the developing hindbrain region between 5s and 22s, but fades thereafter (Fig. 1C, 3A–D, F, G). Use of *krox20* probe to mark rhombomeres (r) 3 and 5 revealed that hindbrain *dtng* expression is in r2 and r4–6 and is especially strong in r6 (Fig. 3A, F). This expression pattern persists at 10s–15s (Fig. 3B–D, F). The expression of *dtng* is detected throughout the dorsoventral axis of the rhombomeres (Fig. 3F, inset). Expression is also detected in migrating neural crest overlying r6 at 10–13s, but although retained in rhombomeres, expression declines in crest as it migrates away from the neural tube (Fig. 3B). Expression in rhombomeres becomes concentrated away from rhombomere boundaries, and then declines. As the levels of *dtng* mRNA declines, it persists most highly in r4 around 22s (Fig. 3G). By 26 hpf, no *dtng* is detected in the hindbrain (Fig. 3N). None of the other genes was found to have clear-cut hindbrain expression patterns. Thus, *dtng* is an early

marker of rhombomere formation; Zfin reports no gene with this expression pattern in the hindbrain (www.zfin.org).

From around 6s until around 15s, *dtng* is expressed in the ventral part of the developing diencephalon (Fig. 3C, E, F). This midbrain expression is somewhat stronger in the midline and the left side (Fig. 3E and F). We found no evidence in the literature to left-right asymmetry in this region of the developing brain. Midbrain expression declines after 15s and is no longer detected at later stages (data not shown). *Dtna* mRNA is widespread within the developing brain around 13s (Fig. 1Q), and it becomes more evident in the forebrain by 20s (Fig. 3H). From this stage a strong expression is detected in the hypothalamus, as shown by dual staining for *dtna* and *pitx3*, a marker for lens, ventral posterior diencephalon and pituitary (Dutta et al., 2005; Fig. 3H-L). By 26 hpf, other parts of the forebrain, such as the telencephalon, also show detectable levels of *dtna* mRNA (Fig. 3K and L). Interestingly, as in zebrafish, analysis of the spatiotemporal expression pattern of mouse α -*dystrobrevin* showed correlation with induction of various differentiation processes in the CNS and decline at later stages (Rees et al., 2007).

Drp2 mRNA is diffuse in the brain (Fig. 3P), as seen in amniotes (Dixon et al., 1997). In contrast, we have not detected *dtmb*, *dmd* or *utrn* mRNA in the developing brain (Fig. 3M, O and data not shown). *R-utrn* was detected in the lateral line neuromasts from 24 hpf at least until 48 hpf (Fig. 1O and 2L' and data not shown).

Taken together, we find three clear instances of co-expression of dystrophin and dystrobrevin family members: a) widespread *drp2* and *dtna* expression in the brain, b) *utrn* and *dtmb* expression in pronephric duct and c) *dmd* (*dystrophin*) and *dtng* co-localisation at the vertical myosepta. Aside from these, there are multiple examples of strong and specific expression of members of one family in the absence of an obvious partner from the other family.

2.0 Experimental procedures

2.1 Zebrafish lines and maintenance

All embryos were King's wild type background. Zebrafish mutant lines *smo*^{b641} (Barresi et al., 2000) was maintained on King's wild type background. Staging and husbandry were as described (Westerfield, 1995).

2.2 Bioinformatics and cloning

Probes to the following *Danio rerio* Genbank sequences were used: *dmd* (*dystrophin*), bases 3027-3495 of AF339031; *utrn* (pan-*utrophin*), bases 10524-11019 of EF473649; *r-utrn* (rod-*utrophin*), bases 2508-3185 of EF473649; *drp2*, bases 378-1065 of DQ443728; *dtna* (α -*dystrobrevin*), bases 1892-2354 of DQ516344; *dtmb* (β -*dystrobrevin*), bases 1616-2204 of DQ516345; *dtng* (γ -*dystrobrevin*), 1866-2570 of DQ516346. All probes correspond to 3'-untranslated regions or parts of the coding sequences encoding paralogue-specific peptides, except for *R-utrn* (rod-*utrophin*) and *drp2*, which correspond to regions encoding highly divergent parts of the respective spectrin-like rod domains.

2.3 Semi quantitative RT-PCR

Total RNA from embryos of 16 hpf, 24 hpf, 3d and 5d was made by using TRIzol (Invitrogen). Approximately 1 μ g of total RNA was used to produce the first cDNA strand using specific primers followed by a nested PCR preformed in the linear phase of the reaction. Sequences for primers are available on request.

2.4 In situ mRNA hybridisation and immunohistochemistry

Embryos were fixed in 4% paraformaldehyde, stored in methanol at -20°C and processed for in situ mRNA hybridisation as described (Mann et al., 2006). Additional probes were: *pitx3* (Dutta et al., 2005), *krox20* (*egr2b*, (Oxtoby and Jowett, 1993) and *myod* (used for staging embryos; (Weinberg et al., 1996). Embryos were mounted in glycerol and visualized on Zeiss Axiophot and Axiocam. Antibody labelling of wholemount embryos was as follows: embryos were fixed overnight in methanol at -20°C , rehydrated in PBTx (PBS 0.25% Triton X-100), blocked in PBTx containing 5% horse/goat serum and incubated with primary antibody in block overnight at 4°C . Embryos were then washed in PBTx, incubated with secondary antibody in block overnight at 4°C , either HRP-conjugated horse anti-mouse (Vector) followed with PBTx wash, and detection with diaminobenzidine, or incubated with Alexa 568 conjugated goat anti-mouse (Molecular Probes), PBTx, washed in PBTx, mounted in Citiflour (Agar) and analysed on LSM510 confocal microscope. Primary antibodies used were dystrophin (Sigma, MANDRA-1, 1:100), β -sarcoglycan (Novocastra, NCL-L-b-SARC, 1:50), and utrophin (Novocastra, utrophin-N terminus, 1:20).

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Figure 1. Differential expression of dystrobrevin and dystrophin genes in early stages

A. EST frequencies of the dystrobrevin and dystrophin genes from human, mouse and zebrafish as a percentage of total dystrophin and dystrobrevin family ESTs of each species. **B.** Semi-quantitative RT-PCR for mRNA samples from 16hpf (14s), 24hpf, 3d and 5d to the genes: *dmd*, *dp116*, *utr*, *G-utr*, *drp2*, *dtna*, *dtnb*, *dtng* and *dytn* normalized to β -actin. **C-V.** In situ mRNA hybridisation for *dmd* (C-E), *dtng* (F-I), *utr* (J), *R-utr* (K-P), *dtna* (Q and R), *dtnb* (S and T) and *drp2* (U and V) in dorsal flatmounts, anterior to top (C, F-H, J, K, Q, S, U), lateral flatmounts, anterior to left (E, I, O, P, R, T, V), lateral flatmounts, anterior to top, dorsal to right (D, L, N) or transverse sections, dorsal to top (C', E', F', H', I', M, O', R', T', V'). **C-E.** *Dtng* mRNA is expressed strongly in the hindbrain (red arrowheads, C) and in the notochord at 13s (arrows in C and C') but is restricted to the posterior notochord by 22s (D, arrow). At 24 hpf, no notochord expression is detected and transcripts are localised to somite borders (E and E'). **F-I.** *Dmd* is expressed strongly in the axial region at tb and 5s stage (F, F', G). By 13s, *dmd* is strong in the chordo-neural hinge (arrow in H) and is expressed in the floor plate (H', white arrowhead) and the hypochord (H', green arrowhead). Weak *dmd* expression is detected in somite cells (H, yellow arrowheads). At 24 hpf, *dmd* transcripts are detected only in the myotome concentrated at somite borders (I, I'). **J-O.** *Utr* (J) and *R-utr* (K-O) are expressed in the pronephric duct from around 13s to 24 hpf (arrowheads). At 22s and 24 hpf expression is noted at muscle fibre ends at somite borders. *Smo* embryos lacking slow cells have similar expression in muscle and pronephric duct (P). Note the expression in the lateral line primordia (blue arrowhead). **Q-R.** At 13s, *dtna* mRNA shows a diffuse widespread signal (Q and Q'). No specific expression is detected at 24 hpf in the somite area (R and R'). **S-V.** Little specific *dtnb* expression (S and S') or *drp2* (U and U') was found at 13s and at the somitic area at 24 hpf (*dtnb*: T and T'; *drp2*: V and V').

Figure 2. Expression of dystrobrevin and dystrophin genes in muscle, kidney and gut development

A-L. In situ mRNA hybridisation for *dmd* (A-E), *dtng* (F-H), *dtna* (I), *drp2* (J), *dtnb* (K and K'), *R-utrn* (L and L') in lateral flatmounts, anterior to left (A-L), or transverse sections, dorsal to top (K', L'). **A-H.** Expression of *dmd* and *dtng* mRNA in muscle begins to accumulate at ends of recently differentiated fibres (arrows in A and F) at the somite borders from 22s, and persist at that location until after hatching (B, C, E, G, H). By 3d, some head muscles express *dmd* (yellow arrowheads, D). **I-K.** At 48hpf, low mRNA levels of *dtna*, *dtnb* and *drp2* are detected in muscle fibres in somites, but *dtnb* is also present in the pronephric duct (K and K', arrowheads). **L.** At 48 hpf, *R-utrn* mRNA is expressed strongly in the pronephric duct (black arrowheads) and in the lateral line neuromasts (red arrowheads, L and L'-right panel) and is diffuse in muscle. **M-U.** Wholemount immunohistochemistry for utrophin (M-O), dystrophin (P-R) and β -sarcoglycan (S-U) in lateral flatmounts, anterior to left, in either diaminobenzidine staining (M-O), confocal stacks of one side of somites 12-14 (M', O', P', R', S', U'), a single confocal scan (O''), or transverse section, dorsal to top (U'). By 25 hpf dystrophin (P, P') and β -sarcoglycan (S, S') proteins are detected in muscle fibres concentrated at fibre ends, whereas utrophin protein is more evenly spread in muscle fibres. After 48 hpf, utrophin protein (N-O') labels fibres uniformly. Note the costameric expression of utrophin in slow fibres (white arrows, O''). Between 48 hpf and 5d, dystrophin (Q-R') and β -sarcoglycan (T-U') proteins are spread in muscle fibres, but remain concentrated mainly at fibres ends. Note the immunoreaction in the gut at 5d with all three proteins (arrowheads in O, R, U, U'). All bars 20 μ m.

Figure 3. Expression of dystrobrevin and dystrophin genes in head development

In situ mRNA hybridisation in dorsal flatmounts, anterior to left (A, B, D, F, J, K-right panel and L-right panel), wholemounts in lateral view, anterior to top (C, G, H, M-P) or to left (E-left panel, I, K and L-left panel), and transverse sections, dorsal to top (E-right panel, F-inset). **A-G.** *Dtnb* mRNA is expressed in r2 and r4-6 from 5s-15s whereas *krox20* (red) is expressed in r3 and r5 (A-D, F). Expression is throughout the dorso-ventral axis of the rhombomere (F, inset), including neural crest precursors within and as they are leaving the neural tube (B, blue arrowheads at r6). By 22s, expression of *dtnb* has declined in rhombomeres leaving the highest signal in r4 and away from rhombomere borders (G), and is lost by 26 hpf (N). Expression is also detected in the diencephalon between 5-15s concentrated at the left side (C, E, F, yellow arrowheads). Transverse section at the level of the dotted line (E) shows that expression is ventral and stronger at the midline and left of it. **H-L.** Low level of *dtna* mRNA expression is detected throughout the brain with stronger expression in the hypothalamus from 20s to at least 26 hpf (black arrowheads) whereas *pitx3* (red) stains the lens, posterior diencephalon and pituitary. Note *dtna* expression in the telencephalon at 26 hpf (green arrowheads). **M-P.** *Drp2* (M) is widely expressed in the developing brain at 26 hpf, whereas little *dtnb*, *dtna* and *dmd* brain expression is detected. r=rhombomere, vd=ventral diencephalon; h=hypothalamus; t=telencephalon; le=lens, pdn=posterior diencephalon, pt=pituitary.

Table 1
Summary of expression of dystrobrevin and dystrophin genes during zebrafish embryonic development

Tissue	Stage (hpf)	Gene						
		<i>dtna</i>	<i>dtub</i>	<i>dtng</i>	<i>dmd</i>	<i>utrn</i>	<i>drp2</i>	<i>dyn</i>
Muscle	<24 >24	- -	- -	- +++	+ +++	++ -	- -	- -
Notochord/Hypochord	<24 >24	- -	- -	+++ -	+++ -	- -	- -	- -
Floor plate	<24 >24	- -	- -	- -	++ -	- -	- -	- -
Pronephric duct	<24 >24	- -	- ++	- -	- -	+++ +++	- -	- -
Gut	<24 >24	- -	- -	- -	- ++	- ++	- -	- -
Lateral line	<24 >24	- -	- -	- -	- -	+++ +++	- -	- -
Hindbrain	<24	-	-	+++	-	-	+	-
Midbrain	<24	+	-	++	-	-	+	-
Forebrain	<24	++	-	-	-	-	+	-

(-) No indication of a specific signal. +, ++, and +++ indicate weak, medium, and strong hybridization signals, respectively. For each tissue, the first row describes expression during somitogenesis (<24), and the lower row describes expression after 24 hpf (>24).