Published in final edited form as:

Gene Expr Patterns. 2013 January ; 13(1-2): 30–37. doi:10.1016/j.gep.2012.09.002.

Comparative gene expression analysis of the *fmnl* family of formins during zebrafish development and implications for tissue specific functions

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Abstract

Fmnlins belong to the Formin family, catalysts of linear Actin polymerization with mostly unknown roles *in vivo*. In cell culture Fmnlins are involved in cell migration and adhesion and the formation of different types of protrusions including filopodia and blebs, suggesting important roles during development. Moreover, Fmnlins can act downstream of Rac and Cdc42, mediators of cytoskeletal changes as targets of important pathways required for shaping tissues. The zebrafish genome encodes five Fmnlins. Here we report their tissue specific expression patterns during early development and pharyngula stages. The *fmnl*s show overlapping and distinct expression patterns, which suggest that they could regulate similar processes during development, but may also have independent functions. In particular, we find a strong maternal contribution of all *fmnl*s, but distinct expression patterns in the developing brain eye, ear, heart and vascular system.

Keywords

Actin; vascular system; visual system; otic vesicles; brain

1-Results and discussion

During embryogenesis, tissues are shaped by complex cellular interactions and movements to ensure organ primordia are properly specified and positioned (1). The underlying complex movement of tissues requires precise coordination of signals with individual cell behaviors in part through modulation of the Actin cytoskeleton. Actin nucleation is a key event in the assembly of long filaments, which are required for many cellular activities including cell division, migration, and polarization (2). Formins are important catalysts of linear Actin polymerization (3). However, the developmental contributions of individual members of this large protein family are not well understood since only a few Formins have been examined.

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The Formins are subdivided into seven subfamilies that include the founding member, Cappuccino, which is crucial for anteroposterior and dorsoventral patterning in *Drosophila* (4). Other well known members include Diaphanous and the Disheveled-associated activators of morphogenesis (DAAM), the latter of which function as components of the non-canonical Wnt/Fz-Planar Cell Polarity (Wnt/PCP) pathway, a major regulator of polarized cell behaviors required for cell migration during morphogenesis (5). All Formins share key structural domains (2, 6) (Fig. 1A), including a FH1 (a Formin homology domain 1 that binds profilin often in complexes with Actin monomers) and the catalytic FH2 domain. Diaphanous related formins (Drf) also contain a Rho-family GTPase binding domain (GBD), a Diaphanous inhibitory domain/Dimerization domain (DID/DD, a.k.a. FH3 domain) and a C-terminal Diaphanous autoregulatory domain (DAD). The activation mechanism for Formins is not well understood; often, Drfs are auto-inhibited upon binding of the DAD domain to the DID domain (Fig. 1A) (6–8), until the proteins are activated by GTPase binding or phosphorylation of the DAD domain. Formin homo and heterodimers are thought to represent the active form of these proteins (2) as either mediators of Actin nucleation or extension.

A less known Formin subfamily is the Formin-related in Leukocytes (Frl in *Drosophila* and originally in mice, now more commonly known as Fmnl in vertebrates), which control actin nucleation or polymerization via Rac or Cdc42 regulated mechanisms (9–11). While *Drosophila* has one *frl* gene, mammals have three and zebrafish have five. Although Fmnl3 are composed of the same functional domains as the other Formins, our knowledge of how they contribute to early development remains limited to a recent study reporting a role for Fmnl3 in trunk angiogenesis in zebrafish (12). However, the extent to which individual Fmnl3 have cell type specific functions or are redundant remains to be determined. To determine whether individual zebrafish *fmnl*s (*1a*, *1b*, *2a*, *2b* and *3*) show temporal or tissue specific expression patterns during early development and morphogenesis, we examined their expression profiles by RT-PCR and *in situ* hybridization.

Our detailed expression studies will guide future functional work toward understanding the function of Fmnl3 during development. These studies reveal that *fmnl*s show overlapping expression patterns during early development except *fmnl2a* (Fig. 3), which becomes distinct from late gastrulation onwards. At later stages, the *fmnl*s are all expressed in the visual, otic and vascular systems, but cell type specific patterns are also apparent (Fig. 4 and 5). For example *fmnl1b* is detected in the lens, *fmnl2a* in the central part of the retina, and *fmnl3* in the cristae of the otic vesicles (Fig. 4).

1.1 Zebrafish *fmnl*s and their phylogenetic relationships with other *formins*

As mentioned, flies possess a single *frl* (13), mammals have three distinct *fmnl*s (14) and zebrafish have five *fmnl* genes. To clarify the relationship between individual Formins of human, mouse, fly and fish, we have compared the protein sequence of the FH2 domain, which is the defining domain of the Formin superfamily (6). Fmnl3 of each species are phylogenetically related and cluster together in the phylogram as a single subbranch that includes *Drosophila frl*, but no ‘non-Fmnl’ Formins (Fig. 1B), such as Cappuccino, Diaphanous, or DAAM. A similar relationship was observed when we compared the full coding sequence (data not shown).

As zebrafish *fmnl*s have received different names depending on whether the human (*fmnl*) or early mouse (*frl*) nomenclature was used, we have summarized the accession numbers and the names of *fmnl*s in humans, mice, and zebrafish in Table 1. Based on sequence comparison and phylogenetic analyses zebrafish have a single *fmnl3* gene and duplicated *fmnl1* and *fmnl2* genes, (Table 1, Fig 1B, Zebrafish Genome Project Assembly ZV7, Wellcome trust Sanger Institute).

1.2 *fmnl*s show dynamic expression patterns during zebrafish development

To determine if the five zebrafish *fmnl*s show distinct temporal expression profiles, we used RT-PCR to examine their expression during embryogenesis. The *fmnl* mRNAs were detected at all stages examined albeit with variable abundance (Fig. 2). Specifically, *fmnl1a* was maternally expressed and became more abundant in early and late gastrulae (6 and 10 hours postfertilization, hpf), while *fmnl1b* showed substantial maternal expression, which persisted with a lower abundance thereafter. *fmnl2a* was expressed maternally and in early gastrulae and was more abundant later in morphogenesis, while *fmnl2b* expression was highly expressed during maternal stages and persisted throughout gastrulation and larval stages. Intriguingly, at mid-gastrula (8 hpf) a second splice variant of *fmnl2b* was detected. This variant of *fmnl2b* includes an additional exon in between exon 11 and exon 12 that was not amplified from no-RT controls (Fig. 2; see sequence in methods). *fmnl3* was expressed in blastulae (1 and 4 hpf) when only maternal transcripts are present and became more abundant in late gastrulae and larvae (10, 24, 48 hpf).

Several functions have been attributed to Fmnl s such as cell migration and cell adhesion (15), important phenomena during early development. Our results suggest that all zebrafish *fmnl*s are expressed throughout early development and morphogenesis. Although temporal analysis alone did not reveal or exclude distinct functions of *fmnl*s, they could be divided based on peaks in their expression profiles: *fmnl1b* is uniformly abundant, *fmnl2b* appears most abundant during maternal stages and pharyngula stages, *fmnl1a* is low at maternal stages, is more abundant in gastrula, and thereafter is highly expressed through pharyngula stages. *fmnl2a* and *fmnl3* are maternally expressed and appear most abundant during pharyngula stages of morphogenesis.

1.3 *fmnl*s are ubiquitously expressed during early development and gastrulation

Our RT-PCR analysis indicates that the *fmnl*s are expressed throughout embryogenesis. However, some Formin family members are known to be expressed in a discrete subset of tissues, such as FMN, which is expressed in the developing nervous system and kidneys of mammals (15–17) and in the oocytes of *Drosophila* (4). To investigate whether zebrafish *fmnl*s also show tissue specific expression patterns we generated ribo-probes and analyzed the distribution of *fmnl* mRNAs at several developmental stages. All *fmnl*s were maternally provided and showed similar ubiquitous patterns at stages when the zebrafish embryo undergoes extensive actin remodeling and rapid cell divisions (Fig. 3A,B). Another Formin, Dia, has been shown to contribute to mitosis through Actin dependent (18) and independent (19) mechanisms. During late blastula and early gastrulation (6–10hpf; Fig. 3C–F), *fmnl*s remained ubiquitously expressed during stages when cell adhesion and coordinated polarized cell behaviors contribute to organization of the embryonic body plan (20, 21). The first detectable difference between expression patterns of the individual *fmnl*s was observed at tailbud stage when *fmnl2a* expression becomes prominent in the otic primordium (asterisks in Fig. 3E''), in the neuroectoderm border (red arrow in the dorsal view in Fig. 3F'') in a pattern reminiscent of *dlx3* expression, and in the hatching gland (white arrow in Fig. 3E''). This unique expression pattern might reflect a more significant role for *fmnl2a* in these tissues of the late gastrulae.

The robust expression of *fmnl*s during gastrulation is consistent with putative roles for Fmnl s in regulating cell migration *in vivo*. For example, The balance between Actin-based protrusions (e.g. filopodia) and other protrusions (e.g. blebs) is important for proper gastrulation and when disrupted leads to cell migration defects (22). Consistent with this notion, Fmnl3 has been described to be involved in the formation of filopodia (23), Fmnl1 has been implicated in polarized membrane blebbing (24), and Fmnl2 has been shown to drive Actin-based protrusions downstream of Cdc42 in cell culture (10). Although the *fmnl*

RNAs are widely expressed, we cannot exclude the possibility that individual *Fmnls* proteins show more restricted localization or activation to fulfill tissue specific functions.

1.4 Overlapping and distinct expression patterns of *fmnls* during pharyngula stages

Morphogenesis of the embryonic body plan and organ primordia continues after gastrulation; therefore, we conducted whole mount *in situ* staining to investigate whether zebrafish *fmnls* show distinct expression patterns during pharyngula stages (24 and 48 hpf). Although *fmn11a*, *1b*, *2b* were more widely expressed, all *fmnls* partially overlapped in their expression patterns (Fig. 4–E; Fig. 7L, M). At 24 hpf, all the *fmnls* were more prominently expressed in anterior regions of the embryo. Specifically, common domains of enhanced expression included the vitreal region of the retina (Fig 4A–E and A'–E'), which gives rise to the ganglion cell layer at later stages, and the lens, except for *fmn13* (Fig. 7A–D). Furthermore, some *fmnls* showed distinct expression patterns within the retina. *fmn12a* expression was more pronounced in the dorsal region of the retina compared to the vitreal region (arrowhead in Fig. 4C'). Within the forebrain, *fmn12a* was robust in the epiphysis (Fig. 7C). All the *fmnls* were enriched in a fibrous-like pattern within the cephalic floor plate of the hindbrain (Fig. 4A'–E), but *fmn11b* was also highly expressed in the dorsal hindbrain (arrowhead in Fig. 4B''). At this early pharyngula stage, consistent with chicken FMN1 expression (25), *fmn11a*, *fmn12a*, *fmn12b*, and *fmn13* were strongly expressed in distinct regions of the otic vesicle (asterisk in Fig. 4C'', arrowhead in Fig. 4E''). Tissue specific expression of *fmn12a* and *fmn13* within the retina and the otic vesicles is consistent with the possibility that *fmnls* differentially contribute to certain processes, including lens and otic vesicle development.

fmnls were detected within the trunk and the tail regions of the embryo (Fig. 4A'''–E''' and a–e) with *fmn11a*, *fmn12a* and *3* displaying more pronounced expression in more rostral regions (Fig. 4A''', 4C''' and 4E''') and *fmn11b*, and *2b* more robust in caudal somites (Fig. 4b, 4d). All *fmnls* were robust along the myotome boundaries (arrows in Fig. 5A–E). *fmn12b* was also abundant in the hatching gland (arrowhead in Fig. 4D), and also is present in the heart. Expression of *fmn12a* and *3* was robust in the intersegmental vessels (asterisks in Fig. 5C, E), in the ventral aorta (Fig. 4c, e and 5E) and *fmn13* in other vessels within the head of the embryo (Fig. 7E). Consistent with the expression pattern of *fmn13*, we and others have observed a role for *fmn13* in zebrafish angiogenesis via morpholino mediated interference (12) (and A.S-L., A.J. and F.L.M. unpublished). Thus, it is tempting to speculate that the other *fmnls* will also have nonredundant tissue specific requirements. For example, *fmn12b* might play a specific role in heart development like the closely related Formin, DAAM1, which modulates cytoskeletal function in cardiomyocytes of the mouse via a RhoA, Rac1 and Cdc42 dependent mechanism (26).

At 48 hpf, *fmn1* expression patterns become more refined, particularly within the brain (Fig. 7M). *fmn11a*, *1b* and *2b* remain widely expressed with strong expression in the head (Fig. 6A, B and D; Fig. 7F, G and I). While all are expressed in the optic nerve of the retina (Fig. 7F'–J'), *fmn11b* and *fmn13* appear more robustly expressed there (Fig. 6B' and E'), which might reflect a role in axon pathfinding or outgrowth. In support of this notion, *fmn11b* colocalizes with α -Tubulin (Fig. 7K). *fmn1* expression in the visual system is not limited to the optic nerve, as *fmnls* are also expressed in the optic commissures (*fmn11a*, *1b* and *2b*, Fig. 6A'–C'), the optic chiasm (*fmn12a*; Fig. 6C' and *fmn13*; Fig. 6E') or the ganglion cell layer (*fmn11a*; Fig. 7F' and *fmn13*; Fig. 7J'). Interestingly, *fmn12a* was distinctly expressed within the central part of the outer most layer of the retina (Fig. 6C'), the region undergoing cell differentiation at this stage. *fmnls* are also very robust in the optic tectum (Fig. 7F, G, I, J). These patterns are consistent with potential dedicated functions of *fmnls* to specific aspects of visual development, including retinal and tectum differentiation.

In the posterior brain of the pharyngula, we observed common domains of expression within the basal plate of the hindbrain and the otic vesicle (Fig 6A''–E'' and Fig 7F–J). However, as within the eye, *fmnl* expression within the otic vesicle was not completely overlapping. For example, *fmnl2a* was most prominently expressed within the otic vesicle (Fig. 6C''; Fig. 7H) while *fmnl3* was more robust in the cristae (Fig. 6E''), actin-rich structures within the developing ear (27). Other structures in the anterior part of the embryos that also present *fmnl*s expression are the branchial arches (*fmnl1a*; Fig. 7F), the pharynx (*fmnl1a*; Fig. 7F; *fmnl2b*; Fig. 7I and *Fmnl3*; Fig. 7J), the liver (*fmnl2a*; Fig. 7H), and the gut (*fmnl3*; Fig. 7J).

In the trunk only *fmnl1a*, *1b*, *2b* and *3* (Fig. 5F, G, I, J; Fig. 6A''', B''', D''', E'''), but not *fmnl2a* (Fig. 5H and Fig. 6C and C''') were expressed, although *fmnl2b* expression pattern was very diffuse (Fig. 5I) *fmnl3* was also detected in the aorta and the vascular system of the head (Fig. 6E and 6E''' and Fig 7J). *fmnl1a* and *2b* were also expressed in the heart (Fig. 6). At this stage, among the *fmnl*s, *fmnl1b* was most prominent in the somites (Fig. 6B''').

Our expression analysis and the recent report demonstrating an endothelial cell development defect in *fmnl3* morphants (12) suggest that the zebrafish *fmnl*s play an important role during vertebrate development. Moreover, the overlapping and distinct expression patterns throughout development described here point to redundant as well as specific functions of the different *fmnl*s. Future work is required to elucidate the extent of this redundancy and the individual contributions of each *Fmnl* to the development of structures such as the otic and optic vesicles, the optic nerve projections, the optic commissures, and the developing brain, heart, and vascular system.

2-Experimental Procedures

2.1-Animals

AB strain zebrafish embryos were generated by natural pair-wise mating. They were staged and reared according to standard procedures (28). Specimens were anaesthetized with ethyl 3-aminobenzoate methanesulphonate salt (Sigma, St Louis, MO) before they were fixed in paraformaldehyde 4%. The stages used were: maternal stages (2, 4 hpf), gastrulation (6, 8, 10 hpf) and pharyngula (24, 48 hpf).

All procedures and experimental protocols were in accordance to NIH guidelines and approved by the Einstein IACUC under protocol #20100509.

2.2-Phylogram

FASTA protein sequences corresponding to the FH2 domain were obtained from Genbank or the Ensembl website (www.ensembl.org) and analyzed using the Satchmo online software provided by the University of Berkely (<http://phylogenomics.berkeley.edu/q/satchmo>) (30). Accession or ID numbers are as follows: Capu: NP_722950; dFhos: NP_729410; dDAAM: AAF45601; zDAAM1: XP_707353; dFrl: NP_001137948 (CG32138); huFMNL1: NP_005883; mFmnl1: NP_001071166; zFmnl1a: XP_685133; zFmnl1b: XP_001336442; huFMNL2: NP_443137; mFmnl2: XP_288949; zFmnl2a: XP_001345195; zFmnl2b: XP_002662354; huFMNL3: EAW58086; mFmnl3: XP_128263; zFmnl3: XP_001920583; dFrom3 (Inf): BAC76439; dDia: T13170; mDia1: NP_031884; zDia2:XP_683813.

2.3-RT-PCR

Total RNA was extracted using Trizol Reagent (Invitrogen) and cDNA synthesis was carried out by reverse transcription using the Applied SuperScript® III Reverse Transcriptase (Invitrogen) according to manufacturer instructions.

The concentration of cDNA was determined by measuring the absorbance at 260 nm with a Nanodrop ND-1000 Spectrophotometer and the PCR was performed using the Gotaq Hot Start Polymerase (Promega). The following primers were used:

<i>fmnl1a</i>	CAGATCCAGGCCTATTTGGA	TCCAGGCTTCCAGAGACTGT
<i>fmnl1b</i>	AGAGCGTCCTCATCCTCAAA	CACGAGTGCTGCTTATCCA
<i>fmnl2a</i>	CCAGGACTAAGGCTCTGGTG	CAGAGCTCCGACATCAAACA
<i>fmnl2b</i>	CGAGATCATCCTTGCTGCT	TTTTGGTCTCTGCGTCTCT
<i>fmnl3</i>	CCCCAAAAGTACCCTAAT	GAATGGACGTCAATGGAAGG
EF1 α	CTGGTTCAAGGGATGGAAGA	CACACGACCCACAGGTACAG

PCR products were amplified using an Eppendorf mastercycler epgredient machine, with the following conditions: 2 minutes at 94°C followed by 35 cycles of 15 seconds at 94°C and 30 seconds at 57°C and 1 minute at 72°C and a final extension of 10 minutes at 72°C. Samples without reverse transcriptase were used as negative controls.

The sequence of the alternative splice variant of *fmnl2b*:

Exon11:GTGGCGTGCATGCAGTTCATCAATATTGTGGTGCA
 CTCGGTGGAGGACATGAATTTTCAGAGTTCACCTTCAGTATGACTTCACCAAAC
 TCTCTCTGGACGACTATTTGGAG-Exon11a:GTATTAACTTTTTCAAAGTATCTT
 GCATTACGCTCCCTCAGAACTCACTTCCCTGGGGTTTGTGTTTTGTCCGCA G)-
 Exon12:AGGCTGAAGCATACGGAGAGCGATAAACTCAAAGTGCAGATCC...

2.4-In situ hybridization and histology

In situ hybridization was performed according to established protocols (29). Digoxigenin-labeled RNA probes were synthesized using a DIG labeling kit (Roche) using the PCR fragments after subcloning into pSCA (Agilent). For Anti-sense probes, plasmid cDNA were linearized using XhoI for *fmnl1b*, *2a*, *2b* and *3* and with NotI for *fmnl1a* and the ribo-probe was synthesized using T7 polymerase. For sense (control) probes, plasmid cDNA were linearized using AscI for *fmnl1a*, *2a* and *3*, with HindIII for *fmnl2b* and with KpnI for *fmnl1b* and the ribo-probe was synthesized using Sp6 polymerase for *fmnl1a*, *2a* and *3*, T7 polymerase for *fmnl2b* and T3 polymerase for *fmnl1b*. Probes were detected with anti-DIG-AP antibody (1:5000, Roche) and BM purple substrate (Roche). For all the stages whole-mount ISH was carried out. Histological sections were obtained for a subset of the 24 and 48 hpf embryos.

Sense probes were used as negative controls and no staining were observed (data not shown).

Coronal cryosections were obtained as previously described (30). Briefly: 24 hpf and 48 hpf old embryos were cryoprotected in 15% sucrose in PBS for 12 hours at 4°C and then in 30% sucrose at room temperature during 2 hours. Embryos were embedded in OCT and 18 μ m sections were obtained using a Leica cryostat.

2.4-Immunohistochemistry

α -acetylated Tubulin (Sigma T6793) staining was performed on sections after the *in situ* hybridization as previously described (31) and using an Alexa-488 secondary antibody.

2.4-Image analysis

Whole-mount embryos were examined under a Olympus SZ61 microscope with an Olympus America camera. Digital images were taken using Picture Frame software. Cryosections were analyzed on a Axioskop2 plus (Zeiss) with a AxioCam MRc camera (Zeiss) and images were acquired using the Carl Zeiss AxioVision Rel. 4.6 software. Sharpness, contrast, and brightness were adjusted to reflect the appearance seen through the microscope. For final adjustments Adobe® Photoshop® CS3 extended Adobe® Illustrator® CS3 were used.

Acknowledgments

Research of the Marlow and Jenny labs is funded by grants NIH RO1 GM1089979 (F.L.M.) and RO1 GM088202 (A.J.) and start up funds to FLM and AJ, respectively. We thank the Einstein Cancer Center supported Histopathology resource for use of the cryotome. We thank P. Campbell and Dr. L. Feng for their valuable comments on the manuscript and Marlow and Jenny lab members for discussion.

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Highlights

Phylogenetic relationships between Fmnl Formins, conserved catalysts of linear actin

Fmnls are maternally provided and widely expressed in early zebrafish embryos

Histological analysis of zebrafish *fmnls* shows distinct and common expression domains

Fmnls show tissue specific expression in the vascular, visual and otic systems

Figure 1.

A) Schematic of the protein domains of a typical Fmnl. B) Phylogenetic relationship between the FH2 domain of individual Fmnls in human, mouse, zebrafish and fly as well as selected other formins. The length of the branches represents the degree of change in FH2 domain sequence. DAD: Diaphanous autoregulatory domain; DID/DD: Diaphanous inhibitory domain/Dimerization domain; FH: Formin homology domain; GBD: GTPase binding domain.

Figure 2.

Temporal analysis of zebrafish *fmnl*s using RT-PCR. * indicates alternative splice variant of *fmnl2b* detected in mid-gastrula stage embryos. This variant includes an additional exon between exons 11 and 12.

Figure 3.

Expression of *fmn1* transcripts in blastula and gastrula. (A–B) *fmn1*s are maternally provided (1–4 hpf) and (C–F) expression persists during gastrulation (5–10 hpf). (E,F) Tailbud stage (Tb). The head is indicated by an arrow and the tailbud by an arrowhead. *fmn12a* is robustly expressed in the otic vesicle primordia (asterisk in E''), the neuroectoderm border (red arrow in F'') and in the hatching gland (white arrow in 3E''). A: anterior; AnVi: anterior view; D: dorsal; DoVi: dorsal view; LaVi: lateral view; P: posterior; V: ventral.

Figure 4.

Expression of *fmnls* at 24 hpf reveals overlapping and distinct patterns. A–E) Whole mount lateral views. Colored triangles indicate level of cross sections: black indicates forebrain (fb; A'–E'), red the level of the hindbrain (hb; A''–E''), blue the sections through the trunk (tr; A'''–E'''), and green the sections through the tail (tl; a–e). *fmn1a* and *fmn1b* are expressed in the ventral hindbrain in a fibrous-like pattern (arrows in A'' and B''). *fmn1b* is expressed in the dorsal hindbrain (arrowhead in B''); *fmn2a* in the dorsal retina (r) (arrowhead in C'), otic vesicle (ov) (asterisk in C'') and somites; *fmn2b* in the hatching gland (arrowhead in D), the heart (h) and the lens and *fmn13* in the aorta (a), the ov (arrowhead in E'') and somites (s). Orientation is conserved for all the whole mount and section images. C: caudal; D: dorsal; mb: midbrain; mo: medulla oblongata; n: notochord; iv: intersomitic vessels; R: rostral; V: ventral.

Figure 5.

Expression of *fmnl*s in the trunk of larvae at 24 (A–E) and 48 (F–J) hpf. At 24 hpf *fmnl*s were robust in the myotome boundaries (arrows) and *fmnl2a* and 3 were also expressed in the intersomitic vessels (asterisks) and *fmnl3* in the aorta (a) (E). At 48 hpf *fmnl1a*, 1*b* and 2*b* were robust in the notochord (n) (F, G, I) and *fmnl3* is still present in the aorta and the intersomitic vessels (asterisks in J).

Figure 6.

Expression of *fmnls* at 48 hpf reveals more distinct patterns than at earlier stages. A–E) Lateral views of whole-mount embryos. Colored triangles indicate level of cross sections: black indicates forebrain (fb; A'–E'), red the level of the hindbrain (hb; A''–E''), and blue the sections through the trunk (tr; A'''–E'''). Colored arrowheads indicate the level cross sections in A'–E') black arrows indicate forebrain (fb), A''–E'') red arrowhead and A'''–E''') blue arrowhead indicating sections through the trunk (tr). *fmnl1a*, *1b* and *2a* are enriched in the head (A, B, D). *fmnl1a* and *1b* are robust in somites (A''' and B'''). *fmnl1b*, *2b* and *3* are highly expressed in the visual system including the optic commissure (oc), optic nerve (on) and lens (l) (B', D', E'). *fmnl2a* is expressed in the medial region of the retina (C'). Within the otic vesicle (ov), *fmnl2a* is broadly expressed (C''), while (E'') *fmnl3* has more prominent expression in the cristae (c). a: aorta; C: caudal; gcl: ganglion cell layer; D: dorsal; mb: midbrain; mo: medulla oblongata; n: notochord; och: optic chiasm r: retina iv: intersomitic vessels; R: rostral; r: retina; tl: tail; V: ventral.

Figure 7.

Expression of *fmnls* in the anterior part of the larvae. At 24 hpf, *fmn1a*, *1b* and *2b* are widely expressed (A, B, D). *fmn2a* is robust in the eye and in the epiphysis (e) (C). *fmn3* show enhanced expression in the posterior part of the hindbrain (hb) and in the aorta (a) (E). At 48 hpf, *fmn1a*, *1b* and *2b* are enhanced in the posterior part of the midbrain (mb) and the floor plate (fp) of the hb. *fmn2a* and *3* are also expressed in the floor plate. *fmn1a*, *2b* and *3* are expressed in the heart (F, G, J) and all *fmnls* are expressed in the optic nerve (on) (F'–J'), this staining colocalized with α -Tubulin (K for *fmn1b*). In L and M, a summary of the expression pattern of all *fmnls* is shown (not all the domains are represented). C: caudal; D: dorsal; fb: forebrain; h: heart; hp: hypothalamus; l: lens; R: rostral; r: retina; oc: optic commissure; tr: trunk; V: ventral.

Table 1
Summary of zebrafish *fmm1s* and their homologs in human and mouse. In flies there is only one *Frl*.

Zebrafish name	Zebrafish Chromosome	Other names	Zebrafish Ensembl ID	Human name	Human Ensembl ID	Mouse name	Mouse Ensembl ID	References
<i>Fmm11a</i>	3	<i>zFrl1B/Fmm11</i> (1 of 2)	ENSDARG000000055713	<i>Fmm11</i>	ENSG00000184922	<i>Fmm11</i>	ENSMUSG000000055805	(14, 24)
<i>Fmm11b</i>	24	<i>zFrl1A/Fmm11</i> (2 of 2)	ENSDARG000000075519					
<i>Fmm12a</i>	9	<i>zFrl3</i>	ENSDARG000000012586	<i>Fmm12</i>	ENSG00000157827	<i>Fmm12</i>	ENSMUSG000000036053	(10, 14)
<i>Fmm12b</i>	6		ENSDARG000000075041					
<i>Fmm13</i>	23	<i>zFrl2</i>	ENSDARG000000004372	<i>Fmm13</i>	ENSG00000161791	<i>Fmm13</i>	ENSMUSG000000023008	(11, 12, 14, 23)