Conserved developmental expression of Fezf in chordates and Drosophila and the origin of the Zona Limitans Intrathalamica (ZLI) brain organizer

Manuel Irimia1*, Cristina Piñeiro2, Ignacio Maeso1, José Luis Gómez-Skarmeta2*, Fernando Casares2*, Jordi Garcia-Fernàndez1*

Abstract

Background: The zona limitans intrathalamica (ZLI) and the isthmus organizer (IsO) are two major secondary organizers of vertebrate brain development. These organizers are located at the interface of the expression domains of key patterning genes (Fezf-Irx and Otx-Gbx, respectively). To gain insights into the evolutionary origin of the ZLI, we studied Fezf in bilaterians.

Results: In this paper, we identified a conserved sequence motif (Fezf box) in all bilaterians. We report the expression pattern of Fezf in amphioxus and Drosophila and compare it with those of Gbx, Otx and Irx. We found that the relative expression patterns of these genes in vertebrates are fully conserved in amphioxus and flies, indicating that the genetic subdivisions defining the location of both secondary organizers in early vertebrate brain development were probably present in the last common ancestor of extant bilaterians. However, in contrast to vertebrates, we found that Irx-defective flies do not show an affected Fezf expression pattern.

Conclusions: The absence of expression of the corresponding morphogens from cells at these conserved genetic boundaries in invertebrates suggests that the organizing properties might have evolved specifically in the vertebrate lineage by the recruitment of key morphogens to these conserved genetic locations.

Background

Secondary morphogenetic organizers are located at the boundaries of major vertebrate brain compartments, and play essential roles in the development of the highly complex vertebrate brain. The two main brain internal organizers are the isthmus organizer (IsO) and the zona limitans intrathalamica (ZLI). The IsO is located in the midbrain-hindbrain boundary (MHB), at the abutting expression domains of Otx and Gbx, and the ZLI develops within the diencephalon, between the prethalamus and thalamus, at the boundary of Fezf and Irx gene expression domains (Figure 1A). As is typical for organizers, cells from these structures are the source of diffusible signaling factors that determine the further development of the adjacent cellular compartments. ZLI cells characteristically secrete Shh [1,2], whereas the IsO typically releases Fgf8 and Wnt1 [3].

Bona fide IsO and ZLI organizers are present in all vertebrates, including basal living agnathans [4,5]; however, the absence of the key morphogens at analogous topological positions [6-9] suggests that comparable signaling centers are not present in invertebrates with a central nervous system (CNS), including amphioxus, a basal chordate considered to be the best living proxy to the vertebrate-invertebrate ancestor [5]. Like vertebrates, amphioxus has a dorsal hollow neural tube that forms from a neural plate. However, the amphioxus brain is relatively simple, consisting only of a putative non-subdivided diencephalon, a Hox-patterned hindbrain and perhaps a small midbrain [10].

Despite the lack of internal brain organizers in invertebrates, previous observations suggested that the interface between the abutting expression domains of the
Fezf and expression abuts that of the most anterior part of the brain, and its caudal in vertebrates [13-15]. reported to have a primary role in establishing the ZLI Otx/otd and the two organizers physically related to each other ori-rior-posterior brain subdivision and the ZLI, and how about the evolutionary origin of the other major ante-bilaterians [11,12]. By contrast, much less is known positioning of the MHB (Figure 1), is ancestral to all vertebrates. To gain insights into these questions, we characterized the Fezf gene in the basal chordate amphioxus and in the protostome Drosophila melanogaster. We analyzed their developmental expression pat-terns and compared them with those of the Irx, Otx and Gbx genes. Strikingly, we found that the relative expres-sion of Fezf, Irx, Otx and Gbx genes in the CNS is fully conserved between these species, suggesting a wide-spread involvement of these genes in early molecular patterning of the bilaterian CNS.

Results and Discussion
Fezf is highly conserved across phyla and is ancestral to bilaterians
Fezf is a transcription factor of the C2H2 zinc finger family, containing six zinc fingers. Using in silico analysis, we identified putative Fezf orthologs in all studied metazoans (see Methods), including non-bilaterians. The orthology of the different putative Fezf genes is robustly supported by phylogenetic analysis (Figure 2A). The coding sequences of the zinc finger domain are highly conserved between different groups, showing typically > 75% identity at the amino acid level. In addition to the zinc finger domain, non-bilaterians Fezf proteins have a co-repressor SNAG domain, typical of other related zinc finger gene families, such as Snail and Gfi [16]. This domain was probably present at the origin of Fezf gene family, but has been lost in all studied bilaterians, with the excep-tion of amphioxus, for which we could identify a puta-tive SNAG domain in silico, although reverse transcription PCR experiments showed that this domain is not included in the Fezf transcripts during development of either Branchiostoma floridae or Branchiostoma lanceolatum. Multiple convergent secondary losses of the SNAG domain have also been reported in the Snail/Scratch superfamily [16,17] and it has been proposed that these losses are associated with the acquisition of different conserved domains that carry out a co-repressor function, such as the CtBP-binding site or the NT box [16]. Consistent with this hypo-thesis, we identified a highly conserved sequence motif near the N-terminus of all studied bilaterian Fezf pro-teins, which we have termed ‘Fezf box’ (Figure 2B) and which seems to be exclusive to the Fezf gene family. The clear inverse relation between the presence of the Fezf box (in bilaterians) and the SNAG domain (in non-bilaterians and related genes) suggests that this previously unidentified conserved motif might also function as a co-repressor domain.

Otx/otd and Gbx/unpg genes, which determines the positioning of the MHB (Figure 1), is ancestral to all bilaterians [11,12]. By contrast, much less is known about the evolutionary origin of the other major ante-rior-posterior brain subdivision and the ZLI, and how the two organizers physically related to each other ori-ginally. Recently, the zinc-finger Fezf gene family was reported to have a primary role in establishing the ZLI in vertebrates [13-15]. Fezf is expressed exclusively in the most anterior part of the brain, and its caudal expression abuts that of Irx genes. The interface of the Fezf and Irx expression domains delineates the border
Figure 2 Phylogenetic relationships, Fezf box, and exon-intron structure of Fezf genes across metazoans. (A) Phylogenetic tree of the putative Fezf orthologs identified in different metazoan genomes generated by Bayesian inference. The orthology of the genes is supported by a posterior probability of 1. (B) Consensus sequence of the conserved Fezf box located near the C-terminal of all bilaterian Fezf orthologs. (C) Alignment of the zinc finger domains of some representative species showing intron positions and intron phases (colored numbers). Only vertebrates and Ciona intestinalis show lineage-specific introns in these domains.
In addition to the canonical Fezf genes, we also found ‘Fezf-like’ genes in cnidarians and sea urchin (NveFezf3, NveFezf12 and NveFezf30 and SpuFezli [18]); these genes branch at basal positions of the phylogenetic tree (Figure 2A) and contain a SNAG domain but not a Fezf box.

Finally, we also studied the exon-intron structure within the zinc finger domain, where the sequence can be confidently aligned. Surprisingly, whereas in nearly all species no introns are found within the zinc finger domain, all vertebrate genes have three introns (one conserved with the tunicate Ciona) that seem to be lineage-specific gains (Figure 2C). This is unexpected, considering the high conservation of intron positions from cnidarians to vertebrates in the deuterostome line [19-23] and the generally low rate of intron gains along these lineages.

**Expression of Fezf, Irx and Gbx in amphioxus**

To gain insights into the evolutionary origin of the vertebrate ZLI, we analyzed the developmental expression of the single Fezf gene of the basal chordate amphioxus (B. lanceolatum). As in vertebrates, Fezf expression starts at the beginning of neurulation, and its expression is highly restricted to the most anterior part of the neural plate (the six to seven anterior-most rows of cells) (Figure 3A, B). This restricted anterior neural domain continues to the larval stages (Figure 3C, D), at which point the expression is found only in the cerebral vesicle, the most anterior part of the amphioxus larval neural tube. We next compared Fezf expression to Irx and Gbx genes at neurula stage, which in vertebrates mark the posterior boundaries of the presumptive ZLI and MHB, respectively. Strikingly, we found that the relative expression of Fezf, Irx and Gbx genes in the neural plate is fully conserved between amphioxus and vertebrates (Figure 4A, B), indicating that these genetic interfaces, which contribute to delineate these major brain subdivisions, were present before the origin of vertebrates. In both Xenopus and amphioxus, the expression of Fezf abuts that of Irx, and there is a conserved gap between the expression domains of Fezf and Gbx that shows Irx expression (Figure 4A, B), consistent with an ancestrally conserved anterior-posterior topology of the MHB positioning relative to the ZLI. Moreover, the abutting expression of the Fezf and Irx genes constitutes a conserved genetic subdivision within the amphioxus presumptive diencephalon, raising the intriguing possibility of potential equivalents of proto-prethalamic and a proto-thalamic regions in the primitive chordate brain, consistent with other observations [24,25]. Further investigation will be required to assess to what extent these structures are homologous and functionally equivalent to their vertebrate counterparts or whether they correspond to distinct amphioxus novelties.

**Expression of Fezf in flies**

To further investigate the evolutionary origin of these early genetic brain subdivisions, we examined the expression patterns of Fezf and Irx homologous in the Drosophila developing CNS. dFezf/Earmuff/CG31670 has been recently shown to maintain the restricted developmental potential of intermediate neural progenitors in Drosophila [26], and its embryonic expression pattern has been documented previously [27]. As in chordates, dFezf expression is restricted to the most anterior part of the fly CNS throughout early CNS development (Figure 3E-I). Fezf expression starts in blastoderm embryos as a dorsal and lateral stripe in the anterior (neurogenic procephalic) region of the blastoderm (Figure 3E,F). Characteristically, the lateral ends of this stripe widen, making the pattern resemble earmuffs (Figure 3G). In early germband extension-stage embryos, the stripe is split at the dorsal midline (Figure 3G), generating bilaterally symmetrical domains (Figure 3G-J). During later embryogenesis, dFezf-expressing cells delaminate and cluster to form part of the brain hemispheres (Figure 3H-J). Importantly, the expression domain of mirror (mirr), the earliest fly Irx expressed gene, also abuts that of dFezf (Figure 4C). Our results, along with the fact that in Drosophila the orthologs of Otx and Gbx also show complementary expression domains (Figure 4C, [12]), and the presence of the conserved gap between the expression domains of fly Fezf and Gbx orthologs, suggest that this simple initial genoarchitectural plan, which broadly subdivides the vertebrate nervous system, was present in the last common ancestor of extant bilateral animals.

Significantly, Fezf and Irx in vertebrates regulate each other in a mutually exclusive manner. In knockout or knockdown mutants for these genes in different vertebrate species, there is a shift in the expression limit of the counterpart gene, either anteriorly (in the case of Irx [13,14]) or posteriorly (in the case of Fezf [15]). To assess whether this situation was at least partially conserved in flies, we also analyzed the expression of dFezf in iroDFM3 mutant embryos, which lack the Irx genes [28]. In stark contrast to vertebrates, we did not find any noticeable caudal shift in the posterior limit of dFezf expression (data not shown).

**Early complex brains**

The strongly restricted expression of Fezf to the anterior forebrain in vertebrates is an indication of its crucial role in the patterning of the vertebrate brain. The presence of deeply conserved Fezf orthologs in all studied
metazoans, from placozoans to vertebrates, thus raises the question of whether Fezf might play a similar conserved role throughout animal phylogeny, or whether it has been recruited for different developmental functions in the different phyla. We show that in two distantly related invertebrate groups with a centralized CNS Fezf orthologs are also expressed in a strongly restricted manner in the developing anterior CNS, suggesting that the ancestral function of Fezf in bilaterians might well be related to the patterning of the CNS. Furthermore, the conserved relative expression with other key patterning genes (Irx, Gbx and Otx) at early neurulation stages suggest that all these genes may help to define broad conserved regions within the neural ectoderm as a whole in different bilaterian organisms [11,12,29,30].

Based on several similarities in patterning gene expression and function, Reichert and collaborators suggested that the last common ancestor of extant bilaterians,
Urbilateria, had a tripartite brain, and that *Drosophila* and vertebrate brains had a comparable anterior-posterior patterning \[12,31,32\]. These authors proposed a model with three domains consisting (from anterior to posterior) of: (1) forebrain/midbrain, (2) an intervening MHB region and (3) a hindbrain. These three structures are characterized by the specific expression of the *Otx*, *Pax2/5/8* and *Hox* genes, respectively \[12,31\]. Our results complement and expand this model, adding an extra conserved genetic subdivision to the forebrain/protocerebrum of the studied species.

**Origin of the ZLI secondary organizer**

In addition to *Fezf-Irx*, other sets of genes with mutually exclusive expression patterns have been proposed to be involved in the development of the ZLI in vertebrates. Based on misexpression analysis, it was first suggested that the mutual repression between *Irx* genes and *Six3* (expressed at early stages in the whole anterior forebrain anlage, limited caudally by the anterior boundary of *Irx* genes) (Figure 1B[33]) contributed to the establishment of the ZLI and other diencephalic subdivisions \[34\]. In amphioxus early neurula, *BfSix3/6* is expressed in the anterior-most part of the neural plate, with a posterior boundary seemingly consistent with that of *Fezf-Irx* \[24\]. However, the role of *Six3* in establishing the ZLI has recently been challenged, because, in contrast to *Fezf*, *Six3* expression is very dynamic and regresses rostrally, both in vertebrates and amphioxus \[24,33,34\], leaving a region free of *Irx* and *Six3*. *Six3* function might thus be
related to cell proliferation rather than to neural patterning at these stages [24].

Another important pair of genes with mutually exclusive expression patterns thought to be involved in ZLI formation in vertebrates are Lfng and Wnt8b [1]. Lfng is expressed widely in the chick prosencephalon, with the exception of a wedge-shaped area (presumptive ZLI), where Wnt8b is expressed (Figure 1B, [1]). This Wnt8b-positive/Lfng-negative region is where the ZLI will develop. In amphioxus, however, the single BfFng gene is expressed throughout the anterior-most part of the neural plate in early neurula, apparently with no discontinuity [35], whereas Bf/Wnt8 is not expressed in the CNS at early developmental stages [36] (Figure 1B).

Taken together, these results suggest that, although the genetic boundary determining the location of the ZLI in vertebrates was present in proximal chordate ancestors, some of the components of the putative ZLI gene network [1] were not yet assembled. Accordingly, molecules secreted as secondary organizers in vertebrates have not been found in the amphioxus developing brain [7,9]. Thus, it is likely that in vertebrate ancestors, the ZLI secondary organizers evolved through the recruitment of the expression of key morphogens to the cells located in the interface of these conserved major genetic domains, defined by the abutting expressions of Fezf-Irx. Presumably, this would have led to the development of new subdivisions and brain structures, possibly allowing the increase in proliferation and complexity of present-day vertebrate brains. However, it is also possible that internal brain organizers evolved before the vertebrates originated, and were then lost in the studied invertebrate chordates by reductive evolution. Equivalent signaling centers have not been reported in any invertebrate to date; however, it is still possible that a more thorough study of other invertebrates, such as hemichordates, which show a wide conservation of relative gene expression patterns with vertebrates [37], or basal slow-evolving protostomes, for which there are no molecular data yet available, will help to clarify the origins of the vertebrate brain complexity.

Methods

In silico Identification and comparison of Fezf genes across metazoans

Using the previously described vertebrate Fezf genes as queries, we performed tBLASTN and/or BLASTP searches against the genomes of Branchiostoma floridanae JGI v1.0, Trichoplax adhaerens Grell-BS-1999 v1.0, Nematostella vectensis JGI v1.0, Ciona intestinalis JGI v2.0, Daphnia pulex JGI v1.0, Lottia gigantea JGI v1.0 and Capitella teleta JGI v1.0, using the JGI website (http://genome.jgi-psf.org/euk_home.html) and of Strongylocentrotus purpuratus Build 2.1, Tribolium castaneum Build 2.1, Nasonia vitripennis Build 1.1, Drosophila melanogaster Build Fb5.3, Homo sapiens Build GRCh37, Mus musculus Build 37.1, Danio rerio Build Zv8, using the NCBI website (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). For Saccoglossus kowalevskii we performed a tBLASTN search against the traces at NCBI and then manually assembled the genomic locus.

We then downloaded each corresponding genomic region and build different gene models using GenomeScan [38] and GeneWise2 [39] software as necessary. We compared these predictions with expressed sequence tags and existent gene models when available. Annotation and comparison of intron positions and phases across zinc finger domains was performed as previously described [40,41].

The amino acid sequences for the zinc finger domains were aligned using ClustalW [42] and the resulting alignment was manually curated. Phylogenetic trees were then generated by the Bayesian method, using the software MrBayes 3.1.2 [43,44], with the model Dayhoff +Gamma, recommended by ProtTest 1.4 [45-47], under the Akaike information and the Bayesian information criterions. Two independent runs were performed, each with four chains. For convention, convergence was reached when the value for the standard deviation of split frequencies stayed below 0.01. Burn-in was determined by plotting parameters across all runs for a given analysis: all trees before stationarity and convergence were discarded, and consensus trees were calculated for the remaining trees (from at least 1,000,000 generations).

Fezf box consensus was decided by the program Sequence Logo online (http://genome.tugraz.at/Logo/) using a multiple alignment for all studied species containing a Fezf box.

Cloning of European amphioxus Fezf, Irx and Gbx genes

Primer pairs were designed to span the whole length coding sequences of the B. floridanae Fezf [18] and Gbx [11] genes, if possible. A liquid cDNA library from different developmental stages of the European amphioxus (Branchiostoma lanceolatum) was screened by PCR using the B. floridanae Fezf primers. B. lanceolatum Fezf and Gbx were cloned, sequenced and submitted to NCBI (accession numbers HM245959, HM245960; primer sequences: Fezf_L: ATGGCAATGTTCGGA ACCCTTG, Fezf_R: TTACTCTGCGGCTGGAAGTG, Gbx_L: TGAAAATGCAGCGGCACAGC, Gbx_R: ATGCTGACTCCTCATGGCGAA). For Bllrbx, we used the previously reported full-length sequence [48]. Neural plate expression patterns for Irx and Gbx in B. lanceolatum were consistent with those reported in B. floridanae [11,29]. To assess whether the putative SNAG domain was included in the transcripts we used the following...
The Drosophila hybridization signal was developed as described above. The deficiency iroDFM3 templates.

**In situ hybridization in the different species, antibody staining and Drosophila strains**

Antisense RNA probes were prepared from cDNAs using digoxigenin or fluorescein (Boehringer Mannheim GmbH, Mannheim, Germany) as labels. The Drosophila Fezf cDNA (GH 14092) corresponding to the CG31670 was obtained from the Drosophila Genome Resources.

**Xenopus specimens** were prepared, hybridized and stained as previously described [49,50]. For in situ hybridization of European amphioxus, we used a modified version of the protocol previously described [51] (see Additional file 1). Importantly, the hybridization temperature was 65°C, and antibodies were incubated for 3 to 4 hours, followed by overnight washes in MABT buffer (100 mM maleic acid, 150 mM NaCl, 0.1% Tween-20, pH 8) to reduce background. Detection was done with alkaline phosphatase-conjugated anti-digoxigenin (DIG) or anti-fluorescein antibodies. Alkaline phosphatase reaction products were visualized with nitroblue tetrazolium chloride (NBT)-5-bromo-4-chloro-3-indolyl-phosphate p-toluidine salt (BCIP) (purple color), 2-(4-iodophenyl)-5-(4-nitro-phenyl)-3-phenyltetrazolium chloride (INT)-BCIP (red) or BCIP only (cyan). Drosophila embryos were collected on yeasted apple juice-agar plates [52]. Pretreatment of embryos and hybridization in situ were performed as previously described [53], with some modifications: proteinase K treatment was avoided and incubations with anti-DIG (1:1000) were performed for 1 hour at room temperature. For double in situ hybridization and immunostaining, the rabbit anti-β-galactosidase (Cappel) antibody was incubated with the anti-DIG. First, β-galactosidase detection was carried out as described previously [54], then the in situ hybridization signal was developed as described above. The Drosophila strain used were min<sup>880-lacZ</sup> and the Irx deficiency iro<sup>DFM3</sup> [55,56]. The deficiency iro<sup>DFM3</sup> was balanced over the 'blue' balancer Tm6B, P(35UZ)BD1, Tb<sup>1</sup> (Flybase: http://flybase.org/). Embryos were simultaneously hybridized with probes against dFezf and anti-β-galactosidase transcripts, and homozygous iro<sup>DFM3</sup> embryos were those not transcribing β-galactosidase. Embryos were dehydrated and mounted as previously described [57].

**Additional material**

**Additional file 1: Branchiostoma lanceolatum ISH protocol**

Detailed protocol used for whole-mount in situ hybridization in the European amphioxus B. lanceolatum embryos.

**Acknowledgements**

We thank Senda Jimenez-Delgado for help on the experimental work, Jose Luis Ferrán for helpful comments and discussions, and Isabel Almudí for help on image processing. MI, IM and JGF were funded by grants BFU2005-00252 and BMC2008-05776 from the Spanish Ministerio de Educación y Ciencia (MEC), MI and IM held FPI and FPU fellowships, respectively and JGF the ICREA Academia Prize; CP and FC were funded by grants BFU2006-00349/BMC (IMEC) and CVI 2658 (Junta de Andalucía) and JLGS by grants BFU2007-60042/BMC, Petri PET2007_0158, CSD2007-00008 (MEC) and CVI 3488 (Junta de Andalucía).

**Authors’ contributions**

MI conceived the study, carried out the expression experiments in amphioxus and participated in the sequence analyses. CP generated the Drosophila data. IM participated in the sequence analyses and performed the Xenopus and amphioxus ISH. JLGS conceived and participated in the design and coordination of the experiments and generated Xenopus data. FC coordinated the Drosophila experiments. JGF participated in the design and coordination of the project. MI, JLGS, FC and JGF wrote the draft manuscript, and all authors read, discussed and approved the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Received: 11 March 2010 Accepted: 1 September 2010 Published: 1 September 2010**

**References**

1. Kiecker C, Lumsden A: Compartments and their boundaries in vertebrate brain development. Nat Rev Neurosci 2003, 6:553-564.
2. Scholpp S, Wolf O, Brand M, Lumsden A: Hedgehog signalling from the zona limitans intrahyalamica orchestrates patterning of the zebrafish diencephalon. Development 2006, 133:855-864.
3. Martinez S: The isthmic organizer and brain regionalization. Int J Dev Biol 2001, 45:367-371.
4. Osorio J, Mazan S, Retaux S: Organisation of the lamprey (Lampetra fluviatilis) embryonic brain: Insights from LIM-homeodomain, Pax and hedgehog genes. Dev Biol 2005, 288:100-112.
5. Murakami Y, Uchida K, Rijli FM, Kuratani S: Evolution of the brain developmental plan: Insights from agnathans. Dev Biol 2005, 280:249-259.
6. Lowe CJ, Terasaki M, Wu M, Freeman RM, Runft L, Kwan K, Haigo S, Aronowicz J, Lander E, Gruber C, Smith M, Kirschner M, Gerhart J: Dorsalventral patterning in hemichordates: insights into early chordate evolution. PLoS Biol 2006, 4:e291.
7. Shmiedl SM: The evolution of the hedgehog gene family in chordates: insights from amphioxus hedgehog. Dev Genes and Evol 1999, 209:40-47.
8. Meulemans D, Bronner-Fraser M: Insights from amphioxus into the evolution of vertebrate cartilage. PLoS One 2007, 2:e787.
9. Holland LZ, Short S: Gene duplication, co-option and recruitment during the origin of the vertebrate brain from the invertebrate chordate brain. Brain Behav Evol 2008, 72:91-105.
10. Holland LZ, Satoh N, Azumi K, Benito-Gutiérrez E, Bronner-Fraser M, Brunet F, Butts T, Candiani S, Dishaw LD, Ferrier DEK, Garcia-Fernández J
23. Putnam N, Butts T, Ferrier DEK, Furlong RF, Hellsten U, Kawashima T, Large-scale comparison of intron positions. Dev Biol 2006, 295:40-51.

24. Hirth F, Kammereimler L, Frei E, Waldorf U, Noll M, Reichert H. An urbilaterian origin of the tripartite brain: developmental genetic insights from Drosophila. Development 2003, 130:2365-2373.

25. Hirata T, Nakazawa M, Muraoka O, Nakayama R, Suda Y, Hibi M. Zinc-finger genes Fez and Fez-like function in the establishment of diencephalon subdivisions. Development 2006, 133:3994-4004.

26. Jeong J-F, Einhardt Z, Mathur P, Chen L, Lee S, Kawakami K, Guo S. Patterning the zebrafish diencephalon by the conserved zinc-finger protein Fez. Development 2007, 134:127-136.

27. Rodriguez-Seguel J, Alarcon P, Gomez-Skarmeta JL. The Xenopus lrx genes are essential for neural patterning and define the border between prethalamicus and thalamus through mutual antagonism with the anterior repressors Fez and Anx. Dev Biol 2009, 329:258-268.

28. Marullo-Gimeno A, Nieto MA. Evolutionary history of the Snail/Scratch superfamily. Trends Genet 2009, 25:248-252.

29. Kerner P, Hung J, Bejaque J, Le Gouar M, Balavoine G, Vervoort M. Zinc-finger genes Fez and Fez-like function in the establishment of diencephalon subdivisions. Development 2006, 133:3994-4004.

30. Shimoishi SM, CH22 zinc finger genes of the Gb, Zic, KLF, SP, Wlims' tumour, Hucklebee, Snail, Ovo, Spalt, Odd, Blimp-1, Fez and related gene families from Branchiostoma florbidae. Dev Genes Evol 2008, 218:639-649.

31. Sullivan JC, Reitzel AM, Finnerty JR. The amphioxus genome illuminates vertebrate neurogenetics in Drosophila. Brain Res Bull 2005, 66:691-694.

32. Koyabashi D, Koyabashi M, Matthias K, Ogura T, Nakafuku M, Shimamura K. Early subdivisions in the neural plate define distinct competence for inductive signals. Development 2002, 129:83-93.

33. Rodríguez-Seguel J, Alarcón P, Gómez-Skarmeta JL. Characterisation of an amphioxus Fringe gene and the evolution of the vertebrate segmentation clock. Dev Genes Evol 2003, 213:505-509.

34. Shimoishi SM. Zinc finger genes of the Gb, Zic, KLF, SP, Wlims' tumour, Hucklebee, Snail, Ovo, Spalt, Odd, Blimp-1, Fez and related gene families from Branchiostoma florbidae. Dev Genes Evol 2008, 218:639-649.

35. Sullivan JC, Reitzel AM, Finnerty JR. A high percentage of introns in human genes were present early in animal evolution: evidence from the basal metazoan Nematostella vectensis. Genome Inform 2006, 17:219-229.

36. Coulombe-Huntington J, Majewski J. Characterization of intron loss events in mammals. Genome Res 2007, 17:23-32.

37. Roy SW, Fedorov A, Gilbert W. Large-scale comparison of intron positions in mammalian genomes shows intron loss but no gain. Proc Natl Acad Sci USA 2003, 100:7158-7162.

38. Putnam NH, Sistavasta M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov V, Jurka J, Genikhovich G, Grigoriev IV, Lucas SM, Steele RE, Finnerty JR, Technau U, Martindale MQ, Rohksar D. Sea anemone genome reveals ancestral metazoan gene repertoire and genomic organization. Science 2007, 317:86-94.

39. Putnam N, Butts T, Ferrer DEK, Furlong RF, Hellsten U, Kawashima T, Robinson-Rechavi M, Shoguchi E, Terry A, Wu M, Charalambous C, Sibley L, Sudarsanam P, Capra JH, Alikhan F, Bafna V, Bork P, Charbonneau S, Kasif S, Zwieb LC, Miller W, Pincus T, Withler R, Alizadeh A, Smit AFJ, Zimin A, Haussler D, Haussler D. Aniello S, Irimia M, Maeso I, Pascual-Anaya J, Jiménez-Delgado S, Bertrand S, García-Fernández J. Gene expansion and retention leads to a diverse tyrosine kinase superfamily in amphioxus. Mol Biol Evol 2008, 25:1841-1854.

40. Higgins DG, Thompson JD, Gibson TJ. Using CLUSTAL for several sequence alignments. Methods Enzymol 1996, 266:383-402.

41. D'Ansello S, Irimia M, Maeso I, Parcual-Aranay J, Jiménez-Delgado S, Bertrand S, García-Fernández J. An improved whole mount method for in situ hybridization: Iroquois homeobox genes across metazoans. Development 2008, 135:353-364.

42. Hsieh FY, Lim LP, Burge CB. Computational inference of homologous gene structures in the human genome. Genome Res 2001, 11:803-816.

43. Birney E, Durbin R. Using GeneWise in the Drosophila annotation experiment. Genome Res 2000, 10:547-548.

44. Marullo-Gimeno A, Nieto MA. Evolutionary history of the Snail/Scratch superfamily. Trends Genet 2009, 25:248-252.

45. Kerner P, Hung J, Bejaque J, Le Gouar M, Balavoine G, Vervoort M. Insights into the evolution of the snail superfamiy from metazoan wide molecular phylogenies and expression data in annelids. BMC Evol Biol 2009, 9:94.

46. Abascal F, Zardoya R. The amphioxus genome and the evolution of the chordate karyotype. Protoc. 2003, 19:751-755.

47. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference using Monte Carlo Markov chain simulation. Bioinformatics 2005, 21:84-90.

48. Drummond A, Stimmier K, PAML: an object-oriented programming library for molecular evolution and phylogenetics. Bioinformatics 2001, 17:S15-15.

49. Abascal F, Zardoya R, Posada D. ProtTest: selection of best-fit models of protein evolution. Bioinformatics 2005, 21:2104-2105.

50. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003, 52:696-704.

51. Irimia M, Maeso I, Garcia-Fernandez J. Convergent evolution of clustering of Iroquois homeobox genes across metazoa. Mol Biol Evol 2008, 25:1521-1525.

52. Harland R. In situ hybridization: an improved whole mount method for Xenopus embryos. Methods Cell Biol 1991, 36:683-695.

53. Tena JJ, Neto A, de la Calle-Mustienes E, Bras-Pereira C, Casares F, Gomez-Skarmeta JL. Odd-skipped genes encode repressors that control kidney development. Development 2007, 134:193-204.
55. Gómez-Skarmeta JL, Modolell J: Araucan and caupolican provide a link between compartment subdivisions and patterning of sensory organs and veins in the Drosophila wing. Genes Dev 1996, 10:2935-2946.
56. McNeill H, Yang CH, Brodsky M, Ungos J, Siman MA: Mirror encodes a novel PBX-class homeoprotein that functions in the definition of the dorso-ventral border of the Drosophila eye. Genes Dev 1997, 11:1073-1082.
57. Hartenstein V, Posakony JW: The development adult sensilla on the wing and notum of Drosophila melanogaster. Development 1989, 107:389-405.
58. Holland LZ: Chordate roots of the vertebrate nervous system: expanding the molecular toolkit. Nat Rev Neurosci 2009, 10:736-746.
59. Campos-Ortega J, Hartenstein V: The Embryonic Development of Drosophila melanogaster. Heidelberg: Springer-Verlag 1997.

Cite this article as: Irimia et al.: Conserved developmental expression of Fezf in chordates and Drosophila and the origin of the Zona Limitans Intrathalamica (ZLI) brain organizer. EvoDevo 2010 1:7.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit