Evolution of the Vertebrate Paralemmin Gene Family: Ancient Origin of Gene Duplicates Suggests Distinct Functions

Greta Hultqvist1,*, Daniel Ocampo Daza2, Dan Larhammar2, Manfred W. Kilimann1

1 Department of Neuroscience, Unit of Molecular Cell Biology, Uppsala University, Uppsala, Sweden, 2 Department of Neuroscience, Unit of Pharmacology, Uppsala University, Uppsala, Sweden

Abstract
Paralemmin-1 is a protein implicated in plasma membrane dynamics, the development of filopodia, neurites and dendritic spines, as well as the invasiveness and metastatic potential of cancer cells. However, little is known about its mode of action, or about the biological functions of the other paralemmin isoforms: paralemmin-2, paralemmin-3 and palmdelphin. We describe here evolutionary analyses of the paralemmin gene family in a broad range of vertebrate species. Our results suggest that the four paralemmin isoform genes (PALM1, PALM2, PALM3 and PALMD) arose by quadruplication of an ancestral gene in the two early vertebrate genome duplications. Paralemmin-1 and palmdelphin were further duplicated in the teleost fish specific genome duplication. We identified a unique sequence motif common to all paralemmins, consisting of 11 highly conserved residues of which four are invariant. A single full-length paralemmin homolog with this motif was identified in the genome of the sea lamprey Petromyzon marinus and an isolated putative paralemmin motif could be detected in the genome of the lancelet Branchiostoma floridae. This allows us to conclude that the paralemmin gene family arose early and has been maintained throughout vertebrate evolution, suggesting functional diversification and specific biological roles of the paralemmin isoforms. The paralemmin genes have also maintained specific features of gene organisation and sequence. This includes the occurrence of closely linked downstream genes, initially identified as a readthrough fusion protein with mammalian paralemmin-2 (Palm2-AKAP2). We have found evidence for such an arrangement for paralemmin-1 and -2 in several vertebrate genomes, as well as for palmdelphin and paralemmin-3 in teleost fish genomes, and suggest the name paralemmin downstream genes (PDG) for this new gene family. Thus, our findings point to ancient roles for paralemmins and distinct biological functions of the gene duplicates.

Introduction
Paralemmins are a protein family with four previously characterized isoforms in humans and mice: paralemmin-1, paralemmin-2, paralemmin-3 and palmdelphin. The first three are anchored to the plasma membrane through prenylation and di-palmitoylation of a C-terminal cysteine cluster (CaaX motif), whereas palmdelphin is predominantly expressed as a splice variant that lacks the CaaX motif, thereby becoming a cytosolic protein [1]. Paralemmin-1 stimulates cell expansion and the extension of filopodia and processes in fibroblasts, and the formation of filopodia, neurites and dendritic spines in neurons [2,3,4]. Increased expression of paralemmin-1 has been correlated with tumor progression, invasion or metastatic potential [5,6,7]. Paralemmins associate with lipid rafts and have been proposed to function as adaptors between membrane proteins or with the cortical cytoskeleton [4]. This notion is supported by observations of binding between paralemmin-1 and the dopamine receptor D3 [8] and between paralemmin-3 and SIGIRR (single immunoglobulin IL-1 receptor-related molecule) [9]. Human and mouse paralemmin-2 can also be expressed as a fusion protein with the product of a downstream gene, the A kinase anchor protein 2 gene (AKAP2) through transcriptional readthrough and differential splicing [10].

The presence of four paralemmin genes (PALM1, PALM2, PALM3 and PALMD) on four distinct chromosomes in mammals suggested that this gene family might have arisen from a single ancestral gene through the two rounds of tetraploidization early in vertebrate evolution referred to as 2R [11,12,13]. The lineage leading to the lancelets diverged from the rest of the chordate lineage before the 2R events, but shares several attributes common to all chordates like a hollow dorsal neural tube and a notochord [14]. These facts, together with the sequenced genome of the Florida lancelet Branchiostoma floridae [11,15], make this lineage highly interesting in the evolution of complex nervous systems. It has been suggested that 2R provided many new genes that have contributed to vertebrate features such as a complex nervous system, the neural crest, jaws, limbs etc. [16]. A third tetra-
pliodization event, called 3R, occurred in the teleost fish lineage approximately 350 million years ago [17,18,19], which might have generated additional paralemmin diversity. Many gene families, essential for the function and development of the brain and nervous system, have been found to have expanded in this way [20,21,22,23,24,25,26].

There are several reasons why the paralemmin gene family merits closer evolutionary study of sequence features. The known paralemmin proteins are characterized by their predicted intrinsic disorder and extensive sequence regions with low inter-species conservation. Conserved sequence features common to all paralemmins have not been characterized until the present study. These may be utilized to explore interactions of paralemmins with other proteins involved in the many functions of the paralemmins. Further, the C-termini of paralemmin-2 and palmdelphin show differential splicing and the PALM2 and AKA2 genes produce a peculiar fusion product, why it is interesting to investigate if similar features are found in other paralemmin gene family members.

Here we have investigated the evolution of the paralemmin family by analyzing the genomes of a wide selection of vertebrate species, as well as the lancelet Branchiostoma floridae. To determine the evolutionary relationships between the family members, we have also analyzed gene families that show conserved synteny with the paralemmin genes. In addition to the sometimes co-transcribed AKA2, we included the neighboring families of sphingosine-1-phosphate receptors (SIPR), plasticity related genes (PRG) (also called lipid phosphate phosphatase related proteins), ATP-binding cassette subfamily A (ABCA), calponin (CNV), and polypyrimidine tract-binding protein (PTBP) in the analysis.

The combined information from sequence-based phylogenies as well as chromosomal locations of the paralemmin genes and the adjacent gene families allow us to conclude that the paralemmin family expanded in the early vertebrate genome duplications and thus that all four paralemmin isoforms have been retained for more than 450 million years of vertebrate evolution, suggesting a unique functional role for each family member.

Methods

Database searches and identification of paralemmin genes

The human paralemmin family sequences were used as queries in tblastn searches [27] of the Ensembl database [28] (www.ensembl.org) in the following vertebrate genomes: human (Homo sapiens), mouse (Mus musculus), chicken (Gallus gallus), Western clawed frog (Silurana Xenopus tropicalis), zebrafish (Danio rerio), medaka (Oryzias latipes), fugu (Takifugu rubripes), three-spined stickleback (Gasterosteus aculeatus), green spotted pufferfish (Tetraodon nigroviridis) and sea lamprey (Petromyzon marinus). In order to appropriately root the phylogenetic trees, corresponding searches were made in the Ensembl genome databases for a tunicate (Ciona intestinalis) and a nematode (Caenorhabditis elegans) or fruitfly (Drosophila melanogaster), as well as in the lancelet (Branchiostoma floridae) in the National Center for Biotechnology Information (NCBI) databases at http://www.ncbi.nlm.nih.gov. In Ensembl, the protein predictions representing the best BLAST hits were collected and their chromosomal locations were noted. For short, incomplete or divergent protein predictions, better predictions were manually curated from the corresponding genomic sequence with regard to consensus start and stop codons, splice donor and acceptor sites and sequence similarity to other identified family members. Expressed sequence tags (ESTs) curated and aligned by the Ensembl database were also considered. The InterPro database of protein domain predictions (www.ebi.ac.uk/interpro) was used to identify conserved protein domains. Ensembl searches were initiated in database versions 53 (July 2009) and 56 (September 2009), and simultaneously in the pre.ensembl.org database for the sea lamprey genome. All sequences and database identifiers were verified against the most updated genome assembly versions as shown in Ensembl database version 66 (February 2012). This information can be found in Table S1.

To identify putative paralemmin family members in the lancelet genome with greater certainty, a protein blast search was performed using the pattern hit initiated algorithm (PHI-BLAST) in the NCBI non-redundant protein sequence database. The identified zebrafish palmdelphin-B (see Results) was used as query and the conserved amino acid motif KX[KR]XXR[ED]XWL[ML], identified from a preliminary alignment of the identified vertebrate paralemmin homologs, was entered as PHI-pattern.

Identification of neighboring gene families

Protein families, as defined by the automatic Ensembl database protein family predictions, which had members closer than 5 Mb to at least three different parlemmins in the human genome were considered for synteny analyses. The protein sequences corresponding to the best gene predictions for these families were collected and their chromosomal locations noted. These sequences were used for tblastn searches in order to identify additional family members. The AKA2 gene and its protein family members were also included in the analysis since AKA2 has been reported to be transcribed together with paralemmin-2 [10]. These protein families were analyzed as described for the paralemmins with regard to species representation (except green spotted pufferfish), sequence alignment and phylogenetic analyses.

Sequence alignment and phylogenetic analyses

The identified protein predictions from the database searches were used to produce amino acid alignments using the ClustalW [29] tool with standard settings in Jalview2.4 [30]. Green spotted pufferfish sequences were included in the phylogenetic analyses of the paralemmin gene family, but not of neighboring gene families due to the close evolutionary relatedness of this species and fugu. The final alignments were manually inspected using Jalview with regard to incomplete protein sequence predictions and poorly aligned sequence stretches. Details on the sequence curation and alignment editing process can be given upon request.

Two bootstrapped phylogenetic methods were applied on the alignments: a neighbor joining (NJ) analysis [31] and a phylogenetic maximum likelihood (PhyML) analysis [32]. The NJ tree construction method (with 1000 bootstrap replicates) was applied with standard settings (Gonnet weight matrix, gap opening penalty 10.0 and gap extension penalty 0.20) in ClustalX 2.0 [29]. The PhyML method was applied using the web-application of the PhyML 3.0 algorithm available at http://www.atgc-montpellier.fr/phyml/ with the following settings: amino acid frequencies (equilibrium frequencies), proportion of invariable sites and gamma shape parameters were estimated from the datasets; the number of substitution rate categories was set to 8; BIONJ was chosen to create the starting tree and both the NNI and SPR tree improvement methods were used to estimate the best topology; both tree topology and branch length optimization were chosen. For branch support a bootstrap analysis with 100 replicates was chosen. The best amino acid substitution models for the PhyML analyses were estimated from the alignments using ProtTest 1.4 [33]. Models were tested with no add-ons and assuming eight gamma rate categories, the optimization strategy was set to slow and the BIONJ strategy was selected for the random input tree.
The JTT model was assumed for all PhyML analyses based on the ProTest results.

The paralemmin gene family tree was not rooted since no complete invertebrate paralemmin sequence could be identified. Regarding the neighboring gene families, identified nematode sequences were used if such a sequence could be found, if not, identified lancelet sequences were used. If no invertebrate protein prediction could be found, the trees were unrooted.

EST sequence analysis of read-through transcripts

To detect possible read-through transcripts similar to the Palm2-AKAP2, the chromosome locations of all identified paralemmin family members were analysed with regard to the expressed sequence tags (ESTs) that are automatically identified and aligned to the genomic sequence by the Ensembl database. These EST sequences are gathered from the NCBI dbEST database at http://www.ncbi.nlm.nih.gov/dbEST.

Results

Sequence identification of paralemmins

The BLAST searches of the genome databases, using the amino acid sequences of the known paralemmins in human and mouse (Palm1–A, -B, -C, and Palm3) as queries enabled us to identify paralemmin sequences in several other vertebrates. In tetrapods, aside from human and mouse, the chicken and Western clawed frog genomes were searched. We could identify Palm1, Palm2 and Palm3 sequences in all four genomes, while Palm3 sequence could only be identified in the human, mouse and Western clawed frog genomes. The lack of a Palm3 sequence in the chicken genome could be due to a genome sequencing or assembly error. Therefore we also searched the zebra finch (Taeniopygia guttata), duck (Anas platyrhynchos) and turkey (Meleagris gallopavo) genomes, but no Palm3 sequence could be identified in these bird genomes either, indicating that Palm3 has been lost from the bird lineage.

In the teleost fish genomes up to six different paralemmin sequences could be identified, including Palm2, Palm3 and duplicates of Palm1 and Palm4. However, there appear to be several differential losses of paralemmin genes in the teleost genomes. We have named the teleost-specific duplicates paralemmin-1A and paralemmin-1B (Palm1-A and -B) and Palm4-A and -B (Palm2-A and -B) because their chromosomal locations suggest origin in 3R (described below), and such duplicates are usually designated with A and B. The greatest diversity could be found in the zebrafish genome with six paralemmin genes. The identified paralemmin genes and their chromosome locations are shown in Table 1. The accession numbers of all identified gene predictions can be found in Table S1. A partial Palm1-A-like sequence could be identified in the green spotted pufferfish including only exon F3 and a short segment of exon G (see Figure 1), similarly only a single partial Palm3-like sequence could be identified in medaka. Due to genome sequencing or assembly errors in these genome assemblies, these sequences could not be predicted in their entirety and were therefore not included in the phylogenetic analyses.

Partial sequences of at least three different paralemmin genes were found in the elephant shark (Callorhinchus milii) genome database, but since they were found on very short genomic scaffolds and covered different regions of the proteins, they could not be included in alignments for phylogenetic analyses (data not shown). More importantly, one paralemmin sequence could be found in the jawless vertebrate genome of sea lamprey, Petromyzon marinus, representing a lineage that is known to have diverged before the origin of jawed vertebrates.

When all the identified vertebrate paralemmin sequences were aligned, a conserved motif in the N-terminal region became apparent (see below). A pattern-hit initiated Blast (PHI-blast) search with this motif (KK[KR][XXR][ED][XXL][ML]) in the lancelet generated one hit on genomic scaffold 302 (assembly v1.0) and a putative exon containing this motif could be predicted (see Discussion). The Blast-hit can be accessed in the Branchiostoma floridae v1.0 browser available at http://genome.jgi-psf.org/ (last accessed May 24, 2012) in the region scaffold302:530570-530600 (minus strand). Positive hits for paralemmin sequences could also be found in any other invertebrate genome such as the extensively analyzed genomes of the tunicate Ciona intestinalis, the sea urchin Strongylocentrotus purpuratus, the fruitfly Drosophila melanogaster, and the nematode Caenorhabditis elegans.

Paralemmin gene organization and sequence features

The identification and annotation of paralemmin genes in the different vertebrate genomes revealed that they have a common gene organization (Figure 1). The 5′ parts of the genes contain six to eight small exons (A–F3) that are followed by a large 3′ exon (G). This mirrors the previously known gene organization of Palm1, Palm2 and Palm3 in human and mouse [10]. Still, paralemmin sequences diverge substantially between species and isoforms. For instance, the amino acid sequence of paralemmin-3 is almost twice as long as that of paralemmin-1, a difference that is largely due to an earlier start for the last large exon of Palm3 (exon G in Figure 1). The corresponding exon of Palm3 also starts earlier than in Palm1 and Palm2. Because of these differences, it was important to identify conserved sequence features for all identified paralemmin genes in order be able to use the relevant sequence regions for the phylogenetic analysis. We found regions of sequence similarity in the first exons (A–E) and the end of the downstream exon that we call exon H. The amino acid sequence of exon H encoded by this exon is highly conserved in all identified paralemmin sequences except teleost Palm3, the lancelet homolog and stickleback Palm2 (Fig. 2A). The M and I residues are not conserved across all sequences while the F and the following G residue are completely conserved in our alignment. This stretch, encoded by exon G, has been named the MIF motif [10]. The C-terminal CaaX motif could also be identified in all sequences except teleost Palm3-A sequences (Figure 2A). In fact, the teleost Palm3-A sequences show no apparent sequence conservation between them in the C-terminal. The alternatively spliced C-terminal KKVI motif could be identified in all tetrapod Palm3 sequences, but not in any teleost Palm3s (Fig. 2B). This KKVI motif is encoded by an additional downstream exon that we call exon H. The amino acid sequence encoded by this exon is highly conserved in all identified Palm3 sequences; only one out of 14 positions deviates (Fig. 2B).

Palm3 has two splice variants; one contains the CaaX motif and the other uses exon H with the KKVI motif. In the alignment
used for the phylogenetic analyses, the splice variant with the CaaX motif was used due to higher sequence conservation within the PALM genes, even though the cytosolic PALMD splice variant without the CaaX is the more frequent variant. The alignments in Figure 2 show both splice variants.

**Phylogenetic analysis of the paralemmin gene family**

Our phylogenetic analyses of the paralemmin sequences using both the distance-based neighbor joining method (NJ) and the phylogenetic maximum likelihood method (PhyML) concurrently show that the paralemmins form four distinct clusters: PALM1, PALM2, PALM3 and PALMD (Figure 3). The PALM2 and PALMD clusters are well supported in both analyses, however, the lower bootstrap support for the PALM1 branch in the NJ tree and the PALM3 branch in the PhyML tree likely reflect a larger degree of sequence divergence within these clusters. Notably, the internal topology of the PALM1 cluster could not be resolved with high bootstrap support using either method, indicating a larger degree

![Figure 1. Conserved organization of paralemmin isoform genes. Exon sizes are roughly proportional relative to each other, whereas intron sizes are schematic. Kinked lines indicate differential splicing. Human (h-) and teleost fish (tf-) genes are shown as examples. doi:10.1371/journal.pone.0041850.g001](image)

**Table 1. Identified PALM genes and their chromosomal locations.**

| PALM1-A | PALM1-B | PALM2 | PALM3 | PALMD-A | PALMD-B |
|---------|---------|-------|-------|---------|---------|
| Human (Hsa) | 19: 0.71\* | 9: 112.54\* | 19: 14.17 | 1: 100.11 |
| Mouse (Mmu) | 10: 79.26\* | 4: 57.58\* | 8: 86.55 | 3: 116.67 |
| Chicken (Gga) | 28: 2.25\* | Z: 64.23\* | - | 8: 12.87\* |
| Western clawed frog (Xtr) | S_289: 1.39\* | S_229: 1.52\* | S_649: 0.15 | S_252: 0.88 |
| Zebrafish (Dre) | 11: 14.44\* | 2: 26.38 | 10: 4.91\* | 6: 9.59\* | 24: 30.85\* | 2: 18.92\* |
| Medaka (Ola) | - | 22: 4.42 | 12: 18.32\* | S_6823:1bae | U_236: 1.11\* | - |
| Fugu (Tru) | S_269:0.17\* | S_158: 0.27 | S_409: 0.14\* | S_207:0.31\* | - | S_285:0.14\* |
| Stickleback (Gac) | VIII: 17.39\* | - | XIV: 9.36\* | 0X: 17.53\* | S_95:0.36\* | III: 5.24\* |
| Green spotted pufferfish (Tnl) | S_10234:14.32\* | 10: 9.63 | 4: 0.98\* | 18: 8.82\* | - | S_7729: 58.61\* |
| Lamprey (Pma) | GL477367: 9.36 Kb | | | | | |
| Lancelet (Bre) | Scaffold 302 | | | | | |

*PALM genes with an identified adjacent AKAP2 homolog/paralemmin downstream gene (PDG). For a detailed list of PDG gene locations see Table S1. In the case of Medaka, no PALM1-A or PALMD-B gene was found, but PDG1A and PDG4B genes could be predicted in the expected respective locations. PDG3B genes were found in stickleback and in medaka but are not listed in this table.

**Table 1. Identified PALM genes and their chromosomal locations.**

- **T**he identified PDG genes are located on the opposite strand to these PALMD-B genes.
- **K**nown Palm2-AKAP2 fusion protein [10].
- **R**ead-through ESTs of PALM2-A and PDG4B detected. See Results.
- **T**he identified partial PALM1-A like sequence in Green spotted pufferfish and partial PALM3 sequence in medaka were not used in the phylogenetic analyses. See Results.

Chromosome assignments are given where possible, “s...” denotes assignment to unassembled scaffolds and “u...” denotes assignment to unassembled ultracontigs. Locations are given in megabases, unless specified. Information about genomic assembly versions can be found in Table S1.

doi:10.1371/journal.pone.0041850.t001
of divergence between the PALM1 sequences in the different species. The teleost-specific duplicates PALMD-A and PALMD-B form well-supported branches within the PALMD cluster with both phylogenetic methods. With the topology within the PALM1 cluster being less resolved, there is not a clear teleost-specific PALM1-A and PALM1-B divergence, although the PALM1-B sequences cluster together with high bootstrap support in both trees (Figure 3). The identified lamprey sequence could not be assigned to a specific subtype in either analysis, although it seems to be more similar to PALM1 and PALM2.

The phylogenetic trees in Figure 3 show that PALM3 is the least conserved of the paralemmin isoforms, as indicated by the longer branch-lengths within this cluster. Sequence conservation between the species included in the analysis and with the other paralemmin isoforms is low, only showing high identity within the paralemmin and CaaX motifs (Figure 2). The MIF motif described above could not be identified in the PALM3 sequences.

Identification of paralemmin downstream gene family (PDG)

The gene located 3' to PALM2, AKAP2, has been reported to give rise to a fusion protein (Palm2-AKAP2). Therefore we were interested in identifying homologs of this gene downstream of other paralemmin genes in the studied genomes. We found genes downstream of PALM1 that have sequence similarity to AKAP2 in most studied genomes (Table 1). In teleost fish genomes, AKAP2 homologs could only be found downstream of PALM1-A but not PALM1-B genes. In the green spotted pufferfish genome it was not possible to find homologs of AKAP2 downstream of either PALM1-A or -B.

Furthermore, we identified AKAP2 homologs downstream of PALM3, PALMD-A and PALMD-B in the teleost genomes, but not downstream of tetrapod PALM3 and PALMD genes. No AKAP2 homologs could be identified in the sea lamprey or elephant shark genomes.

We have called this family of AKAP2 homologs, paralemmin downstream genes (PDG). Our phylogenetic analysis of PDG sequences shows four well-supported clades corresponding to the chromosomal locations adjacent to PALM1, PALM2, PALM3 and PALMD genes (Figure 5); therefore we have assigned the names PDG1, PDG2 (AKAP2), PDG3 and PDG4 (located downstream of palmdelphin, the fourth paralemmin family member) to the identified sequences. The PDG genes, including AKAP2, are summarized in Table 1 and their chromosome locations are shown in Table S1. The amino acid alignment of the PDG sequences is shown in Figure S6. Although there is significant sequence conservation between species and between the duplicates, we could only identify one region with reasonably high conservation in the C-terminal, starting around position 1290 of the alignment (Figure S6). This region includes two invariant positions (WE) as well as two positions representing conservative substitutions across all homologs in all studied species, with additional positions showing isoform-specific conservation. We have marked the small motif of 11 amino acids encompassing the most conserved positions as the PDG motif in Figure S6. Additionally there is a region around positions 1127–1160 rich in positively and negatively charged amino acid residues showing a high degree of isoform-specific conservation.

To investigate whether the paralemmin gene family expanded through the early vertebrate genome duplications, and if the additional teleost duplicates are the result of the 3R event, we identified gene families with members adjacent to the paralemmin genes and performed phylogenetic analyses. The identified families are: sphingosine-1-phosphate receptors (S1PR), plasticity related genes (PRG) (also called lipid phosphatase related proteins), ATP-binding cassette sub-family A (ABCA), calponin (CNN), and polypyrimidine tract-binding protein (PTBP). This selection of neighboring families represents all Ensembl protein family predictions with members closer than 5 Mb to at least three paralemmin genes in the human genome. Additionally, homologs of the sometimes co-transcribed AKAP2 were identified by sequence homology and chromosomal location adjacent to a paralemmin gene (see below). The identified blocks of conserved synteny around the paralemmin genes in human, chicken, zebrafish and stickleback are shown in Figure 4. Phylogenetic trees using the PhyML method were made for each protein family (Figure 5, Figures S1, S2, S3, S4, and S5), which allowed us to assign new sequence predictions their correct subtype names within the families. The accession IDs of the identified Ensembl protein family predictions as well as all identified family members are shown in Table S1. The assigned sequence names of newly predicted family members are written in a separate column. Three identified sequences were not included in the phylogenetic analyses because of gaps in the genomic read or spurious regions of poor sequence alignment: PRG2 and ABCA7 in chicken, and PRG4A in stickleback. These boxes are shown in Figure 4 with a dashed frame.
Discussion

Evolution of the paralemmin gene family

The paralemmin family of proteins consists of four members encoded by four different genes, \( \text{PALM1}, \text{PALM2}, \text{PALM3}, \text{PALM4} \), in the investigated mammalian genomes and in the genome of the frog \( \text{Xenopus tropicalis} \). It consists of three members in the chicken genome as the \( \text{PALM3} \) gene appears to have been lost from the avian lineage. These results are summarized in Table 1. Due to considerable sequence divergence between the four isoforms, and the absence of invertebrate orthologs, their evolutionary history was difficult to deduce solely based upon sequence information and sequence-based phylogenetic analyses. In previous studies of other gene families it has been possible to resolve the evolution by combining sequence data with information on the chromosomal locations of the genes across a wide selection of vertebrate genomes. This includes investigating the conserved synteny of gene families adjacent to the \text{PALM} genes as well as the possible paralogy relationships between the chromosome segments. Thereby one can also use the phylogenetic analyses and chromosomal data of the adjacent gene families to see if these share the same evolutionary history and may perhaps give a clearer picture of the events.

By analyzing both phylogenetic trees and chromosomal data, we conclude that the four tetrapod paralemmins arose from a single ancestral gene in the two basal vertebrate tetraploidizations, 2R, prior to the origin of jawed vertebrates. Although our phylogenetic analyses of the paralemmins are unrooted (Figure 3) because no true invertebrate ortholog could be identified, the time-frame for the divergence of the four paralemmin isoforms clusters is shown by the rooted phylogenetic analyses of the neighboring gene families, which support the divergence of the four vertebrate paralemmin lineages or clades in the same timeframe as 2R: before the divergence of lobe-finned fish (including tetrapods) and ray-finned fish, and after the emergence of vertebrates.

Our discovery of a sequence fragment with paralemmin features in the lancelet was surprising because paralemmins are considered vertebrate specific [1,34]. While this finding potentially supports the presence of an ancestral \text{PALM} gene in the common ancestor of the lancelet and vertebrates, the identified sequence only constitutes a putative prediction of exon D (Figure 1) with sequence similarity to the identified paralemmin-motif and sheds little light on the structure of paralemmin before the emergence of vertebrates. The identified sequence can be seen in Figure 2A aligned slightly below the rest of the alignment. The sequence includes most of the paralemmin motif, with three of the four invariant positions and all three positions showing conservative substitutions. The fourth invariant position is on another exon in the other species.

It is not possible to determine the orthology relationships of the single paralemmin sequence that was found in the genome of the sea lamprey \( \text{Petromyzon marinus} \). In the phylogenetic maximum likelihood tree there is not enough phylogenetic resolution to confidently assign it to a paralemmin subtype (bootstrap value 25), although it seems to be more similar to \text{PALM1} and \text{PALM2} (Figure 3B), while the neighbor joining tree suggests it represents an ancestral lineage including both \text{PALM1} and \text{PALM2} (Figure 3A) with somewhat higher support. It is not clear whether the lineage leading to the sea lamprey underwent the 2R tetraploidization or possibly only one of them [35,36].

Interestingly, none of the neighboring families in the blocks of conserved synteny around \text{PALM3} have orthologs in the bird genomes that were investigated (Figure 5, Figures S1, S2, S3, S4, and S5), summarized in Figure 4), indicating that \text{PALM3} was lost as part of a larger block in this lineage. Our phylogenetic analyses (Figure 3) also suggest that the tetrapod \text{PALM3} sequences evolve more rapidly than the other isoform genes, indicating a lower conservative selection pressure. The larger distances between the sequences in the tree, point towards an increased rate of substitutions in this lineage.

In addition to the expansion in 2R, the teleost-specific tetraploidization, 3R, produced additional isoforms of \text{PALM1} and \text{PALM4} (Table 1, Figure 3). These duplicates, which we have called \text{PALM1-A} and \text{PALM1-B} and \text{PALM4-A} and \text{PALM4-B}, could all be found in the zebrafish and medaka genomes. In the stickleback, fugu and green-spotted pufferfish genomes there appear to have been differential losses (Table 1, Figure 3).

The support for the origin of the four paralemmin isoform genes by the early vertebrate whole genome duplications, and the subsequent expansions in the teleost-specific tetraploidization, comes from the analysis of conserved synteny blocks around the \text{PALM} genes (Figure 4) as well as the phylogenetic analyses of their chromosomal neighbors (Figure 5, Figures S1, S2, S3, S4, and S5). Our synteny analyses of the regions spanning 5 Mb around each paralemmin gene identified six additional gene families that seemed to belong to the same paralogon, i.e. that quadruplicated as part of the same chromosomal block as the ancestral paralemmin gene and would thus share the same evolutionary history. The phylogenetic analyses of these gene families, using identified lancelet, tunicate or nematode \text{Caenorhabditis elegans} sequences as outgroup, are shown in Figure 5 and Figures S1, S2, S3, S4, and S5. The phylogenetic trees of the \text{Amp} and \text{PALM3} cassette subfamily A (\text{ABCA}), \text{calponin} (\text{CNN}), \text{polyprimidin}ic tract-binding protein (\text{PTBP}) and paralemmin downstream gene (\text{PDG}) families are consistent with the phylogenetic analyses of the paralemmins (Figure 3), and support their concomitant duplications as part of the same chromosome block. However, there have been differential gene losses: The \text{CNN} family lacks paralogs neighboring \text{PALM2} genes, while the \text{ABCA} and \text{PTBP} families lack paralogs neighboring \text{PALM3} genes (Figures S1, S2, S3, S4, and S5). The phylogenies of the sphingosine-1-phosphatase related proteins (\text{SIPR}) and plasticity related genes (\text{PRG}) families are more difficult to resolve. The phylogenetic tree of the \text{PRG} family is not fully consistent with the phylogenetic analysis of the paralemmins (Figure S4). However, this is likely due to a local gene duplication before the basal tetraploidization. The \text{PRG} family has genes neighboring all four paralemmin isoform genes in all of the investigated genomes, and their chromosomal locations are consistent with the expansion of the family as part of the same chromosome block as the \text{PALM} genes (Figure 4, Table S1). As for the \text{SIPR} family, there appears to have been a local duplication after the basal tetraploidization as well as several translocations in the teleost lineage. Additionally, no homolog could be identified in the investigated invertebrate genomes, which makes the relative dating of the duplication events difficult (Figure S3). However,
Evolution of the Vertebrate Paralemmin Gene Family

Human

- **PALM1**
  - Chromosome 19
    - PALM1: 0.71 MB
    - PRG1: 0.75 MB
    - PRB1: 0.80 MB
    - CNN2: 0.97 MB
    - ABCA7: 1.04 MB
    - S1PR4: 3.18 MB

Chicken

- **PALM1**
  - Chromosome 28
    - S1PR4: 0.09 MB

Zebrfish

- **PALM1-A**
  - Chromosome 11
    - ABCA7: 5.67 MB
    - PRG2A: 14.39 MB
    - PALM1-A: 14.44 MB
    - PRG1: 14.52 MB
    - PTB1B: 14.56 MB

- **PALM1-B**
  - Chromosome 2
    - CNN2: 23.31 MB
    - PRG2A: 26.33 MB
    - PALM1-B: 26.38 MB
    - PTB1A: 26.43 MB

Stickleback

- **PALM1-A**
  - Chromosome 11
    - ABCA7: 9.61 MB
    - S1PR4: 13.30 MB
    - PRG2A: 14.74 MB
    - PALM1-A: 17.36 MB
    - PRG1: 17.39 MB
    - PTB1B: 17.38 MB
    - PTB1A: 17.48 MB

- **PALM1-B**
  - Chromosome 2
    - CNN2: 10.31 MB
    - PRG2A: 10.35 MB

- **PALM2**
  - Chromosome 9
    - S1PR3: 91.60 MB
    - PRG2: 103.79 MB
    - ABCA7: 107.54 MB
    - PALM2: 112.54 MB
    - PALM2: 112.81 MB
    - ROU1: 114.98 MB

- **PALM3**
  - Chromosome 19
    - S1PR2: 10.62 MB
    - S1PR5: 11.33 MB
    - CNN1: 11.65 MB
    - PALM3: 14.17 MB

- **PALM3-A**
  - Chromosome 10
    - S1PR5B: 4.57 MB
    - PALM2: 4.91 MB
    - PRG3: 4.88 MB
    - ROU1: 11.23 MB

- **PALM3-B**
  - Chromosome 6
    - S1PR5A: 4.02 MB
    - PALM2: 9.36 MB
    - S1PR2: 9.32 MB
    - PRG3: 9.38 MB

- **PALM3-C**
  - Chromosome 6
    - S1PR5A: 4.02 MB
    - S1PR2: 9.36 MB

- **PALM4**
  - Chromosome 1
    - ABCA4: 94.46 MB
    - CNN3: 95.39 MB
    - PTB2: 97.19 MB
    - PRG5: 99.36 MB
    - PRG1: 99.73 MB
    - PALM2: 100.11 MB
    - S1PR1: 101.70 MB

  - Chromosome 8
    - PALM2: 12.87 MB
    - S1PR1: 12.35 MB
    - PRG1: 12.95 MB
    - PRG5: 13.00 MB
    - PTB2: 13.67 MB
    - CNN3: 14.10 MB
    - ABCA4: 14.35 MB

- **PALM-A**
  - Chromosome 2
    - S1PR1: 29.48 MB
    - S1PR2: 30.65 MB
    - PRG1: 30.93 MB
    - PRG5: 30.98 MB
    - PTB2: 31.36 MB
    - PTB2: 31.68 MB

- **PALM-B**
  - Chromosome 3
    - S1PR5: 13.61 MB
    - S1PR5A: 13.73 MB
    - S1PR5A: 19.18 MB
    - S1PR5: 55.47 MB

  - Chromosome 24
    - S1PR5B: 29.48 MB
    - S1PR1: 30.65 MB
    - PRG1: 30.93 MB
    - PTB2: 31.36 MB

  - Scaff 37
    - S1PR1: 29.48 MB

  - Scaff 95
    - S1PR2: 30.65 MB
    - PRG1: 30.93 MB
    - PTB2: 31.36 MB

  - Gill
    - PALM2: 14.87 MB
    - ABCA4: 14.94 MB
    - PTB2: 18.29 MB
    - PRG1: 18.84 MB
    - PALM2: 18.92 MB
    - PDG4B: 18.94 MB
    - ABCA4: 11.52 MB
there are homologs neighboring all four paralemmin isoform genes in all tetrapod genomes, except that of the Western clawed frog where mapping data is not available, which indicates that the family expanded in the same time-frame and chromosomal block as the PALM genes (Table S1), but that secondary events complicate the tree topology (Figure S3).

Together with the synteny analyses (summarized in Figure 4), these phylogenetic data support duplication of these chromosomal regions in the basal tetraploidizations or are at least consistent with such a scenario. The neighboring gene families also support further duplications in the teleost 3R event, as detailed in the supporting information. Our proposed evolutionary scenario is presented in Figure 6.

The chromosomal regions that harbor the PALM genes have previously been identified as belonging to a paralogy group, i.e. regions that are derived from the two early vertebrate rounds of tetraploidization, 2R. In the analysis of the lancelet genome and the evolution of the vertebrate karyotype [37] the PALM-bearing segments of human chromosomes 1 (91.24–102.06 Mb), 9 (93.15–114.84 Mb) and two segments of chromosome 19 (1–9.86 Mb and 9.36–15.44 Mb) were found to represent four ancient blocks of conserved linkage. These segments were also found to correspond to the same ancestral chromosome before 2R in a reconstruction of the ancestral vertebrate genome and evolution of vertebrate chromosomes [13]. The two segments of chromosome 19, which harbor PALM1, and PALM3, respectively, likely represent a fusion event in the lineage leading to humans. In the mouse genome the PALM1 and PALM3 genes are located on different chromosomes, 10 and 8 respectively (Table 1). This is consistent with a large-scale rearrangement of synteny regions between the mouse and human genome (see for instance the homology map at http://www.ncbi.nlm.nih.gov/projects/homology/maps/human/chr19/, last accessed May 23, 2012).

After the divergence of the paralemmin gene family, the paralemmin isoform genes also evolved differential expression profiles. In mice, palmdelphin protein is broadly expressed in virtually all tissues analyzed, with highest expression in the heart [1], whereas the paralemmin-1 protein and mRNA are detected in most tissues but at much more differential levels than palmdelphin, and by far highest expression in the brain [4]. Paralemmin-2, Palm2-AKAP2 and paralemmin-3 have yet other patterns of tissue expression; each one of these genes is most highly expressed in a different tissue with the brain being the most highly expressed.

Sequence features of the paralemmin proteins

Detailed inspection of all paralemmin sequences allowed us to identify a few well-conserved sequence motifs. Exon D encodes a stretch of 11 highly conserved residues, of which four are invariant and three represent conservative substitutions, which we have called the paralemmin motif (Figures 1 and 2). Because this domain is so well conserved across species and paralemmin isoforms it is likely to be associated with a conserved function. Other highly conserved features in the paralemmins are the MIF motif encoded by exon G and the CaaX/KKVI motifs at the C-terminus. The MIF motif consists of 7 residues that show a high degree of conservation in all paralemmins except paralemmin-3 and surprisingly the stickleback paralemmin-2 (Figure 2). The paralemmin-3 sequences differ between species in this stretch, indicating absence of conservative selection pressure. However, occasional sequence similarity between species downstream of this point suggests that the paralemmin-3 sequences have changed by amino acid substitution rather than insertion or deletion.

The CaaX motif that attaches the parlemmins to the cell membrane by a lipid anchor is conserved in all paralemmins except palmdelphin-A in teleosts. We have not found any conserved C-terminal motif for palmdelphin-A; it has neither the CaaX nor the KKVI motif, whereas in palmdelphin-B we identified the CaaX motif (Figure 2). This shows that the loss of the palmdelphin-A CaaX motif happened after the teleost 3R event. In mammals, the single palmdelphin gene can give rise to either a membrane-anchored or a cytosolic variant of palmdelphin through two different splice variants using either the CaaX membrane-anchoring motif or the conserved KKVI motif encoded by exon H (Figures 1 and 2). The cytosolic variant is the predominant expression product of mammalian palmdelphin [10]. Based on the differing sequence features, it is possible that in teleost fish this specialization is accounted for by two separate proteins; one of the 3R duplicates, palmdelphin-A, is the cytosolic form, even though they seem to lack the alternatively spliced exon H, while the other, palmdelphin-B, is the membrane-anchored version of the protein. The overall identity of zebrafish palmdelphin-A and palmdelphin-B is 41%, suggesting that perhaps also other functions have become subdivided between the two gene products. The conserved KKVI motif in the tetrapod palmdelphins is also preceded by an extended highly conserved stretch, with 13 out of the last 14 amino acids between human, mouse, chicken and frog identical, indicating functional importance perhaps for the binding of glutamine synthetase [1].

The C-terminus of palmdelphin-B in teleost fish has only two cysteines among the last 7 amino acids while all the other family members with CaaX boxes have three (Figure 2). The most C-terminal cysteine is prenylated and the preceding cysteine is palmitoylated to constitute a membrane anchor. It has been reported that mutagenesis of any of these three cysteines in paralemmin-1 caused the protein to lose its ability to increase the number of filopodia and spines in transfected cells [2]. Therefore, if palmdelphin-B has lost one lipidation site, it may be expected to have acquired different membrane interaction properties than the family members that do have three cysteines.

The paralemmin downstream genes (PDG)

It was previously reported that the AKAP2 gene immediately downstream of PALM2 in human, mouse and rat can be co-transcribed with PALM2 to generate a fusion protein [10,38]. This opens an intriguing possibility regarding co-transcription in other species as well as for the other paralemmins. We searched for AKAP2-related genes downstream of all identified paralemmin family genes in all species’ genomes and constructed a sequence-based phylogenetic tree of this gene family, which we have called
the paralemmin downstream genes. The phylogenetic tree is shown in Figure 5. Although no sequence similar to AKAP2 was previously detected near PALM1 [10], we could now find such genes downstream of all four main paralemmin isoform genes, with notable differential gene losses (see Results, Table 1). The chromosomal locations of the PDG genes as well as the phylogenetic analysis shows that the paralemmin downstream genes have evolved contiguously with the paralemmins. No PDG
genes could be found in the lamprey nor the elephant shark genomes, which diminishes the phylogenetic resolution of this family.

In teleost fishes the chromosomal duplication in 3R that generated duplicates of {\textit{PALM1}} and {\textit{PALMD}} also gave rise to duplicates of the downstream genes to generate {\textit{PDG4A}} and -B flanking {\textit{PALMD}}-A and -B. However, no corresponding {\textit{PDG}} duplication could be observed for {\textit{PALM1}}-A and -B since the {\textit{PDG}} genes adjacent to {\textit{PALM1}}-B appear to have been lost after 3R. The 3R event also generated duplicate {\textit{PDG3A}} and -B genes. While the {\textit{PDG3A}} genes are adjacent to the identified {\textit{PALM3}} genes in all analyzed teleost genomes, the duplicate {\textit{PDG3B}} sequences identified in stickleback and medaka are not adjacent to {\textit{PALM}} genes (summarized in Figure 4). The analyses of the S1PR and PRG families (Figures S3 and S4 respectively) lend support for the generation of {\textit{PDG3A}} and -B as part of a chromosomal block in 3R.

Consensus splice sites potentially allow alternative splicing to generate fusion proteins of {\textit{PALM1-PDG1}} in tetrapods and {\textit{PALM1-A-PDG1A}} in teleost fishes, but no such read-through ESTs could be identified in NCBI’s dbEST database. However, three zebrafish ESTs of {\textit{PALMD-A-PDG4A}} read-through transcripts were found (see Results), suggesting the expression of a fusion protein. In contrast, the {\textit{PDG4B}} gene was found to be
inverted relative to PALMD-B in all studied teleost genomes except zebrafish.

The region around the human AKAP2 (PDG2) amino acid sequence LVQNAIQ (a.a. 573–579) (see Figure S6), constitutes a Protein kinase A regulatory subunit-II (PKA-RII) binding site [39] and is highly conserved in the species orthologs of PDG2 but not in PDG1, PDG3 or PDG4. However, as the RII-binding sites of AKAPs have no clear consensus sequence other than the propensity to form an amphiphilic alpha-helix [40], PKA-RII binding of the other paralogs cannot be excluded by sequence analysis. The molecular and biological properties of AKAP2 and its paralogs, remain unknown. The sequence features described above may offer possibilities to explore the roles of these proteins.

**Conclusions**

Our combined analysis of sequences and chromosome regions leads to the conclusion that the four tetrapod paralemmin genes arose by chromosome duplications in 2R before the origin of jawed vertebrates (Figure 6). Their retention suggests that each of these four paralemmins has a unique function. We have found a single putative paralemmin in the lancelet genome, but in no other invertebrate. Teleost fishes gained duplicates of PALM1 and PALMD in 3R. The zebrafish has retained six of these paralemmin genes. Although no 3R duplicate of PALM3 seems to have been conserved, several of the neighboring genes on the PALM3-bearing chromosome blocks have done so, including the paralemmin downstream genes (PDG).

As paralemmin-1, -2 and -3 are predominantly expressed in the brain and have the ability to induce filopodia and dendritic spines, they may be important for the development and plasticity of complex nervous systems. Differences in exonic organization and important structural motifs suggest that the two fish palmdelphin duplicates have undergone functional changes. Thus, our analyses point to both ancient expansion of the paralemmin family, retention of the members over several hundred million years, and potential functional changes in some family members.

**Supporting Information**

**Table S1** Identified PALM and neighboring gene family sequences. The table includes the respective database identifiers used to predict the sequences, as well as chromosome locations and genome assembly information for all species investigated. (XLS)

**Figure S1** Phylogenetic maximum likelihood tree of the polypyrimidine tract-binding protein (PTBP) family. PTBP genes could be identified neighboring all PALM genes but PALM3. The phylogenetic analysis of this family, as well as the chromosomal data, are consistent with the phylogenetic analysis of the paralemmins (Figure 3) and our proposed duplication scheme (Figure 6). The presence of duplicate CNN1 and CNN3 genes in the zebrafish genome suggests duplication in 3R. However, the chromosomal data only supports such a duplication for the CNN3 genes, here denominated CNN3A and CNN3B (see Figure 6 and Table S1). Since no duplicates could be identified in any other teleost fish genome, the phylogenetic data is inconclusive. Sequence designations are applied as in Figure S1. Colors are applied as in Figure 3. (TIF)

**Figure S3** Phylogenetic maximum likelihood tree of the sphingosine-1-phosphatase related protein (S1PR) family. Members of this family are also known as endothelial differentiation lysophosphatidic acid G-protein coupled receptors (EDG). Since no S1PR-like sequence could be identified in the investigated invertebrate genomes, this tree is presented as an unrooted radial tree. The phylogenetic resolution is not as clear for this tree as for most other identified neighboring families, probably due to relatively low sequence identity within the family as well as independent gene duplications and translocations. Nonetheless, this tree suggests the divergence of four main branches early in vertebrate evolution and S1PR genes could be identified neighboring all paralemmin isoform genes in the tetrapod genomes, excepting the frog (Xenopus tropicalis) genome. This genome assembly is not mapped to chromosomes and the S1PR genes are positioned in different chromosomal scaffolds than the paralemmin genes. The phylogenetic analysis as well as the chromosomal data also suggest that S1PR2 and S1PR5 arose as local duplicates on the PALM3-bearing chromosome block after 2R, and that S1PR5 conserves duplicates from the 3R event, here called S1PR5-A and S1PR5-B. The chromosome locations of the identified S1PR genes in the teleost genomes suggest several gene translocation events in this lineage. Sequence designations are applied as in Figure S1. Colors are applied as in Figure 3. (TIF)

**Figure S4** Phylogenetic maximum likelihood tree of the plasticity related gene (PRG) family. Members of this family are also known as lipid phosphate phosphatase-related proteins (LPPR). PRG genes can be found neighboring all PALM isotype genes in the analyzed genomes. However, the topology of the resulting tree is not fully consistent with the paralemmin trees (Figure 3), likely due to a local duplication event before the 2R events. This is consistent with the chromosomal data and our proposed duplication scheme (Figure 6). The tree is rooted with an identified C. elegans family member to provide a better relative dating for this event. The phylogenetic analysis and chromosomal data taken together also support the duplication of PRG1 and PRG5 genes in 3R as part of the same chromosome block as PALMD, as well as of PRG2 and PRG4 as part of the same chromosome blocks as PALM1 and PALM5 respectively. One putative PRG sequence was identified in the lamprey genome: Although the phylogenetic analysis is inconclusive as to its identity due to the low statistical support within the branch, it seems to be more similar to the PRG1 and PRG2 family members. Two putative PRG sequences were identified in the lancelet genome, however their identity is not resolved in the phylogenetic analysis. It’s possible that they represent an independent duplication in the lancelet lineage. The identified PRG2-like sequence in chicken and PRG4A-like sequence in stickleback were not included in the phylogenetic analysis due to poor sequence quality in the genome databases. Sequence designations are applied as in Figure S1. Colors are applied as in Figure 3. (TIF)
Figure S5 Phylogenetic maximum likelihood tree of the ATP-binding cassette sub-family A (ABCA) family. ABCA genes could be identified neighboring all PALM genes except PALM3. Taken together the phylogenetic analysis and the chromosomal data are consistent with the phylogenetic analysis of the paralemmins (Figure 3) and our proposed duplication scheme (Figure 6). Our analyses also support the duplication of ABCA4 genes in 3R, as part of the same chromosome block as PALM. The identified ABCA7-like sequence in chicken was not included in the phylogenetic analysis due to poor sequence quality in the genome database. Sequence designations are applied as in Figure S1. Colors are applied as in Figure 3. (TIF)

References

1. Hu B, Petrasch-Parwitz E, Laue M, Kilimann M (2005) Molecular characterization and immunohistochemical localization of palmdelphin, a cytosolic isoform of the paralemmin protein family implicated in membrane dynamics. Eur J Cell Biol 84: 853–866.
2. Arstikaitis P, Gauthier-Campbell C, Carolina Gutierrez Herrera R, Huang K, Levston J, et al. (2008) Paralemmin-1, a modulator of filopodia induction is required for spine maturation. Mol Biol Cell 19: 2026–2038.
3. Gauthier-Campbell G, Breid T, Murphy T, El-Husseini A-D (2004) Regulation of dendritic branching and filopodia formation in hippocampal neurons by specific acylated protein motifs. Mol Cell 15: 2005–2217.
4. Kutzleb C, Sanders G, Yamanoto R, Wang X, Lichte B, et al. (1998) Paralemmin, a prenyl-palmityl-anchored phosphoprotein abundant in neurons and implicated in plasma membrane dynamics and cell process formation. J Cell Biol 143: 143–156.
5. Turk CM, Fagan-Solis KD, Williams KE, Gozgit JM, Smith-Schneider S, et al. (2012) Paralemmin-1 is over-expressed in estrogen-receptor positive breast cancers. Cancer Cell Int. 12:17.
6. Han J, Chang H, Giricz O, Lee GY, Baehner FL, et al. (2010) Molecular cloning of single immunoglobulin IL-1 receptor-related molecule: Paralemmin-3. J Biol Chem 285: 878–896, and the C-terminal PDG motif conserved in all isoforms,
7. Chen X, Wu X, Zhao Y, Wang G, Feng J, et al. (2011) A novel binding protein of the paralemmin protein family implicated in membrane dynamics and cAMP signaling. Arch Biochem Biophys 466: 60–68.
8. Basile M, Lin R, Kabbani N, Karpa K, Kilimann M, et al. (2006) Paralemmin interacts with D3 dopamine receptors: implications for membrane localization and cAMP signaling. Arch Biochem Biophys 446: 60–68.
9. Chen X, Wu X, Zhao Y, Wang G, Feng J, et al. (2011) A novel binding protein of single immunoglobulin IL-1 receptor-related molecule: Paralemmin-3. J Biol Chem 285: 878–896, and the C-terminal PDG motif conserved in all isoforms,
10. Hu B, Copeland N, Gilbert D, Jenkins N, Kilimann M (2005) Molecular characterization and immunohistochemical localization of palmdelphin, a cytosolic isoform of the paralemmin protein family implicated in membrane dynamics. Eur J Cell Biol 84: 853–866.
11. Putnam NH, Butts T, Ferrier DEK, Furlong RF, Hellsten U, et al. (2008) The amphioxus genome illuminates vertebrate origins and cephalochordate biology. Genome Res 18: 1100–1111.
12. Turk CM, Fagan-Solis KD, Williams KE, Gozgit JM, Smith-Schneider S, et al. (2012) Paralemmin-1 is over-expressed in estrogen-receptor positive breast cancers. Cancer Cell Int. 12:17.
13. Basile M, Lin R, Kabbani N, Karpa K, Kilimann M, et al. (2006) Paralemmin interacts with D3 dopamine receptors: implications for membrane localization and cAMP signaling. Arch Biochem Biophys 466: 60–68.
14. Chen X, Wu X, Zhao Y, Wang G, Feng J, et al. (2011) A novel binding protein of single immunoglobulin IL-1 receptor-related molecule: Paralemmin-3. J Biol Chem 285: 878–896, and the C-terminal PDG motif conserved in all isoforms,
15. Holland LZ (2009) Chordate roots of the vertebrate nervous system: expanding the molecular toolkit. Nat Rev Neurosci 10: 736–746.
16. Holland LZ, Albatal R, Asami K, Benito-Gutierrez E, Blow MJ, et al. (2008) The amphipathic gene illuminates vertebrate origins and cephalochordate biology. Genome Res 18: 1100–1111.
17. Shindel MD, Holland PW (2000) Vertebrate innovations. Proc Natl Acad Sci U S A 97: 4449–4452.
18. Jaillon O, Aury JM, Brunet F, Petit JL, Stange-Thomann N, et al. (2004) Genome duplication in the teleost fish Tetraodon nigroviridis reveals the extracellular vertebrate K+–caryotype. Nature 431: 946–957.
19. Meyer A, Van de Peer Y (2005) From 2R to 3R, evidence for a fish-specific gene duplication (FSGD). Bioessays 27: 937–945.
20. Widmark J, Sundstrom G, Ocampo Daza D, Larhammar D (2011) Differential evolution of voltage-gated sodium channels in tetrapods and teleost fishes. Mol Biol Evol 28: 859–871.
21. Sundstrom G, Larsson TA, Brenner S, Venkatesh B, Larhammar D (2008) Evolution of the neuropeptide Y family: new genes by chromosome duplications in early vertebrates and in teleost fishes. Gen Comp Endocrinol 155: 705–716.
22. Dreborg S, Sundstrom G, Larsson TA, Larhammar D (2008) Evolution of vertebrate opioid receptors. Proc Natl Acad Sci U S A 105: 15487–15492.
23. Brausch I, Voell J, Scharf M (2009) The endothelin system: evolution of vertebrate-specific ligand-receptor interactions by three rounds of genome duplication. Mol Biol Evol 26: 701–709.
24. Stein RA, Sallows JA (2006) BMC Evol Biol 6: 79.
25. Larsson TA, Olson F, Sundstrom G, Lundin L-G, Brenner S, et al. (2008) Early vertebrate chromosome duplications and the evolution of the neuropeptide Y receptor gene regions. BMC Evol Biol 8: 184.
26. Sundstrom G, Larsson TA, Larhammar D (2008) Phylogenetic and chromosomal analyses of multiple gene families syntenic with vertebrate Hox clusters. BMC Evol Biol 8: 254.
27. Atsulch SF, Gish W, Miller W, Myers EW, Lipman DJ (1996) Basic Local Alignment Search Tool. J Mol Biol 215: 403–410.
28. Fleck P, Arnone MR, Barrell D, Beal K, Brent S, et al. (2011) Ensembl 2011. Nucleic Acids Res 39: D800–D808.
29. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Identification of genes potentially involved in the acquisition of androgen-independent and metastatic tumor growth in an autogenic/chemogenically engineered mouse prostate cancer model. Prostate 67: 83–106.
30. Clamp M, Cuff J, Searle S, Barton G (2004) The Jalview Java alignment editor. Bioinformatics 20: 426–427.
31. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425.
32. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, et al. (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst Biol 59: 307–321.
33. Abascal F, Zardoya R, Posada D (2005) ProtTest: selection of best-fit models of protein evolution. Bioinformatics 21: 2104–2105.
34. Andreu N, Escarellera M, Feather S, Bevenvid K, Wolf A, et al. (2003) PALM, a novel paralemmin-related gene mapping on human chromosome 1q21. Gene 276: 37–40.
35. Ercilla H, Manzon I, Youson J, Laudet V (2002) Analysis of lamprey and hagfish genes reveals a complex history of gene duplications during early vertebrate evolution. Mol Biol Evol 19: 1440–1450.
36. Kuraku S (2006) Insights into Cyclostome Phylogenomics: Pre-2R or Post-2R? Zoolog Sci 25: 960–961.
37. Putnam N, Butts T, Ferrier D, Furlong R, Hellsten U, et al. (2008) The amphipathic gene and the evolution of the chordate K+–caryotype. Nature 453: 1064–1071.
38. Hook PM, van Veen T, Vos M, Heck A (2007) Diversity of cAMP-dependent protein kinase isoforms and their anchoring proteins in mouse ventricular tissue. J Proteome Res 6: 1705–1717.
39. Dong F, Feldmesser M, Casadevall A, Rubin CS (1998) Molecular characterization of a cDNA that encodes six isoforms of a novel murine A kinase anchor protein. J Biol Chem 273: 6535–6541.
40. Skroblin P, Grossmann S, Schäfer G, Rosenhal W, Klussmann E (2010) Mechanisms of protein kinase a anchoring. Int Rev Cell Mol Biol 283: 235–350.