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SHORT COMMUNICATION

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kitb, a second zebrafish ortholog of mouse *Kit*

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Abstract The large numbers of duplicated pairs of genes in zebrafish compared to their mammalian counterparts has lead to the notion that expression of zebrafish co-orthologous pairs in some cases can together describe the expression of their mammalian counterpart. Here, we explore this notion by identification and analysis of a second zebrafish ortholog of the mammalian *Kit* receptor tyrosine kinase (*kitb*). We show that in embryos, *kitb* is expressed in a non-overlapping pattern to that of *kita*, in the anterior ventral mesoderm, Rohon–Beard neurons, the otic vesicle, and trigeminal ganglia. The expression pattern of *kita* and *kitb* in zebrafish together approximates that of *Kit* in mouse, with the exception that neither zebrafish *kit* gene is expressed in primordial germ cells, a site of *kit* expression in the mouse embryo. In addition, zebrafish *kita* is expressed in a site of zebrafish primitive hematopoiesis but not required for blood development, and we fail to detect *kitb* expression in sites of zebrafish hematopoiesis. Thus, the expression and function of zebrafish *kit* genes cannot be described as a simple partition of the expression and function of mouse *Kit*. We discuss the possibility that these unaccounted for expression domains and functions are derived from more ancestral gene duplications and partitioning instead of the relatively recent teleost teleost-specific duplication.

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Introduction

Kit, a receptor tyrosine kinase (RTK) associated with human piebaldism and cancer, has been extensively studied in both model organisms and cell culture systems, resulting in identification of multiple requirements for *Kit* during development. In mammals, *Kit* and its ligand, Steel, are required for the development of primordial germ cells (PGCs), hematopoietic cells, interstitial cells of Cajal (ICCs), and melanocytes (Geissler et al. 1988; Bernex et al. 1996). Specific requirements for *Kit* are best characterized for melanocyte precursors because melanocytes are not required for viability of the organism. Melanocyte precursors require *Kit* for migration to their proper target locations, survival, and differentiation into melanocytes (Bernex et al. 1996). *Kit* may also have a role in proliferation of melanocyte precursors (Mackenzie et al. 1997). Evidence suggests that *Kit* is required for similar functions in the other cell types where it is essential. For instance, PGCs also require *Kit* for differentiation, proliferation, and survival, and weak alleles of mouse *Kit* (*Kit*^{e}) suggest PGCs require *Kit* for migration as well (Buehr et al. 1993; Bernex et al. 1996). Thus, *Kit* is required for similar mechanisms by several different cell types in mammals.

In zebrafish, the requirements for *kit* during development differ from the requirements for *Kit* in mammals. Although zebrafish *kit* (hereafter referred to as *kita*) is expressed in hematopoietic precursors in early development, no defects in blood development are observed in *kita* mutants (*sparse*; Parichy et al. 1999). Furthermore, zebrafish *kita* is not expressed in PGCs, and *kita* null mutant male and female fish are fully fertile, indicating that there is no *kita* requirement in PGC development. A *kita* requirement for the development of the ICCs in zebrafish has not been explored, but *kita* null mutants display no defects in digestion, suggesting the development of these cells in zebrafish does not require *kita*.

One cell type that shares developmental requirements for *Kit* in both fish and mammals is melanocytes. However, whereas *Kit* is required for both migration and survival of melanoblasts or melanocytes in fish and mammals, in zebrafish, *kita* is not required for the differentiation of all melanocytes. Zebrafish *kita* null mutant embryos have approximately 60% of the melanocytes in wild-type fish, a *kita*-differentiation-independent population of melanocytes (Parichy et al. 1999; Mellgren and Johnson 2004). Therefore, whereas melanocytes require a *Kit* gene in both mammals and fish, the specific requirements for *Kit* differ between these two groups.

These divergent requirements for *kit* between fish and mice, combined with a hypothesized whole genome duplication event specific to the teleost lineage (Force et al. 1999; Barbazuk et al. 2000), have led to the hypothesis that a *kit* paralog is acting in those roles where *kit* is not required in zebrafish. Here we show that the *kit* gene has undergone a duplication event in the teleost lineage after the divergence of fish and mammals, resulting in a second extant *Kit* ortholog (*kitb*). However, zebrafish *kitb* is not expressed in all of the expected domains based on the differences in *Kit* requirements between mammals and fish. Instead, we find that *kitb* is expressed in some common domains between mouse and zebrafish, indicating that the *Kit* orthologs may have different functional requirements between the two organisms.

Materials and methods

kitb sequencing and phylogenic tree construction

BLAST searches to identify novel RTK sequences were performed with both protein and cDNA sequences of zebrafish *kita* and related RTK sequences through the Ensembl zebrafish whole genome shotgun sequence blast site (Sanger, UK; http://www.ensembl.org/Danio_rerio/). Once a potential *kit* paralog was identified, ClustalW was used to align the predicted kitb (for partial sequences see EN SDARP00000003199, gi:58760520; see electronic supplementary material Scheme 1 for the full sequence we assembled and used in this analysis) and known kit and fms protein sequences, and the Phylip program (J. Felsenstein, University of Washington) was used to construct trees. We used both the neighbor-joining and maximum- likelihood methods to generate trees, and bootstrap values were determined using both neighbor-joining and maximum -likelihood methods.

For synteny analysis of human and zebrafish *kit* genes, we used the human genome sequence browser from Ensembl (http://www.ensembl.org/Homo_sapiens/) and UCSC (http://www.genome.ucsc.edu/index.html?org=Human) to compare to the Ensembl zebrafish whole genome shotgun project sequence. Because many of the genes annotated in the chromosomal regions surrounding zebrafish *kita* and *kitb* have arbitrary names, we assigned them orthologous names if they showed orthology to known genes in at least two species other than zebrafish (*kita* region: *nmu*, *htp-1*, *gsh2*, *lnx*, *fip1l1*, *scfd2*; *kitb* region: *tyrp1*, *flj20273*, *lap3*, *smc2l*, *fbx10*, *kiaa0368*). For the zebrafish *diaph*-like and *ush3a*-like genes, a definitive ortholog was not identified, so the zebrafish genes were named as being "like" the closest homolog in the other species.

All sequencing reactions were performed with Amersham Sequenase and an ABI 3100 sequencer. Throughout the manuscript, we use standard nomenclature for genes in each organism discussed (mouse *Kit*, human KIT, and zebrafish *kit*), however, when we are talking about one gene in multiple organisms we use the mouse nomenclature (*Kit*) as a default.

In situ hybridizations and Q quantitative PCR

kitb RNA probe template was generated by amplification from cDNA using primers with the T7 RNA polymerase binding site. The following primers were used: forward primer, 5′-TCTTCAGTTCCAAAACAGGCGATGG-3′, and reverse primer, 5′-TAATACGACTCACTATAGGGTACAT GTTTGGTGGTTTCCGACAGC-3′. The underlined sequence is the T7 RNA polymerase recognition sequence. After sequencing of this template, it was used to generate digoxigenin-labeled RNA probe. In situs were performed as previously described (Mellgren and Johnson 2004), and washes were performed by an Abimed in situ robot (Abimed In Situ Pro, Intavis AG, Bergisch Gladbach, Germany). Color development was performed manually. Double-label RNA in situ hybridization combined with antibody labeling was performed as previously described (Cornell and Eisen 2000) with the zn-12 Ab (Trevarrow et al.1990) diluted 1/4000.

Quantitative PCR (Q-PCR) was performed using the iQ real-time PCR machine, SYBR green mix, and the following *kitb* exon 3 primers: forward primer, 5′-TTGAG GGCTGCTACTTCTGC-3′ and reverse primer, 5′-TTTC TGGAGAGGCTGATTGC-3′.

Results

kitb is a second ortholog of mouse *Kit*

To explore the prediction that redundancy between *kit* paralogs in zebrafish may explain the differences in *Kit* requirements between zebrafish and mammals (Mellgren and Johnson 2004), we first identified novel *Kit*-like genes in the zebrafish genome with the following approach. Using known sequences of zebrafish *kita* and other closely related receptor tyrosine kinases, we identified potential *kit*-like gene sequences by performing BLAST searches against the zebrafish whole genome shotgun assembly (Sanger, UK). Sequence alignments generated from this approach allowed us to identify a receptor tyrosine kinase [ensemble ensemble-predicted transcript (ENSDARP00000003199, gi:58760520)] that appeared to be more closely related to zebrafish *kita* than other zebrafish receptor tyrosine kinases. The high level of sequence similarity to *kita* suggests

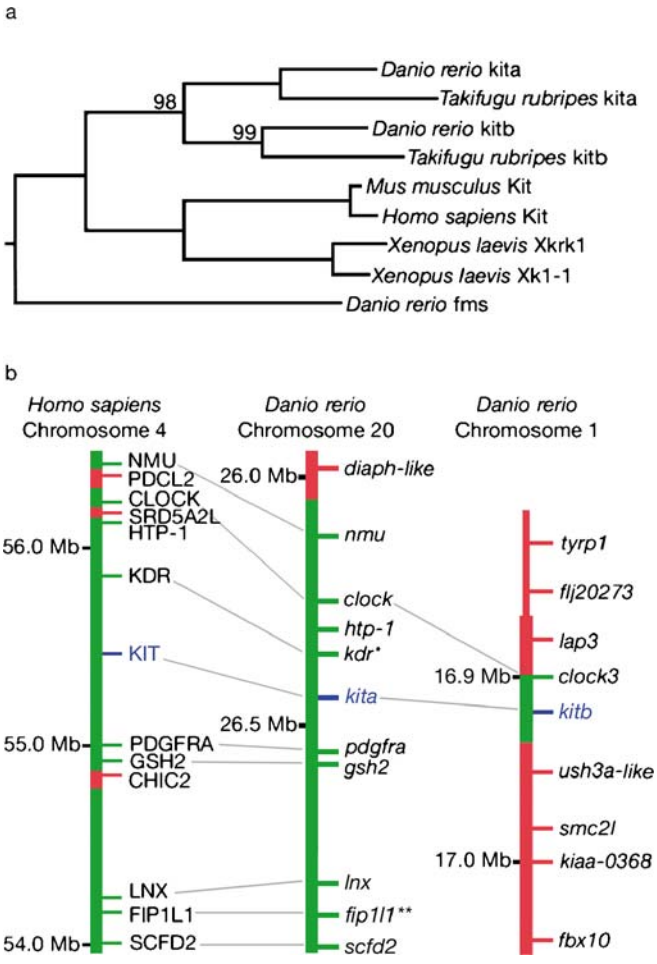

Fig. 1 **a** Phylogeny of kit proteins. Both neighbor-joining and maximum likelihood methods were used to construct trees, with *Danio rerio* (zebrafish) fms as the out-group. The maximum likelihood tree is shown. Bootstrap values using the maximum likelihood method were 100 out of 100 replicates at all nodes, except for the labeled nodes that were 98 out of 100 replicates and 99 out of 100 replicates. The neighbor-joining method resulted in a tree with identical nodes and bootstrap values of 81 out of 100 for the node containing *D. rerio* kitb and *T. rubripes* kitb, and 100 out of 100 replicates at all other nodes. For *T. rubripes* kitb, only a partial protein sequence was available. The predicted protein sequence used included the first tyrosine kinase domain, part of the second tyrosine kinase domain, and part of the transmembrane domain, but lacked the extracellular domain. Accession numbers for all of the protein sequences used to generate this tree are as follows: *Homo sapiens* KIT, gi:1817734; *Mus musculus* Kit, gi:10863917; *T. rubripes* kita, gi:27261157; *T. rubripes* kitb, FRUP00000132220; *D. rerio* kita, gi:18858927; *D. rerio* kitb, ENSDARP00000003199, gi:58760520, electronic supplemental material S1; *D. rerio* fms, gi:8571411; *Xenopus laevis* Xkrk1, gi:763034; *X. laevis* Xkl-1, gi:38303800. **b** Syntenic regions between human and zebrafish *kit* genes as observed in the Ensembl annotated zebrafish genome http://www.ensembl.org/Danio_rerio/). Only regions confirmed by overlapping sequenced BACs (*thick lines*) were used in this analysis, with the exception of the *kitb* chromosomal region represented by a *thin line*. The region represented by a *thin line* is part of the Zv4 scaffold 30 assembly sequence (16.69–16.72 Mb), which contains *lap3*, *flj20273*, and *tyrp1*. Inasmuch as *lap3* is on a BAC (BX546494) that overlaps the BAC containing *clock3* and *kitb*, we interpret this to mean that *tyrp1* and *flj20273* are the next two genes on this chromosome, although the order between them is less certain. The order of the rest of the genes on the *kitb* chromosome is more certain because part of the *clock3* sequence, *kitb*, and the three genes distal to them are on the same sequenced and ordered BAC clone (BX548248). For all three chromosomal regions shown, the *green colored* chromosomal regions are where synteny exists between the human and zebrafish *kit* genes, and the *red regions* denote regions of non-synteny. Note that the human *KIT* region and the zebrafish *kita* region share more synteny than zebrafish *kitb* and human KIT or zebrafish *kita*. This indicates that the *kit* region duplicated after the divergence of fish and mice, followed by a loss of some genes in the *kitb* region. Two of the genes below *kitb* on zebrafish chromosome 1, *smc2l* and *kiaa0368*, are also in the *kitb* region of *T. nigroviridis* (not shown), indicating that the loss or rearrangement of some genes occurred before the speciation of the teleost lineage*We identify this gene as zebrafish *kdr*; however, it should be noted that another gene on zebrafish chromosome 14 was reported to be the human KDR ortholog (Habeck et al. 2002). These genes may be paralogs of each other, or one of them may be a different but related type III RTK, such as *flt-1* or *flt-4*. Identification and classification of all of the type III RTKs in the zebrafish genome as well as their requirements will allow identification of the true ortholog of human KDR**The zebrafish gene *fip1l1* (*zgc*:*103421*) is present in whole genome assembly sequence corresponding to this region in BAC (BX511028), which lacks it. We suggest that the BAC clone has lost this region

that this gene may be a *kita* paralog from a recent duplication event.

To confirm that this newly identified gene is a *kita* paralog and, if so, determine when the duplication of the *kit* locus occurred, we constructed a phylogeny based on protein sequences of both these zebrafish genes and Kit proteins from other vertebrates (Fig. 1a). This phylogeny reveals that the two zebrafish *kit* genes, along with two *kit*-like genes identified in *Takifugu rubripes* and *Tetraodon nigroviridis*, probably arose from a duplication event that occurred after the divergence of teleosts and mammals, but before the divergence of pufferfish and zebrafish. The newly identified gene (*kitb*) shows 55% overall identity with zebrafish *kita* and has an overall similarity of 70% at the amino acid level. The intracellular region of the receptor containing the receptor tyrosine kinase domains shows the most similarity between the two genes, with an identity of 67% and similarity of 79% for the protein sequence encoded by exons 11 through 21. The extracellular and membrane spanning domains are less conserved, with an identity of 45% and similarity of 62% for the protein sequence encoded by exons 1 through 10. Both genes have 21 exons and they are similar in size, except for *kitb* exon 2 that encodes 5 fewer amino acids than *kita* exon 2, *kitb* exon

15 that encodes 11 fewer amino acids than *kita* exon 15, and *kitb* exon 16 that encodes 9 fewer amino acids than *kita* exon 16. Exons 15 and 16 are predicted to encode part of the *kitb* kinase insert domain between the two tyrosine kinase catalytic domains. *kitb* maps to the upper arm of chromosome 1 based on whole genome shotgun assembly data (Zv4 Scaffold 30, 16.9 MB), whereas *kita* is on chromosome 20 (Parichy et al. 1999). We confirmed the position of *kitb* on chromosome 1 by independent mapping on the T51 RH panel (1292.00cR, not shown).

We next looked for synteny between the chromosomal regions containing zebrafish *kita* and zebrafish *kitb*, which would support that these two genes arose during the predicted whole or partial genome duplication event in the teleost lineage after divergence of fish and mammals (Force et al. 1999; Barbazuk et al. 2000). The regions discussed here are present in the genome assembly as sequenced bacterial artificial chromosomes (BACs), allowing for high confidence in order of genes along the chromosomes. Accordingly, we find that the *kita*-containing region and the *kitb*-containing region both have an ortholog of human KIT and human CLOCK, but the *kita* region has two genes in the interval between *kita* and *clock*, *kdr* and *htp-1*, that are not present in the *kitb* region (Fig. 1b). When the zebrafish *kita*-containing region is compared to the human *KIT*-containing region, both chromosomal regions contain *clock*, *kdr*, *kit*, *pdgfra*, and *gsh2* in the same order along the chromosome, with the zebrafish *kita* region lacking only a SRD5AL ortholog in this interval. The larger region of synteny surrounding human KIT and zebrafish *kita* and the more limited region of synteny surrounding zebrafish *kita* and *kitb* support the idea that the *kitb* locus arose by a duplication event specific to the teleost lineage, which was followed by loss or rearrangement of some genes from the *kitb* region. This idea is further supported by comparing the zebrafish *kitb* locus to the *kitb* locus identified in *T. nigroviridis* (chromosome 18, scaffold 9219:3.31 Mb–-3.45 Mb, Jaillon et al. 2004). Like the zebrafish *kitb* region, the *T. nigroviridis kitb* locus contains an ortholog of *kitb* and *clock3* adjacent to each other, as well as the orthologs of *smc2l* and *kiaa0368* in this region (not shown). Whereas the order of these genes in the zebrafish *kitb* region is *clock3*; *kit*; *ush3a*-like; *smc2l*; *kiaa0368*, the gene order in the *Tetraodon kitb* region is *kit*; *clock3*; *smc2l*; *kiaa0368*. The *Tetraodon* ortholog of *ush3a*-like does not appear to be in this region. One possible explanation for these differences is that the *Tetraodon* sequence is misassembled in this region, leading to a different gene order and placement of *ush3a*-like outside of this region. Alternatively, rearrangements on the *Tetraodon* chromosome may have led to the different gene order and localization of *ush3a*-like outside of this region. Despite these differences between the *Tetraodon* and zebrafish *kitb* regions, the similarities in the *kitb* regions of these two teleost species suggests that the whole or partial genome duplication in the teleost ancestor giving rise to the duplicate *kit* genes was followed by a loss of some genes from the *kitb*-containing region, such as *htp-1* and *kdr*, before divergence of zebrafish and pufferfish. These genes may have been lost from the genome or may have been moved to other chromosomal regions by translocation.

kitb is expressed in early embryos during gastrulation, Rohon–Beard neurons, trigeminal ganglia, and the otic vesicle

To determine the possible functions of *kitb* in zebrafish development, we next looked at expression of this gene at different stages of development. We first used quantitative RT-PCR to determine the earliest stage of *kitb* expression. We detected *kitb* transcript at significant levels at 9 hours postfertilization (hpf), followed by increasing expression at 14 hpf and even more expression at 28 hpf (not shown). We failed to detect *kitb* expression at significant levels at 2, 3, 4, and 6 hpf embryos. Thus, *kitb* is first expressed in late gastrula stage embryos, towards the end of epiboly. The *kitb* expression at early stages of development was confirmed by RNA in situ hybridization at 10 hpf, where *kitb* transcript was faintly detected in the anterior ventral mesoderm (8 of 14 embryos, Fig. 2a).

Expression in Rohon–Beard neurons (RBs) was first detected at 15 hpf in a few cells just posterior to the otic vesicle. By 19.5 h, *kitb* expression is in RBs throughout the embryo (Fig. 2b). Cryosections through 19.5 hpf embryos show that the *kitb*-expressing cells are within the neural tube, at a region where RBs are present (Fig. 2c). We confirmed that *kitb* labels RB neurons by performing double double-label staining with the zn-12 antibody (Trevarrow et al. 1990; Cornell and Eisen 2000), which marks RBs, and in situ hybridization with a *kitb* probe (Fig. 2d,f). The double labeling showed that *kitb* is expressed in a subset of RBs, as not all zn-12- labeled cells on the dorsal aspect of the neural tube also expressed *kitb*. Ventral motor neurons that are also labeled by the zn-12 antibody failed to express *kitb* (not shown). Expression of *kitb* in RBs continues to be seen until 26 hpf, but by 42 hpf expression is absent in these cells.

Like many other neural markers that are expressed in RBs, *kitb* is also expressed in the trigeminal ganglia (Fig. 2g). This is revealed in 19.5 hpf embryos, where we observe *kitb* expression in cells between the eye and ear. *kitb* RNA is restricted to the cell bodies of a subset of neurons in the trigeminal ganglia. Confirming that these *kitb*-positive cells are part of the trigeminal ganglia, these cells co-label with zn-12, which is also a marker for trigeminal ganglia at this stage and position (Trevarrow et al. 1990). Expression of *kitb* in the trigeminal ganglia continues through 42 hpf, at which time only a few cells directly posterior and adjacent to the eye express *kitb* (Fig. 2h).

kitb is also expressed in the otic vesicle. Starting at 15 hpf, expression of *kitb* is also seen in two cells in the anterior ventral half of the otic vesicle and in two cells in the posterior ventral part of the otic vesicle (not shown). These cells are in the appropriate position to be the tether cells of the developing ear (Riley 2003). Expression in

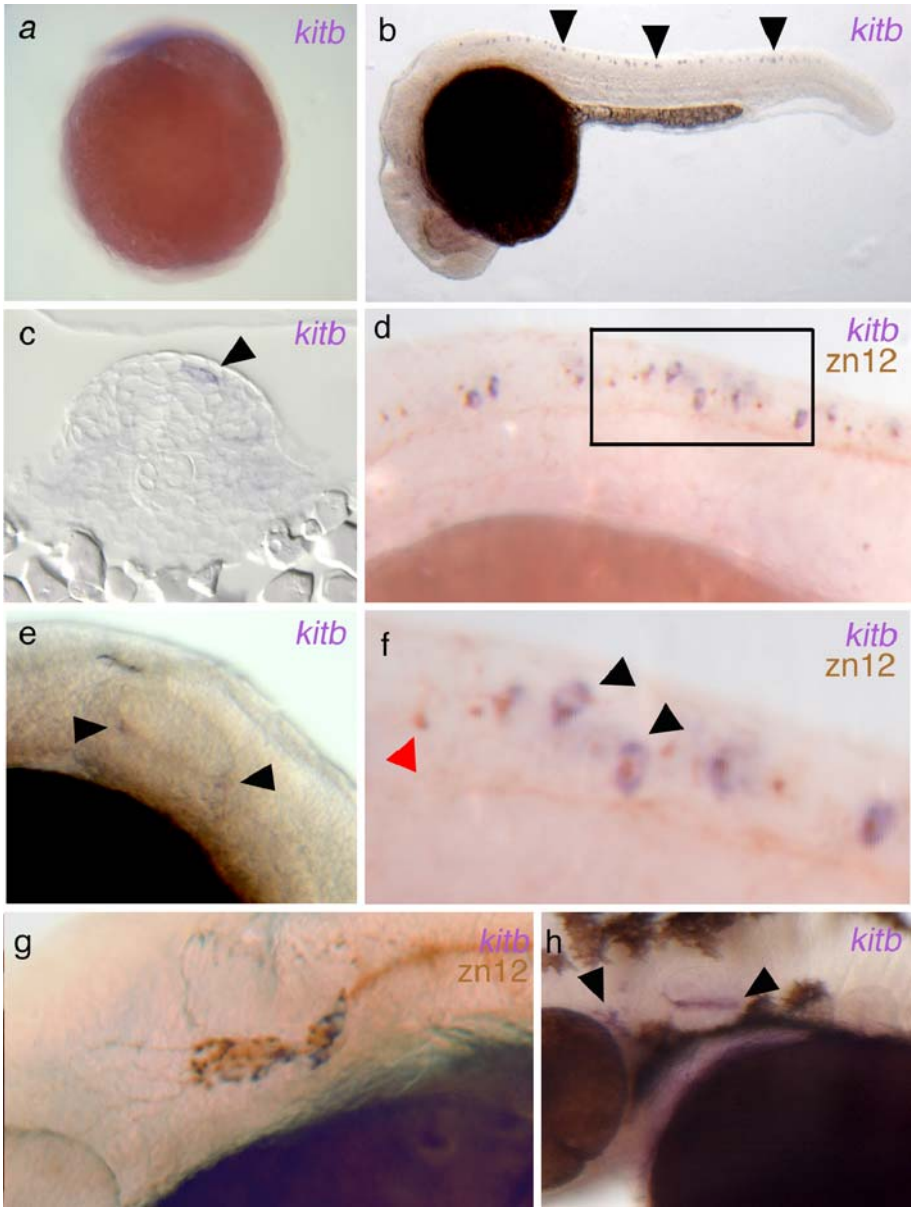

Fig. 2 Expression of *kitb*. RNA in situ hybridizations show *kitb* is expressed in anterior ventral mesoderm of 10 hpf embryos (**a**). The embryo shown is a *kita* mutant embryo; the same expression pattern was observed in wild-type embryos. *kitb* is also expressed in Rohon–Beard neurons throughout the embryo at 19.5 hpf (**b**), and cross sections show the *kitb*-expressing cells are within the neural tube (**c**). **d** and **f** show that these cells within the neural tube can be co-stained with zn-12 antibody and for deoxygenin-labeled *kitb* RNA in situ probe (*black arrowheads*), indicating these are Rohon–Beard neurons. Note that some Rohon–Beard neurons do not co-stain with *kitb* RNA probe (*red arrowhead*). *kitb* is also expressed in a few cells in the anterior and posterior part of the otic vesicle (**e**, *black arrowheads*), and in a subset of cells in the trigeminal ganglia at 24 hpf (**g**). **h** *kitb* staining in a 42 hpf embryo shows expression in a few cells of the trigeminal ganglia and in the ventral wall of the otic vesicle (*black arrowheads*). In **a**, ventral is to the *left* and dorsal is to the *right*. In **b**, **d**, **e**, **f**, **g**, and **h**, anterior is to the *left* and posterior is to the *right*

these presumptive tether cells continues through 24 hpf (Fig. 2e). By 42 hpf, expression in the ear is now found throughout the ventral part of the otic vesicle (Fig. 2h).

Thus, *kitb* is expressed in the gastrulating embryo, Rohon–Beard neurons, trigeminal ganglia, and otic vesicle, and its expression does not overlap with zebrafish *kita* expression (Table 1; Parichy et al. 1999). Despite the many expression domains of *kitb* observed, we failed to detect *kitb* expression in several expected regions. We expected that *kitb* would be expressed in the tissues where *kita* is not required in zebrafish, but where *Kit* is required in mouse. Although some of the *kitb* expression domains we observed are shared with mouse *Kit* and not shared with zebrafish *kita*, we failed to detect *kitb* expression in other domains where we expected *kitb* to be required. Early markers for PGCs such as *vasa* are seen as early as the two-cell stage in zebrafish embryos, and mark PGCs through many of the stages of zebrafish development we have examined (Parichy et al. 1999). However, despite the *Kit* requirement for PGCs in mouse and the lack of requirement

Table 1 *kit* ortholog expression domains (+) or domains lacking *kit* expression (−)

Expression domain	Mouse *Kit*[a]	Zebrafish *kita*[b]	Zebrafish *kitb*	*Xenopus Xkl-1*[c]	*Xenopus Xkrk1*[d]
Neural crest	+	+	−	−	−
Neurons within neural tube[e]	+	−	+	nr	nr
Hematopoietic cells	+	+	−	nr	nr
Primordial germ cells	+	−	−	nr	−
Otic vesicle	−[f]	−	+	+	nr
Trigeminal ganglia	+	−	+	nr	nr
Gastrulating embryo[e]	+	−	+	+	−
Somites	−	−	−	+	−
Apical ectodermal ridge	−	−	−	nr	nr
Lateral line	n/a	+	−	−	+
Interstitial cells of Cajal	+	−	−	nr	nr
Notochord	−	+	−	+	nr
Pineal gland	+	+	−	nr	nr

[a]This table is only a partial listing of all of the mouse *Kit* expression domains, including only those that are shared with any of the other organisms listed. Additional expression domains that are not reported in any of the other organisms listed above include dorsal root ganglia, retinal neurons, lens, respiratory tube endoderm, olfactory epithelium, nasopharynx, trachea, bronchi, foregut cells, stomach epithelium, midgut epithelium, larynx, tongue, thyroid gland, pituitary gland, adrenal gland, vitelline veins, dorsal aorta endothelium, and urogenital system (Bernex et al. 1996)

[b]*kita* expression is reported in Parichy et al. (1999)

[c]*Xenopus sXkl-1* expression is reported in Kao and Bernstein (1995)

[d]*Xenopus Xkrk-1* expression is reported in Baker et al. (1995)

[e]Mouse *Kit* is expressed in a subset of neurons in the neural tube, and zebrafish *kitb* is expressed in Rohon–Beard neurons. It is unknown how these types of neurons are related. Whereas both mouse *Kit* and zebrafish *kitb* are expressed in the gastrulating embryo, mouse *Kit* is expressed in embryonic ectoderm and zebrafish *kitb* is expressed in embryonic ventral mesoderm

[f]Although mouse *Kit* has not been reported to be expressed in the otic vesicle, it is reported to be expressed in the future tympanic membrane and future phyrangeal tympanic membrane

nr None reported, *n/a* not applicable

for *kita* in zebrafish, we failed to observe *kitb* expression in any of the regions where PGCs can be found in any of the stages of development observed (not shown). Similarly, markers for hematopoietic cells are also observed at the multiple stages of development examined (Parichy et al. 1999), but we did not see *kitb* expression in hematopoietic regions either (not shown). We also failed to detect *kitb* expression near the zebrafish gut, where ICCs would localize. Finally, we also failed to observe *kitb* expression in neural crest cells, which include melanocyte precursors, indicating that *kitb* does not play a role in melanocyte development either.

Because we failed to see *kitb* expressed in hematopoietic cells, germ cells, near the gut, or in neural crest (melanocytes) in the stages of development examined, we suggest that *kitb* is not involved in the development of these tissues. One explanation could be that *kitb* is expressed in these tissues at later stages or that it is expressed in these cell types at a lower level undetectable by in situ. Although we cannot rule out this possibility, localization of *kitb* to some domains and failure to detect it in these other domains suggests that *kitb* is not expressed in the tissues where we fail to detect it.

Comparing the other *kitb* expression domains with mouse *Kit* and zebrafish *kita* indicates that *kitb* and *kita* partially recapitulate the expression pattern of mouse *Kit* (Table 1). For instance, we find that *kitb* is expressed in the trigeminal ganglia, and mouse *Kit* is expressed in the trigeminal ganglia as well (Bernex et al. 1996). *kita* is expressed in melanocytes and hematopoietic cells, and mouse *Kit* is expressed in these tissues also. Thus, taken together, *kitb* expression and *kita* expression partially recapitulates the mouse *Kit* expression localization.

Zebrafish *kitb* and mouse *Kit* are also expressed in similar tissues or within the same structure in different subsets of cells. For instance, in zebrafish, *kitb* is expressed in RBs in zebrafish, and in mouse, *Kit* is expressed in a subset of neurons within the neural tube (Hirata et al. 1995; Bernex et al. 1996). Mouse *Kit* is also expressed in dorsal root ganglia, neurons that are functionally related to RBs in that they take over the function of RBs after they undergo programmed cell death in zebrafish. *kitb* is expressed in a subset of cells in the zebrafish otic vesicle, whereas mouse *Kit* is expressed in the future tympanic and pharyngeal tympanic membrane in the mouse ear (Bernex et al. 1996). *kitb* and mouse *Kit* are both expressed in early embryos during gastrulation, although unlike zebrafish *kitb*, mouse *Kit* is expressed in the embryonic ectoderm instead of the embryonic mesoderm (Motro et al. 1991). Interestingly, mesodermal *kitb* expression during gastrulation is shared by the *Xenopus Kit* ortholog *Xkl1-1* (Kao and Bernstein 1995), which is expressed in embryonic ventral mesoderm. Although it is intriguing that the preceding *kitb* and mouse *Kit* expression domains are in similar locations or within the same structures, these domains may not be related evo-

lutionarily, or we lack enough information for now to speculate on how these domains may be evolutionarily related.

The comparison of the complementary expression patterns of zebrafish *kita* and *kitb* with mouse *Kit* tends to support the duplication–degeneration–complementation (DDC) model (Force et al. 1999), which suggests that duplicated genes are retained due to partitioning of the functions of the ancestral gene between the duplicates. If we infer that shared expression of mouse *Kit* with zebrafish *kita* and *kitb* expression represents the ancestral gene expression, we can hypothesize that the ancestral expression pattern was subdivided between the duplicate *kit* genes in zebrafish, and this probably leads to the retention of the two paralogs.

Although mouse and zebrafish *Kit* genes have partially complementary expression patterns, expression domains and functional requirements of *Kit* differ between the two groups in that neither zebrafish *kit* appears to be expressed in PGCs or ICCs, zebrafish *kita* is expressed in hematopoietic cells but not required for hematopoietic cell development, and zebrafish *kitb* is not expressed in hematopoietic cells. An alternative explanation for the divergent functional requirements and expression patterns of *Kit* between mouse and zebrafish that could account for these differences is partitioning of expression domains among paralogs from more ancestral duplications rather than the teleost teleost-specific duplication. This idea, known also as "function shuffling" (McClintock et al. 2002), might suggest that another type III RTK, the result of a gene duplication in an ancestor of fish and mouse, that generated the PDGF receptors and FMS-like tyrosine kinases, is

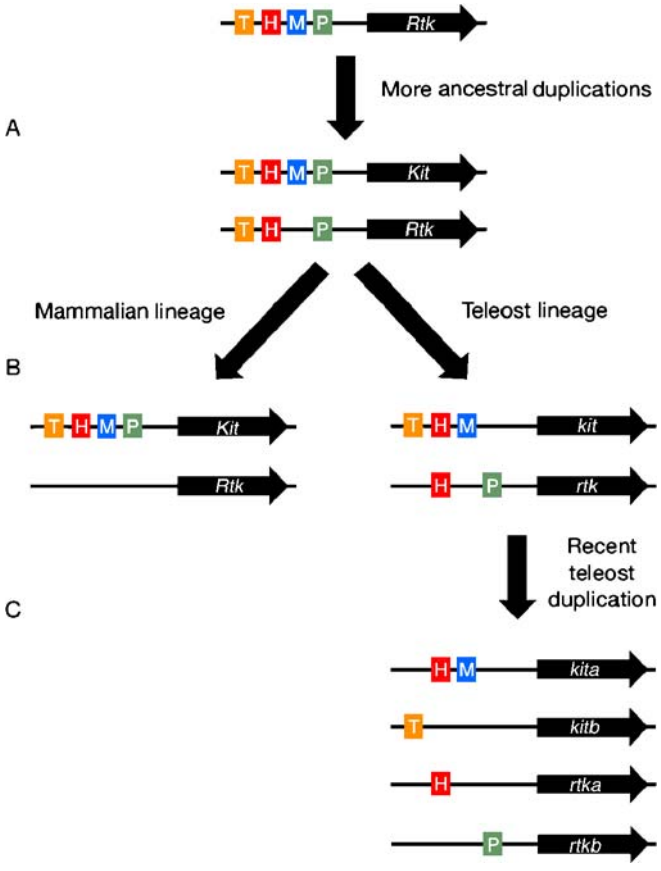

Fig. 3 Regulatory elements can be lost (or partitioned) at multiple stages during successive rounds of gene duplication. The *Kit* receptor tyrosine kinase gene is one of many class III RTKs resulting from a tandem duplication and two rounds of locus or genome duplication before divergence of mammalian and fish lineages. For simplicity, we show a single duplication event, generating a representative of another member of the type III receptor tyrosine kinase family, referred to here as *Rtk*. An additional genome duplication in teleosts resulted in the two zebrafish *kit* genes, *kita* and *kitb*. Loss of regulatory sequences at early stages (**a**), after duplication but before divergence of mammalian and fish lineages, will result in orthologous genes retaining the same regulatory elements (for instance, retention of a melanocyte regulatory element, *M*, in mammalian *Kit* and zebrafish *kita*). Loss of shared regulatory elements at intermediate stages (**b**), after mammalian and fish divergence, may result in genes that were paralogs in the mammalian/fish ancestor retaining similar regulatory elements (for instance, we postulate a primordial germ cell regulatory element, *P*, retained by mouse *Kit* and a paralogous *rtk*). Additional loss or partitioning of regulatory elements may also have occurred after the teleost-specific duplication (**c**). In this model, *color-filled rectangles* represent predicted regulatory domains based on a subset of expression and functional requirements for the *Kit* genes (*T* trigeminal ganglia, *H* hematopoietic cells, *M* melanocytes, *P* primordial germ cells) and *black bars* represent coding sequence of each gene. Because zebrafish (*teleost*) *kita* is expressed in hematopoietic cells but not required for their development, we show that another *rtk* may be expressed in teleost hematopoietic cells and required for their development. Alternatively, the failure to observe this requirement for zebrafish *kita* may be due to functional partitioning caused by changes in protein sequences

required for PGCs, hemotopoetic cells, and ICCs in zebrafish instead of *Kit* (Fig. 3). By examining other type III RTKs and looking for roles in melanocyte, PGC, and hematopoietic cell development, we may find candidates for such ancestrally related genes whose whose functions have been partitioned differently in different species. Furthermore, by continuing to compare expression domains of *kita* and *kitb* in zebrafish to *Kit* expression in other vertebrates, we can begin to decipher which are the ancestral expression domains of *Kit*, and further our understanding of RTK evolution.

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