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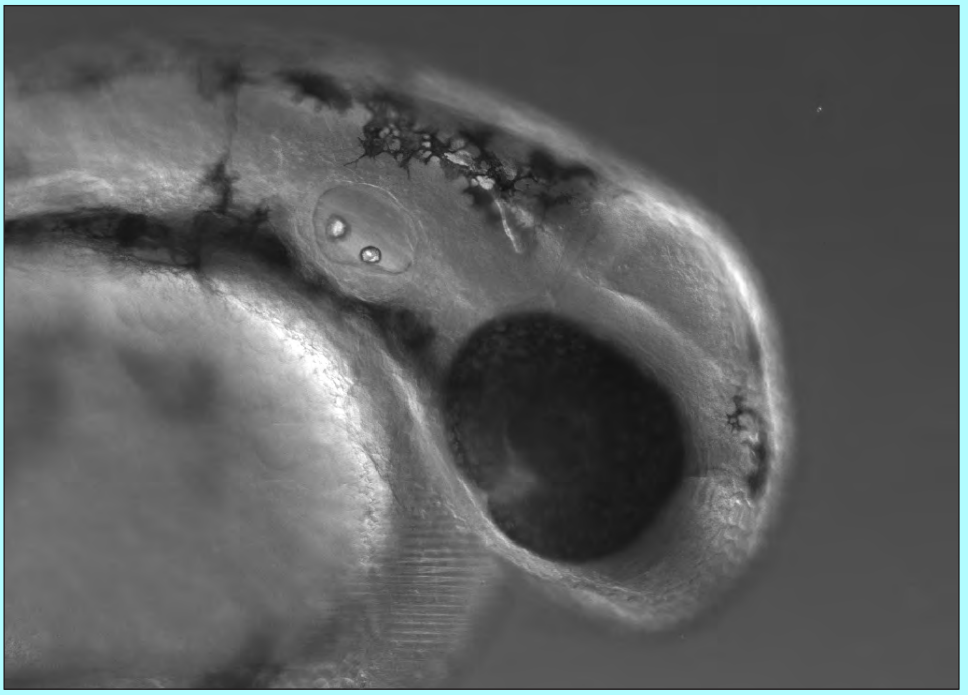

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Cover Photograph: Sagittal view of the head of a 48-hour-old Zebrafish embryo

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Identification and Localization of the Abelson Oncogene in Zebrafish, *Danio rerio*

Richard C. de Triquet[1], Brandon S. Carpenter[2], Ted Zerucha[1,*], and Susan L. Edwards[1]

Abstract - The Abelson oncoprotein (Abl) has multiple molecular functions that affect cell proliferation and morphology. Mutation of the *Abl* gene is the causal event in several types of leukemia and is also linked to the progression of certain lung and breast cancers. Despite extensive literature documenting the protein's function in *Homo sapiens* L. (Human) and the recent increase of cancer research using *Danio rerio* (Zebrafish) as a model system, no research to date has investigated the presence of an *Abl* ortholog in Zebrafish. We have sequenced a Zebrafish *abl1* cDNA clone that exhibits high identity to other vertebrate *Abl* genes and have localized expression of this gene and its protein product by in situ hybridization and immunohistochemistry in developing Zebrafish embryos. Gene expression of *abl1* in regions of the neural plate began at 12 hours post-fertilization (hpf) and continued through 48 hpf. Between 18–36 hpf, *abl1* gene expression was visible in regions of the tail corresponding to the posterior blood island (PBI). We also observed Abl protein expression in the PBI from 24 to 32 hpf. The observed patterns of mRNA and protein expression suggest that Zebrafish *abl1* may be involved with neurulation within the neural plate and hematopoiesis within the PBI. This finding is consistent with what has been reported for homologs of this gene in other vertebrate species.

Introduction

The *Abl* genes encode for several tyrosine kinase isoforms which perform an array of cellular functions that affect the morphology, growth, and migration of specific cells. In *Homo sapiens* L. (Human), a chromosomal translocation involving the *Abl* gene on the long arm of chromosome 9 and the *Bcr* gene on the long arm of chromosome 22 produces the *Bcr-Abl* fusion gene, which codes for a constitutively active kinase (Daley et al. 1990, Kelliher et al. 1990, Lugo et al. 1990, Oda et al. 1995, Scott et al. 1991). Bcr-Abl causes malignant transformation of hematopoietic stem cells (HSC) and progenitor cells through altered cellular adhesion, inhibition of apoptosis, and stimulation of mitogenic pathways, resulting in massive proliferation of the myeloid cell lineage (Deming et al. 2004, Gross and Ren 2000, Mcgahon et al. 1997). This specific fusion kinase is present in >95% of patients with chronic myelogenous leukemia (CML) and approximately 20% of adult acute lymphoblastic leukemia patients (Gross and Ren 2000, Kurzrock et al. 2003, Reinhold et al. 2003, Shtivelman et al. 1985).

Orthologs of the *Abl* gene have been documented in several organisms including the *Caenorhabditis elegans* Maupas (Nematode), *Drosophila melanogaster* Meigen (Common Fruit Fly), *Mus musculus* L. (House Mouse), and Humans (Henkemeyer 1990). Hematopoietic stem cells in mice are transformed by the Bcr-Abl fusion

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kinase (Lin et al. 2005), and functionally inactivating *Abl*1 and *Abl*2 in mice results in death at embryonic day 9. The consequences of inactivating these genes demonstrate these proteins are essential during early development (Koleske et al. 1998). *Abl* has also been implicated in the development of axonal outgrowths in Common Fruit Fly (Plattner et al. 1999) and in the construction of the central nervous system in mice (Koleske et al. 1998). Despite these examples of the utility of studying the Abl gene in non-Human organisms, no research to date has investigated the presence of the *Abl* oncogene in *Danio rerio* Hamilton (Zebrafish).

Zebrafish possess a blood cell repertoire highly similar to Humans, but in Zebrafish, hematopoiesis occurs at different sites during early development and adulthood (Meeker and Trede 2008). The posterior blood island (PBI), a region posterior to the yolk extension, is a major site of erythromyeloid progenitor cell development beginning at 24 hours post-fertilization (hpf) (Bertrand et al. 2007). Currently, research on the blood-related diseases using Zebrafish as a model include MOZ/TIF2-induced acute myeloid leukemia (Zhuravleva et al. 2008), heat-shock-induced T-cell lymphoma (Feng et al. 2007), Notch1-induced T-cell leukemia (Chen et al. 2006), Myc-induced T-cell leukemia (Langenau et al. 2002), and B-cell chronic lymphocytic leukemia (Auer et al. 2007). We report here the sequence of an *Abl* homolog obtained from Zebrafish and describe expression of the *abl* mRNA and abl protein in developing Zebrafish from 2 to 48 hpf at time points corresponding to early hematopoiesis and neurogenesis. Gene and protein expression were characterized using whole-mount in situ hybridization (ISH), immunohistochemistry (IHC), and western blot.

Methods

Zebrafish care and embryo sampling

We housed Zebrafish in a Z-Mod system (Aquatic Habitats, Apopka, FL) and maintained them at 28 °C on a 14-hour light/10-hour dark cycle. We carried out the care of adult Zebrafish in accordance with the Animal Welfare Act Regulations and Standards and the Institute of Laboratory Animal Resources' Guide for the Care and Use of Laboratory Animals. The Appalachian State University Institutional Animal Care and Use Committee (IACUC Reference #12-06) approved procedures for caring for and breeding adult Zebrafish. We obtained embryos by pairwise crosses and staged them according to Kimmel et al. (1995). Embryos were fixed overnight in 4% paraformaldehyde (PFA) in 10 mM phosphate buffered saline (PBS; pH = 7.4) prior to de-chorionation and dehydration in methanol for in situ hybridization (ISH) and immunohistochemistry (IHC).

Sequence analysis and comparison with other species

We purchased a Zebrafish *abl* cDNA clone through Open Biosystems (clone ID 3857626, Accession EB951650, Lafayette, CO). We propagated the gene, which was housed within the pSport1 vector, in the DH5α bacterial strain. Primers were designed by MacVector11.0 and obtained from Eurofins MWG Operon (Huntsville, AL) for use in sequencing reactions. We isolated plasmid DNA for sequencing reactions and ISH by a PureYield Plasmid Maxiprep System (Promega, Madison,

WI). Cornell Life Sciences Core Laboratories Center (Ithaca, NY) and Mount Desert Island Biological Laboratory (Bar Harbor, ME) performed the sequencing reactions. We used the ExPASy proteomics server (http://ca.expasy.org/tools/dna.html) to translate the obtained sequence. We completed phylogenetic tree analysis with Phylogeny.fr (http://www.phylogeny.fr/) using MUSCLE 3.7 for multiple sequence alignment, GBlocks 0.91b for alignment refinement, PhyML 3.0 aLRT for phylogeny, and TreeDyn 198.3 for tree rendering (Dereeper. et al. 2008). We used amino acid sequences from Abl1 orthologs for phylogenetic tree construction: translation of Zebrafish cDNA clone (EB951650), predicted Zebrafish v-*Abl*1 homolog (XP_001337899.1), Human (NP_005148.2, NP_009297.2), House Mouse (NP_001106174.1, NP 033724.2), *Rattus norvegicus* Berkenhout (Norway Rat, ABC46642.1), *Pongo abelii* Lesson (Sumatran Orangutan; XP_002833232.1), *Equus caballus* L. (Horse; XP_001917300.1), *Silurana tropicalis* Gray (formerly *Xenopus topicalis*, Western Clawed Frog; XP_002936228.1), and *Strongylocentrotus purpuratus* Stimpson (Purple Sea Urchin; XP_001203875.1).

Spatial and temporal expression of *Abl1*

We determined the spatial and temporal expression of *abl1* during Zebrafish development by in situ hybridization. We examined the following developmental stages: 2, 12, 18, 24, 32, 36, and 48 hpf. These stages span the cleavage and blastula period to late pharyngula just prior to hatching.

We linearized plasmid DNA (*abl* in pSport1) separately by Not1 and Sal1 restriction enzymes, purified with a Zymo Research Clean kit (Zymo Research, Irvine, CA), quantified them, and confirmed linearization by gel electrophoresis. We used a Roche DIG RNA labeling Kit (Roche Applied Science, Branford, CT) for RNA labeling with digoxigenin-UTP by in vitro transcription using SP6 and T7 RNA Polymerase. Plasmid DNA cut with Sal1 was transcribed with SP6 promoters for antisense experimental probes. Plasmid DNA cut with Not1 was transcribed using T7 promoters for sense negative control probes. Following incubation of the transcription reactions at 37 °C for 2 h, we removed template DNA by incubation with Roche DNAse I recombinant at 37 °C for 30 min. Transcribed probes were then precipitated overnight using 4M lithium chloride and 100% ethanol.

We performed in situ hybridizations utilizing the RNA probes as described by Thisse and Thisse (2008). We progressively rehydrated embryos to 10mM PBS containing 0.1% Tween-20 (PBST), permeabilized with Proteinase K at stage-dependent times, and incubated them in 4% PFA for 20 min. We then pre-hybridized embryos at 70° C for 5 h in hybridization mix (50% formamide, 5xSSC, 50 mg/ml heparin, 500 mg/ml torula yeast, 0.1% Tween-20; pH = 6.2). After pre-hybridization, we incubated embryos overnight at 70° C in hybridization mix containing 50 ng of antisense or sense probe. The following day, we washed embryos with 2X and 0.2X SSC, progressively brought them to PBS Triton, and then incubated them in BSA:PBST:5% sheep serum at room temperature for 1 h. We incubated embryos at 4 °C overnight with a Roche anti-digoxigenin-alkaline phosphatase antibody (α-DIG-AP) at a 1:2000 concentration. We washed embryos with PBST and visual-

ized labeled *abl* mRNA using a staining reaction with nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) and stopped the reaction with TE buffer pH 8. We performed the visualization using a Meiji compound microscope fitted with a Leica DFC320 digital camera.

Antibodies

We obtained the sc-131 polyclonal rabbit antibody specific to the kinase domain of Human *Abl*1 from Santa Cruz Biotechnology, Santa Cruz, CA. The sc-131 antibody recognizes a peptide sequence 99% identical to the Zebrafish abl1 sequence, confirmed via direct communications with Santa Cruz Biotechnology, and is thus also highly likely to be specific for the Zebrafish protein. We conducted additional studies to determine the specificity of the sc-131 antibody and whether staining was consistent with that observed using the monoclonal sc-56887 primary antibody specific to the SH2 domain of *Abl*1 (Santa Cruz Biotechnology, Santa Cruz, CA).

Expression of abl1 protein in Zebrafish embryos

We rehydrated embryos to PBS, and non-specific binding sites were blocked by incubation in Blocking Solution (50 mg/ml Normal Goat Serum (NGS), 6.7 mg/ml tween, and 940 mg/ml PBS) at room temperature for 30 min. Embryos were incubated overnight at 4 °C with a 1:100 dilution of sc-131 (Santa Cruz Biotechnology, Santa Cruz, CA). We then washed the embryos with PBST and incubated them for 1 h at room temperature with 1:1000 goat-α-rabbit-AP antibody (Bio-Rad, Hercules, CA). We stained bound antibody by NBT/BCIP reaction and visualized the samples as described above.

Preparation of whole-cell extracts containing Abl protein

We incubated K562 cells in filter-sterilized Iscove base media containing heat-inactivated fetal calf serum (Atlanta Biologicals, Lawrenceville, GA), sodium-pyruvate, L-glutamine, and penicillin-streptomycin solution. We incubated cells at 37 °C at 5% CO_2. We harvested approximately 2 X 10^7 cells in PBS, pelleted the samples by centrifugation, and resuspended in Freeze/Thaw buffer (250 mM Tris-HCl [pH = 8], 10 mM DTT, and 15% glycerol). We prepared whole-cell extract with repeated cycles of freezing and thawing. We quantified protein concentration in the whole-cell extract using the Bicinchoninic Acid (BCA) protein assay kit (Thermo Fisher Scientific, Rockford, IL).

Expression of abl1 protein in whole-cell extracts from Zebrafish embryos

We homogenized embryos staged at 18, 24, 32, and 36 hpf in ice-cold homogenization buffer (250 mM sucrose, 30 mM Tris, 1 mM EDTA, 0.5% protease inhibitor cocktail (Sigma), and 100 mg/mL phenylmethylsulfonyl fluoride (Sigma; pH 7.8). We centrifuged homogenized samples at 14,000 rpm at 4 °C for 10 min, and quantified supernatants using the BCA protein assay kit. We resolved 75 µg of K562 cell protein and Zebrafish protein at 18, 24, and 36 hpf on a NuSep 4–20% LongLife gel using the mini protean II electrophoresis cell (50 V for 30 min, 90 V for 1.5

hr, Bio-Rad, Hercules, CA). Proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (100 V for 1 hr). We prevented antibody binding to non-specific binding sites by blocking at room temperature with 5% Blotto (5% nonfat dried milk powder in 0.1 M Tris-buffered saline (TBST), 1.21 g/L Tris base, 8.76 g/L NaCl, and 2 ml/L of triton; pH = 7.4). We then incubated membranes in 1:200 sc-131 and primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight. Following 3 consecutive 15-min washes in TBST, we incubated membranes in 1:3000 goat-α-rabbit-AP antibody (Bio-Rad, Hercules, CA) for 1 hr, and imaged them via chemiluminescent reaction with Bio-Rad Immun-Star AP Substrate. We acquired membrane images with an Epson Stylus NX400 Series scanner. We ran negative controls by incubating membrane samples in 5% Blotto in place of primary antibody.

Results

DNA sequencing

We determined that the Zebrafish *abl* cDNA clone obtained from Open Biosystems (ID 3857626, Accession EB951650, Pittsburgh, PA) was 3893 base pairs (bp) in length. This sequence contained a long, open reading frame of 996 bp and 2897 bp of non-translatable region. Our partial clone did not contain a start codon, indicating it was lacking the 5′ region of the gene, but the partial clone did contain the 3′poly-A tail (Fig. 1 and data not shown). BLAST analysis of the translated sequence indicated that it has 99% amino acid identity to two predicted Zebrafish c-*abl*1 oncoprotein homologs: isoform 1 (XP_001337829.1) and isoform 2 (XP_001337899.1). These two isoforms are splice variants of the *abl1* gene, which maps to chromosome 5 in the Zebrafish genome and are 1079 amino acids (aa) and 1060 aa in length, respectively. Phylogenetic analysis clearly shows a distinct relationship between our Zebrafish *abl* clone, the predicted Zebrafish *abl1* (XP_001337899.1), and other *Abl1* homologs, indicating that our clone represents Zebrafish *abl1* (Fig. 2).

Expression of *abl1* gene

There was no evidence of *abl1* gene expression observed at 2 hpf in embryos hybridized with antisense RNA probes (Fig. 3a) or in negative controls using sense RNA probes (Fig. 3b). The first observed *abl1* expression was present at 12 hpf during the bud stage in several regions of the developing neural plate: anteriorly, in regions closest to the mid-sagittal furrow where prechordal plate hypoblast accumulates; posteriorly at the site of early somite development; and within the tailbud (Fig. 3c, d). At 18 hpf, expression continued within anterior regions of the neural plate and the tailbud (Fig. 3f). Ventral views showed that expression within the neural plate was localized closest to the midsagittal furrow (Fig. 3g). At 24 hpf, we observed *abl1* expression anteriorly within the head, through the midsection, and within the PBI (Fig. 3i). Similarly, we saw *abl1* expression anteriorly within the head, through the midsection, and within the PBI at 32 hpf (Fig. 4a–c). At 36 hpf, we continued to observe *abl1* expression within the head (Fig. 4e, f), but expression

Figure 1. Nucleotide and deduced amino acid sequence of the putative Zebrafish *abl1* coding region. Underlining indicates regions we have sequenced from our Zebrafish cDNA clone (clone ID 3857626); other nucleotide sequences were obtained from the predicted *Abl1* homolog on NCBI (XM_001337793.3). The blue underlined region indicates the translatable region of our clone used in protein blasts. Capital letters above nucleotide sequences in the translatable region indicate the amino acid sequence. Red boxes indicate start and stop codons. The green highlighted region represents the coding region for the SH3 domain; the yellow highlighted region represents coding region for the SH2 domain; the red highlighted region represents the coding region of the Kinase domain; and the blue highlighted region represents the coding region for the F-actin binding-domain.

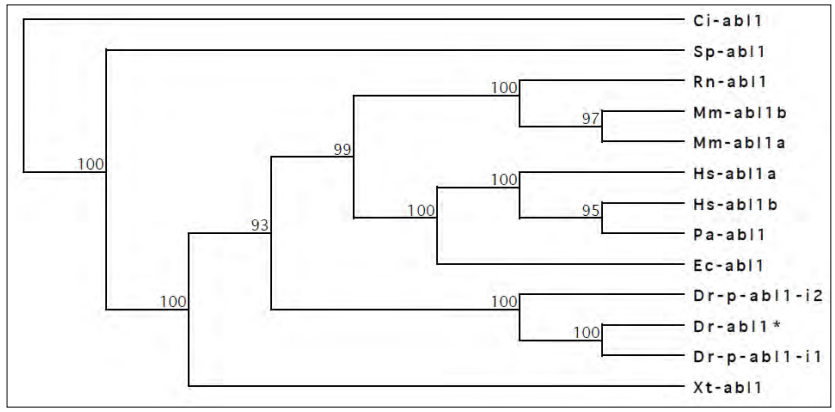

Figure 2. Phylogenetic tree of Abl orthologs. The tree was constructed using MacVector 12.5.1 for multiple sequence alignment, neighbor-joining method. Branch numbers represent percentage of clades grouped following 1000 replications. Several species and the Bcr-Abl fusion kinase were examined: *Ciona intestinalis* L. (Vase Tunicate) Abl1-like (ci-abl1 = XP_002124117.1), Purple Sea Urchin Abl1-like (sp-abl1 = XP_001203875.1), Norway Rat ABL1 (rn-abl1 = ABC46642.1), House Mouse ABL1 isoform b (Mm-abl1b, NP_033724.2), House Mouse ABL1 isoform a (Mm-abl1a, NP_001106174.1), Human ABL1 isoform a (Hs-abl1a, NP_005148.2), Human ABL1 isoform b (Hs-abl1b, NP_009297.2), Sumatran Orangutan ABL1-like (Pa-abl1, XP_002833232.1), Horse ABL1 (ec-abl1 = XP_001917300.1), Zebrafish predicted ABL1-like isoform 2 (dr-p-abl1-i2 = XP_001337899.1), Zebrafish abl1 (dr-abl1* = EB951650), Zebrafish predicted ABL1-like isoform 1 (dr-p-abl1-i1 = XP_001337829.1), Western Clawed Frog ABL1-like (xt-abl1 = XP_002936228.1).

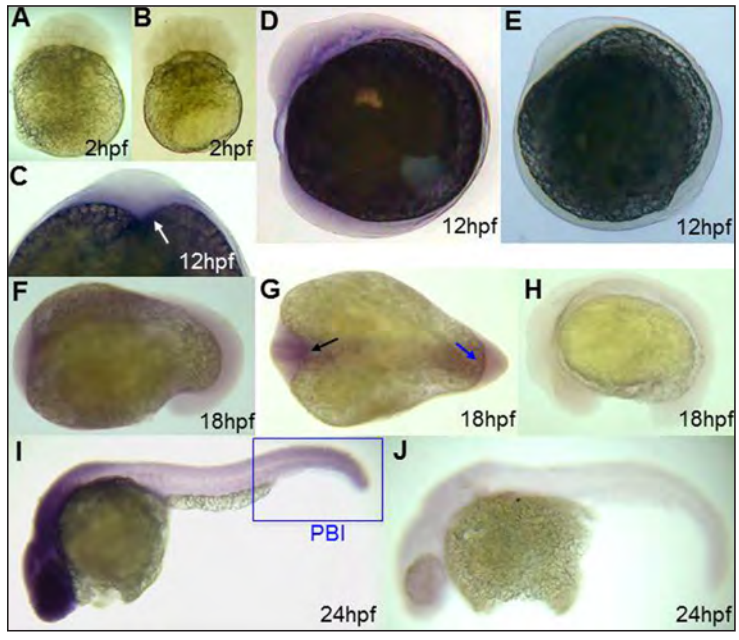

Figure 3. Whole-mount in situ hybridization of *abl* expression from 2–24 hpf in Zebrafish embryos. A = embryos hybridized with antisense probe at 12 hpf, and B = embryos hybridized with sense probe at 2 hpf have no observed expression. C = ventral view at 12 hpf with expression in midsagittal furrow is indicated by awhite arrow, and D = whole embryo view at 12 hpf with observed expression in polster and tail bud. E = negative control at 12 hpf. F = dorsal view at 18 hpf with expression along midsagittal furrow. G = ventral view at 18 hpf with observed expression within polster indicated by a black arrow and within tailbud indicated by a blue arrow. H = negative control at 18 hpf. I = embryo at 24 hpf expression with expression within head regions and within tail regions corresponding to the posterior blood island (PBI) indicated by blue box. J = negative control at 24 hpf.

was diminished within the midsection and tail regions. We observed weaker *abl1* expression along regions of tail immediately above the notochord (Fig. 4g). At 48 hpf, we recorded expression in the head and along the midline (Fig. 4i). Within the head, expression was localized to areas of the eye, telencephalon, midbrain, and within the endoderm most proximal to the yolk (Fig. 4j). Expression of *abl1* along the midline of the tail and midsection was divided into a Y shape above the center of the yolk, and the two lateral branches continued to the inner margins of the developing eyes (Fig. 4k) corresponding to the lateral dorsal aortas and the primitive internal carotid arteries. There was no evidence of expression in any of the negative controls (Figs. 3e, h, j; 4d, h, l).

Expression of abl1 protein

We observed expression of abl1 protein at 18 hpf in the tail bud (Fig. 5a), and at 24 hpf in the PBI and caudal vein plexus (Fig. 5c, d). There was evidence of intense

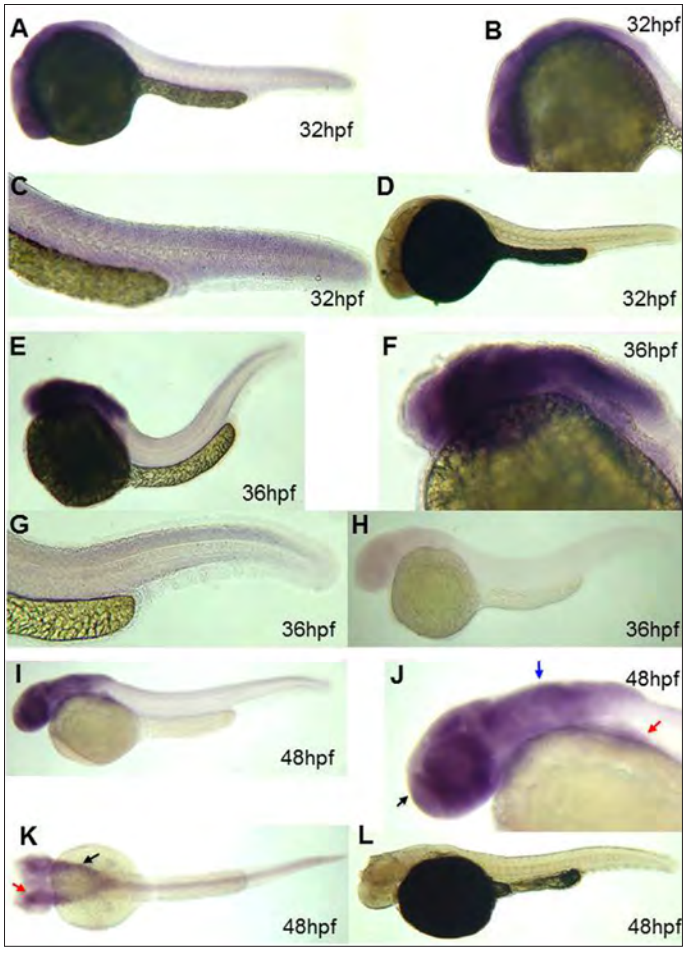

Figure 4. Whole-mount Fin situ hybridization of *abl* expression from 32–48 hpf in Zebrafish embryos using antisense and sense probes (negative control). A = 32 hpf showing expression in the head and tail. B = close-up of head with observed expression. C = close-up of tail at 32 hpf with observed expression in region dorsal and posterior to yolk extension. D = negative control at 32 hpf. E = expression at 36 hpf. F = close up of head at 36 hpf. G = close-up of tail at 36 hpf. H = negative control at 36hpf**.** I, J = **e**mbryo at 48 hpf with expression observed in the head at sites corresponding to the telencephalon (black arrow), myelencephalon (red arrow), and endoderm (blue arrow). K = dorsal view at 48 hpf with expression along midline. The branched expression pattern corresponds to the lateral dorsal aortas indicated by a black arrow and the internal carotid artery indicated by a red arrow. L = negative control at 48 hpf with no observed expression.

immunoreactivity at the tail tip and at regions anteriorly adjacent to the tail tip at 24 hpf (Fig. 5d). Expression within the PBI continued at 32 hpf (Fig. 5f). Negative controls demonstrated a total absence of immunoreactivity of secondary antibody alone (Fig. 5b, e, g).

Expression of abl1 protein in whole-cell extracts from Zebrafish embryos

Initial western blots using sc-131 polyclonal antibody demonstrated specific immunoreactive proteins at ≈ 110 kDa. However, despite repeated attempts, non-specific background remained high. Therefore, we conducted further western blots with the sc-56887 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) specific for the SH2 domain of the Human Abl1 protein. This monoclonal antibody recognizes both isoforms of Zebrafish abl1, because both contain the SH2 domain. Results with the monoclonal sc-56887 antibody ratified the western blot results obtained with the sc-131 polyclonal antibody. As a positive control, we prepared whole cell lysates from the myelogenous leukemia K562 cell line, and the samples showed an immunoreactive protein of approximately 120kD. This size is consistent with the Santa Cruz Biotechnology material datasheet for western analysis of K562 protein extracts probed with the sc-56887 antibody. The Zebrafish

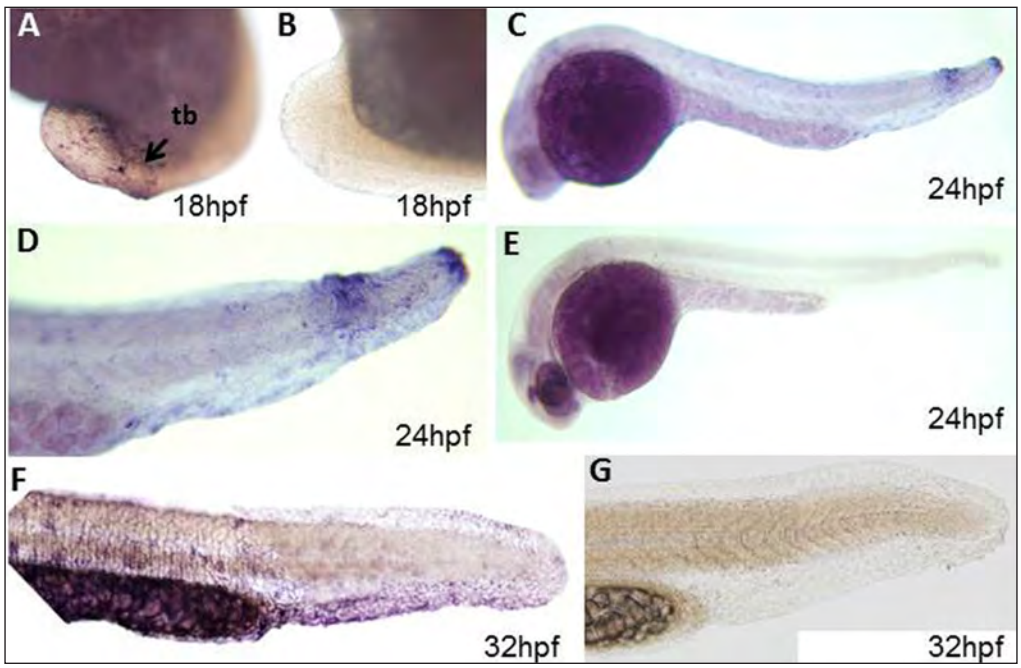

Figure 5. Whole-mount immunohistochemistry of abl expression from 18–32 hpf in Zebrafish embryos. A = 18-hpf embryo showing expression in the tail bud (tb). B = negative control at 18 hpf with no observed expression. C = 24 hpf with expression within posterior regions. D = close-up of the tail at 24 hpf showing punctate expression immediately adjacent to the yolk extension and also within the tail bud. E = negative control at 24 hpf. F = 32-hpf embryo with observed expression within ventral region of the tail immediately posterior to the yolk extension. G = negative control at 32 hpf.

embryos showed an immunoreactive protein of approximately 85 kD at 18, 24, and 36 hpf (Fig. 6).

Discussion

Our results provide DNA, RNA, and protein evidence to support the presence and expression of an *Abl* homolog in Zebrafish. The partial Zebrafish *abl1* cDNA clone we describe demonstrated a 73% identity to the equivalent region of the Human *Abl*1 gene. Zebrafish contain at least 2 *abl* genes, *abl1* and *abl2*, as is observed in other vertebrates. The predicted Zebrafish abl1 and abl2 protein sequences, which are publicly available on the NCBI database, contain several homologous domains including filamentous and globular actin binding sites, the kinase domain, and the SH2 domain. Our partial cDNA clone of the Zebrafish *abl1* gene also contained a coding sequence for the filamentous actin binding domain characteristic of Abl proteins.

The segmentation period in Zebrafish is a key time when the brain rudiment is being sculpted (Kimmel et al. 1995). Prior to this period, during the 64-cell stage at 2 hpf, we observed no *abl1* gene expression. However, beginning at 12 hpf, *abl1* expression was seen anteriorly in regions of the neural plate representing brain primordium and along the inner eye margins (Kimmel et al. 1995). The spatial expression of *abl1* from 12 to 48 hpf documented in this study suggests a link between *abl1* gene expression with neural plate formation and development of the eye. This is further supported by the fact that our spatial expression patterns were consistent with documented functions of *Abl* in other organisms. Previous studies conducted in Common Fruit Fly and mice have demonstrated the importance of the *Abl* gene in axon guidance and synapse formation in the developing central nervous system (Lin et al. 2009, Stevens et al. 2008). In the brain of adult mice, *Abl*2 is found in high amounts and co-localizes with *Abl*1 during neurulation (Koleske et al. 1998). These proteins are also highly active in retinal cells of Common Fruit Fly (Henkemeyer et al. 1990).

Our results also demonstrate that *abl1* expression clusters around the yolk between 18–24 hpf (Fig. 3). Expression in yolk regions closest to the head suggests a possible role in neurulation functions. Expression close to the sinous venosus and posterior to the yolk suggests a possible involvement in hematopoiesis, which

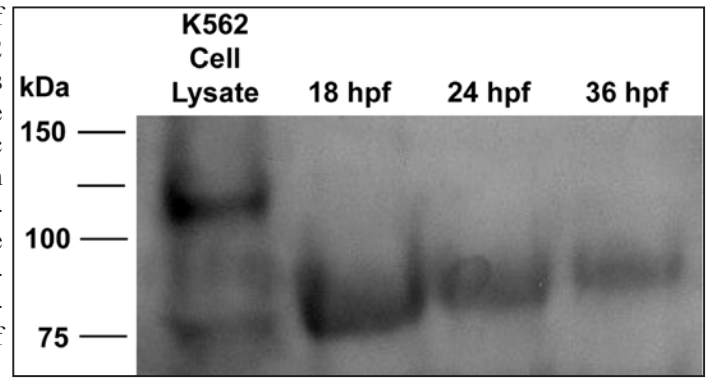

Figure 6. Western blot of quantified protein from K562 cells and Zebrafish embryos at 18, 24, and 36 hpf. The sc-56887 antibody specific to Abl1 of Human origin was used. The lane containing K562 whole cell lysate contains a band of approximately 120 kd, while Zebrafish lanes contain a band of approximately 85 kD.

agrees with documented hematopoietic functions of *Abl* (Gross and Ren 2000, Koleske et al. 1998, Sirvent et al. 2008). The region posterior to the yolk, known as the PBI, harbors hematopoietic progenitor cells from 24 to 48 hpf with peak expression at 30 hpf (Bertrand et al. 2007); *abl1* gene expression patterns observed in this study were similar. Within the PBI, we saw evidence of *abl1* gene expression between 24–36 hpf. Presence of these transcripts was strongest at 24–32 hours which is consistent with the involvement of *abl1* in the emergence or migration of hematopoietic progenitor cells in the PBI.

Later in development, at 48 hpf, *abl1* transcripts were found along the midline of the body above the yolk extension and tail corresponding to the location of the dorsal aorta, a site of HSC emergence. When dorsally viewed, *abl1* expression occurred in a Y-shaped expression pattern with lateral arms pointed toward the head. This pattern is consistent with the splitting of the dorsal aorta into the lateral dorsal aortas at the sinus venosus of the heart. After branching, the lateral dorsal aortas supply the primitive internal carotid artery which lines the inner margins of the developing eye (Isogai et al. 2001), suggesting that *abl1* may be involved with the vascular formation of these vessels.

Protein expression patterns indicated that abl1 functions within the PBI during the emergence of erythromyeloid progenitors and other hematopoietic stem cells.

Immunohistochemistry and western blot results indicated the expression of the abl1 protein from 18 to 36 hpf. Expression of the protein began in the tailbud at 18 hpf and continued to be expressed within the PBI at 24 and 32 hpf. However, expression of the abl1 protein was absent within the anterior regions of the head where gene expression was also observed. There was also intense punctate immunoreactive staining within the tail at 24 hpf. It is possible that this staining in the tail is an artifact, because we only observed the staining pattern in only about 50% of embryos in the same location.

The observed size of abl protein from Zebrafish embryos as determined by western blot studies was approximately 85 kD, which is smaller than the predicted size of 119 kD for abl1 isoform 1. This observed size is not inconsistent with other observations of abl homologs of this protein as several other documented isoforms also have differences in molecular weight: v-Abl is documented at 81.8 kD (NP_955595.1), and one Abl1 protein isoform in Humans has a molecular weight of 41.2 kD (AAI07070). Other Abl isoforms have been found in Human blood cells with weights of 68 and 36 kD (Naldini et al. 1986). It is also possible that the size disparity is due to post-translational modifications or alternative splicing patterns in Zebrafish.

Our investigations of the *Abl* gene and protein in combination with the existing literature support the presence of a conserved *Abl* homolog in Zebrafish, *abl1*. We observed *abl1* expression in regions of early hematopoiesis within the PBI, regions of vascular formation in the head, and neurulation of early brain rudiment. It is likely that *abl1* in Zebrafish displays multiple functions in early morphogenesis and more specific roles later as it does in other species (Sirvent et al. 2008). Investigations of *Abl* function in Humans and mice have been profoundly important

in the development of cancer treatments. The research presented here provides a foundation for the study of *Abl* function in Zebrafish and would allow investigations of *Abl* function in early neurulation and early hematopoiesis that are not possible in other models.

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