blood

1999 94: 2622-2636

Gene Duplication of Zebrafish *JAK2* Homologs Is Accompanied by Divergent Embryonic Expression Patterns: Only *jak2a* Is Expressed During Erythropoiesis

Andrew C. Oates, Alison Brownlie, Stephen J. Pratt, Danielle V. Irvine, Eric C. Liao, Barry H. Paw, Kristen J. Dorian, Stephen L. Johnson, John H. Postlethwait, Leonard I. Zon and Andrew F. Wilks

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

HEMATOPOIESIS

Gene Duplication of Zebrafish *JAK2* Homologs Is Accompanied by Divergent Embryonic Expression Patterns: Only *jak2a* Is Expressed During Erythropoiesis

By Andrew C. Oates, Alison Brownlie, Stephen J. Pratt, Danielle V. Irvine, Eric C. Liao, Barry H. Paw, Kristen J. Dorian, Stephen L. Johnson, John H. Postlethwait, Leonard I. Zon, and Andrew F. Wilks

Members of the JAK family of protein tyrosine kinase (PTK) proteins are required for the transmission of signals from a variety of cell surface receptors, particularly those of the cytokine receptor family. JAK function has been implicated in hematopoiesis and regulation of the immune system, and recent data suggest that the vertebrate *JAK2* gene may play a role in leukemia. We have isolated and characterized *jak* cDNAs from the zebrafish *Danio rerio*. The zebrafish genome possesses 2 *jak2* genes that occupy paralogous chromosome segments in the zebrafish genome, and these segments conserve syntenic relationships with orthologous genes in mammalian genomes, suggesting an ancient duplication in the zebrafish lineage. The *jak2a* gene is expressed at high levels in erythroid precursors of primitive and definitive waves and at a lower level in early central nervous system and developing fin buds. *jak2b* is expressed in the developing lens and nephritic ducts, but not in hematopoietic tissue. The expression of *jak2a* was examined in hematopoietic mutants and found to be disrupted in *cloche* and *spadetail*, suggesting an early role in hematopoiesis. Taken together with recent gene knockout data in the mouse, we suggest that *jak2a* may be functionally equivalent to mammalian *Jak2*, with a role in early erythropoiesis.

CYTOKINES ARE IMPORTANT regulators of proliferation, differentiation, and cell function for a wide range of cells of hematopoietic and other lineages. The JAK/STAT signaling system is required for the function of a number of cytokines, acting to transduce signals from cytokine receptors.[1,2]

JAK tyrosine kinases possess a distinctive structure. Amino acid sequence comparison of the 4 mammalian JAK family members (JAK1, JAK2, JAK3, and TYK2) shows the presence of 7 highly conserved domains (named JH1 through JH7).[3] The N-terminal region of JAK kinases contains structures (JH7-JH3) that confer specific binding activity toward cytokine receptor cytoplasmic domains.[4-9] Further C-terminal, the kinase-related domain (JH2) exhibits significant similarity to a tyrosine kinase domain yet diverges within several critical catalytic motifs.[10] Indeed, this domain appears not to possess phosphotransferase activity[10]; rather, it is required for the stability and binding affinity of associated receptors.[11,12] The C-terminal domain (JH1) contains a protein tyrosine kinase[10] responsible for initiating much of the signaling activity from cytokine receptors that use this family of PTKs. Expression of kinase inactive JAK variants abrogate most if not all aspects of signaling in a dominant negative manner through receptors for cytokines such as erythropoietin (EPO),[13] growth hormone (GH),[5] granulocyte-macrophage colony-stimulating factor (GM-CSF),[14] granulocyte colony-stimulating factor (G-CSF),[15] interleukin-6 (IL-6),[16] interferon α (IFNα),[11] IFNγ,[17] and IL-2.[18] Analysis of the effects of dominant negative JAK variants combined with a series of somatic cell mutants deficient or defective in 1 of the JAK kinases[8,19,20] demonstrates the critical role of the JAK kinase family in cell signaling.

Three lines of evidence link JAK function directly to the regulation of the growth and differentiation of hematopoietic cells in vivo. First, gene targeting of *Jak* loci in the mouse yields hematopoietic phenotypes: *Jak1*-deficient mice exhibit impaired lymphopoiesis,[21] *Jak2* deficiency abolishes definitive erythropoiesis,[22,23] and *Jak3* null mutation results in the murine equivalent of human severe combined immunodeficiency syndrome (SCID).[24-27] Second, the dominant *Drosophila melanogaster JAK* mutant, *hopscotch*$^{Tum\text{-}l}$ causes a lethal leukemia in fruit flies.[28] The protein products of *hop*$^{Tum\text{-}l}$ and a recently identified allele *hop*^{T42} show increased levels of autophosphorylation and the capacity to activate Stat92E.[29-31] Third, constitutive JAK2 catalytic activity can be detected in some acute lymphoblastic leukemia (ALL) cells in humans. Chromosomal translocation in a human T-cell ALL creates a TEL-JAK2 fusion protein capable of oligomerization through the TEL fusion partner, resulting in constitutive activation of JAK2 tyrosine kinase activity.[32] Mice expressing this fusion protein in bone marrow developed a fatal mixed myeloproliferative and T-cell lymphoproliferative disorder.[33] These results indicate that *JAK* genes are required for normal hematopoiesis and that the deregulation of JAK catalytic activity is capable of causing hematopoietic neoplasia.

To further explore the role that the *JAK* family has in controlling blood cell growth, we have isolated and characterized *JAK* genes from the zebrafish, *Danio rerio*. The zebrafish

From the Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Royal Melbourne Hospital, Victoria, Australia; the Howard Hughes Medical Institute, Children's Hospital, Boston, MA; the Department of Genetics, Washington University Medical School, St Louis, MO; and the Institute of Neurosciences, University of Oregon, Eugene, OR.

Submitted November 30, 1998; accepted June 8, 1999.

A.C.O. was supported by an Anti Cancer Council of Victoria Postgraduate Research Scholarship. D.V.I. was supported by an Anti Cancer Council of Victoria Summer Scholarship. E.C.L. is a Pre-Doctoral Fellow of the Howard Hughes Medical Institute. B.H.P. was supported in part by a H.H.M.I. Postdoctoral Fellowship for Physicians. J.H.P. was supported by Grants No. RO1RR10715 and PHS PO1HD22486.

The sequences reported in this manuscript were submitted to the EMBL database and have the following accession numbers: jak1, AJ005689; jak2a, AJ005690; and jak2b, AJ005691.

Address reprint requests to Andrew C. Oates, PhD, Department of Molecular Biology, Princeton University, Washington Road, Princeton, NJ 08544; e-mail: aoates@molbio.princeton.edu.

*The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/99/9408-0039$3.00/0

offers many technical advantages for the developmental analysis of gene function, because embryos are plentiful, develop externally, and are optically transparent. As a vertebrate experimental system, the zebrafish allows strong analogies to be drawn to human biology. For example, in contrast to *Drosophila melanogaster* and *Caenorhabditis elegans*, zebrafish has circulating blood cells of the erythroid, myeloid, and lymphoid lineages.[34,35] Furthermore, a recent mutant screen performed in the zebrafish led to the isolation of mutations in more than 20 complementation groups that disrupt hematopoiesis,[36,37] including a model for human congenital sideroblastic anemia, *sauternes*.[38] Importantly, the possibility of performing modifier screens in the zebrafish offers the means of identifying other factors in these complex developmental processes by a genetic approach.

Our search using degenerate oligonucleotide polymerase chain reaction (PCR) for homologs of *JAK* and *STAT* genes in *D rerio* yielded several genes from both *JAK* (*jak1*, *jak2a*, and *jak2b*) and *STAT* (*stat1* and *stat3*) gene families. The characterization of zebrafish members of the *STAT* family will be reported elsewhere.[38a] We report here in detail on the structure and evolutionary relationships of the zebrafish *JAK2* homologs. We determined the expression of these genes in wild-type and several selected zebrafish mutants and consequently propose a role for *jak2a* in erythropoiesis.

MATERIALS AND METHODS

Isolation of zebrafish jak *homologs.* *JAK*-directed degenerate oligonucleotide primers were designed after Wilks,[39] based around conserved subdomains VIb and IX[40] in the catalytic JH1 domain of *Homo sapiens* JAK1,[10] *Mus musculus* Jak2,[3] *H sapiens* JAK3[41] and TYK2,[42] and *D melanogaster* HOP.[43] Wherever complete degeneracy was required at a nucleotide position in the primers, an inosine residue was incorporated.[44] The sequence of the primers is CCGAATTCCA(C/T)(C/A)GIGA(C/T)(C/T)TIGCIGCI(C/A)GIAA and CCGAATTCIACICC(A/G)(A/T)AI(G/C)(A/T)CCAIAC(G/A)TC. cDNA libraries were plated and screened at high stringency according to standard methods,[45] with PCR products generated by *JAK*-directed degenerate oligonucleotide PCR as described above, and specific for the zebrafish *jak1*, *jak2a*, and *jak2b* genes. Mixed developmental stage *D rerio* cDNA libraries in Lambda Zap (Stratagene, La Jolla, CA), both random and poly-A primed, were a gift of J. Campos-Ortega (University of Köln, Köln, Germany). Lambda Zap cDNA libraries generated from staged embryonic mRNA populations were made by Bob Riggleman and Kathryn Helde and were a kind gift of D. Grunwald (Eccles Institute, University of Utah, Salt Lake City, UT). The ''Contig Manager'' application of the DNAstar suite of programs (DNAstar Inc, Madison, WI) was used to create and monitor contigs from the primary sequence data. cDNA sequences presented herein corresponding to each of the gene transcripts under study were sequenced in both directions to a minimum of 2-fold coverage.

Zebrafish care and strains. Zebrafish were raised and maintained as described.[46] Zebrafish carrying the following mutant alleles of *cloche* (*clo*^{m39}),[47] *spadetail* (*spt*^{b104}),[48] *cabernet* (*cab*^{tl236}), *retsina* (*ret*^{tr217}), *chianti* (*cia*^{tu25f}), *sauternes* (*sau*^{ty121}),[38] *chablis* (*cha*$^{tu245/tu242e}$) (thought to be clonal alleles), *weißherbst* (*weh*^{th238}, *weh*^{tp85c}), *chardonnay* (*cdy*^{te216}), *frascati* (*frs*^{tm130d}, *frs*^{tq223}), *reisling* (*ris*^{tb237}), and *merlot* (*mot*^{tm303c})[36] were studied.

Sequence analysis and evolutionary comparison. Electronic database searches were made by submitting nucleic acid sequence and putative amino acid sequence to the public search facility at the Baylor College of Medicine (Houston, TX; http://hgsc.bcm.tmc.edu/SearchLaunches/) using ''WU-BLAST.''[49] To study the evolutionary relationships between the PTKs and *JAKs* identified, the deduced amino acid sequences of the genes in question were aligned with the ''CLUSTAL'' protein alignment program[50] of the MegAlign application (DNAstar suite) and refined by hand using structural information, where available. These alignments were used to create maximum parsimony phylogenetic trees and distance matrices using the options of that program. The topology of the phylogenetic tree shown was insensitive to the order of sequence addition.

Whole mount in situ hybridization. Embryos were staged according to Kimmel et al.[51] Embryos raised to time points beyond 24 hours postfertilization (hpf) were transferred to E3 embryo medium with 0.003% phenylthiourea (PTU; Sigma, St Louis, MO) to prevent melanization. Riboprobe synthesis and in situ hybridization were performed essentially as described[52] with the following modifications. Riboprobes were purified before use over RNA sephadex G-50 columns (Boehringer Mannheim, Indianapolis, IN). Using estimates of RNA synthesis based on ^{32}P-CTP incorporation, probes were resuspended in HYB^{+} [52] at a concentration of 1 ng/mL for use. Embryos up to 24 hpf were not proteinase K-digested, embryos between 24 hpf and 36 hpf were digested for 10 minutes (10 μg/mL), and embryos greater than 36 hpf were digested for between 20 and 30 minutes (20 μg/mL). Hybridization and washing was performed at temperatures of 65°C to 70°C. Nonspecific antidigoxygenin Fab-AP binding (Boehringer Mannheim; used at a dilution of 1/5,000) was blocked by 2% wt/vol Blocking Reagent (Boehringer Mannheim)/10% heat-inactivated sheep serum/MABT (100 mmol/L Maelic acid, 150 mmol/L NaCl, 0.1% Tween-20, pH 7.5) for 1 hour at room temperature. Color detection reactions used BM purple substrate (Boehringer Mannheim) and were developed for up to 2 days before fixing in 4% paraformaldehyde/PBT (phosphate-buffered saline/0.1% Tween 20). Embryos were either cleared in glycerol or benzyl benzoate:benzyl alcohol (2:1) and photographed using a Leitz Wild T stereo dissection microscope (Leitz, Wetzlar, Germany) or a Nikon Microphot AX compound microscope (Nikon Inc, Melville, NY). The entire *jak2b* cDNA was used to generate a digoxygenin (DIG)-labeled probe, and the *jak2a* riboprobe contained the 5′-most 800 bp encoding the JH7 and JH6 domains.

Generation of DNA polymorphisms and genetic mapping. A bacterial artificial chromosome (BAC) library (Genome Systems, St Louis, MO) containing large insert zebrafish genomic DNA was screened by hybridization to oligonucleotide probes derived from *jak1*, *jak2a*, and *jak2b* cDNA. Clones corresponding to the genomic loci were obtained for *jak1* (96 E18, 100 K4, and 143 O15) and *jak2a* (112 K6), but not *jak2b*. A P1 artificial chromosome (PAC) library (C. Amemiya, Boston University, Boston, MA) was screened by hybridization to an oligonucleotide probe derived from *jak2b* and clones obtained (35 F6 and 58 G17). Sequence information from the ends of each of these genomic clones was determined (data not shown) and, along with the sequence of 3′ UTR regions from cDNA of the *jak* genes, was used to design PCR primer pairs that amplified products from genomic DNA that segregated in a C32xSJD mapping cross.[53] The primers for *jak1* (100 T7-1 GTAGAAGATACAGTCGCCTG, 100 T7-2 GTAAAGCAATATCAATAGAG) give a codominant size polymorphism[54] of 290/270 bp; the *jak2a* codominant size polymorphism (200/220 bp) is from the primer pair j2A.29 (GATCATCCACAGTTCAGCTCC), j2A.30 (TAATGATGAGAGAAACACCCGC); *jak2b* was mapped with a codominant sequence polymorphism[55] in the PCR product generated by the j2B.M1 (AAGAAAGTCTGTCCGCTGTCTTCACATGTC), j2B.M4 (CGCGCCAGCACTGCTAGCATAACAGAAACC) primer pair. Linkage was determined by comparison of a given marker on a C32xSJD haploid panel consisting of 96 individuals that were genotyped against approximately 600 markers for close correlation of segregation patterns[53,56-59] using the program ''MapMaker''[60] (Massachusetts Institute of Technology, Cambridge, MA) and the program ''mapmanager''[61] (Roswell Park Cancer Institute, Buffalo, NY).

Fig 1. ***JAK*** **genes from the zebrafish. (a) Alignment of deduced amino acid sequences for the PTKs identified by PCR from zebrafish cDNA. The alignment was generated using CLUSTAL.[50] The clone name is listed as HD on the right of the sequences, followed by the name of the gene that gave the highest BLAST score in database similarity searches.[49] The JAK-specific sequence elements in Hanks motif VIII are boxed. (b) Comparison of the zebrafish jak2a and human JAK2 protein. The amino acid sequence (single-letter code) of the jak2a protein from zebrafish and the JAK2 protein from human[88] is numbered from the putative initiation methionine. The JAK homology (JH) domains are indicated by brackets and are labeled JH1 through JH7 according to Harpur et al.[3] Conserved motifs in the catalytic JH1 domain are labeled in roman numerals. Conserved motifs in the kinase related domain (JH2) are labeled in roman numerals with the subscript a. A series of conserved residues mutated in murine Jak2 without phenotypic effect in IFN signaling[8] are indicated by an asterisk above the amino acid, and are labeled according to Kohlhuber et al[8] by capital letters. The site of the E665K mutation within JH2 that hyperactivates the catalytic activity[31] of murine Jak2 and** ***D melanogaster*** **HOP is marked with a solid arrowhead. The autophosphorylation site in the JH1 domain of murine Jak2, which is required for catalytic activity,[89] is marked by an open arrowhead. The structure of the variant** ***jak2a*****β cDNA is marked by an arrow at the site of predicted translational termination due to alternate splicing.[62] (c) Phylogeny of the** ***JAK*** **gene family. The amino acid sequences of zebrafish (*Danio rerio, Dr*) jak2a and jak2b were aligned across the known region of jak2b, consisting of most of JH2 and all of JH1, with JAK2 proteins from human (*Homo sapiens, Hs*),[88] pig (*Sus scrofa, Ss*),[90] mouse (*Mus musculus, Mm*),[3] and rat (*Rattus norvegicus, Rn*)[91]; all other members of the *JAK* family from human (*Hs*),[10,41,42] the zebrafish (*Dr*) jak1 sequence (this study), and the sequence of the *D melanogaster* (*Dm*) *JAK* homolog,[42] *hopscotch,* using the CLUSTAL alignment algorithm.[50] This alignment was used to construct a dendrogram with the maximum parsimony options of the DNAstar MegALIGN application to infer the likely genealogy of the *JAK* family. The names of the sequences are displayed to the right of the dendrogram; zebrafish sequences are in bold.**

Assessment of linkage of jak2a *to the* cab *and* mot *mutations.* Homozygous diploid embryos were generated as described[46] from a ABxDAR hybrid mother carrying a single mutant allele of *cab*^{tl236} and from a ABxDAR hybrid mother heterozygous for *mot*^{tm303c} and scored for an erythropoietic phenotype. These embryos were typed for the segregation of the j2A.29/j2A.03 marker from the *jak2a* 3′ UTR with the mutant phenotype, based on a size polymorphism evident between the AB and DAR strains.

RESULTS

Cloning of zebrafish jak2 *genes.* To isolate *JAK* homologs in zebrafish, PCR was used to amplify cDNA derived from mixed-stage embryonic mRNA with *JAK*-directed degenerate primer pairs, yielding 8 distinct PTK fragments corresponding to members from 3 kinase subfamilies (Fig 1a). Sequence comparison of the PCR products to known *JAK* genes suggested that 2 of the PTKs were closest in identity to mammalian *JAK2*; these were termed *jak2a* (HD-1) and *jak2b* (HD-71). A third, HD-9, was most similar to mammalian *JAK1* and was designated *jak1*. This manuscript will focus on the 2 *jak2* genes we detected in the zebrafish. The embryonic expression of *jak1* will be reported elsewhere (Oates and Wilks, manuscript in preparation).

Multiple cDNA clones were recovered from an embryonic cDNA library by DNA hybridization using the PCR product corresponding to *jak2a* as a probe. Conceptual translation of the resulting sequence contig showed an open reading frame (ORF) of 1,095 amino acids with high similarity across the entire coding region to mammalian *JAK2* genes (65% identity to *Mm* JAK2[3]; Fig 1b). One cDNA possessed an internal deletion relative to all other cDNAs, consistent with the omission of an exon due to an alternate splicing event.[62] The longer form of the transcript was named *jak2a*α and the shorter, alternately spliced form was termed *jak2a*β, consistent with the nomenclature for alternately spliced forms of the mammalian *STAT1* and *STAT3* genes.[63,64] Multiple cDNA libraries were screened with a *jak2b* probe and a partial cDNA was isolated 1,967 nucleotides in length containing an ORF of 498 amino acids consisting of the C-terminal region of the protein (data not shown).

Structure of the zebrafish jak2 *transcripts.* Examination of the conceptual translation of both zebrafish *jak2* genes showed a high degree of sequence conservation. The jak2a protein shows approximately 65% identity to the mammalian JAK2 proteins from mouse, human, rat, and pig. As shown in Fig 1b, by comparison to human JAK2, all recognized structural elements found in mammalian JAK2 proteins are present. This high degree of sequence conservation indicated a strong likelihood of functional conservation. Comparison of the predicted amino acid sequence of the majority of the JH2 domain and the entire JH1 domain found in the *jak2b* cDNA with other JAK kinases indicated that it, too, is most closely related to mammalian JAK2 proteins.

The mammalian JAK kinases and *Drosophila* HOP were aligned with the amino acid sequence of jak2a and jak2b over the JH1 and JH2 domains, and a phylogenetic reconstruction of *JAK* gene evolution was derived, as shown in Fig 1c. The zebrafish jak2 proteins are approximately as different from each other (75% identity) as they are from the mammalian JAK2 proteins, indicating an ancient paralogy. jak2b is slightly more similar to the mammalian proteins (average of 78% identity)

b

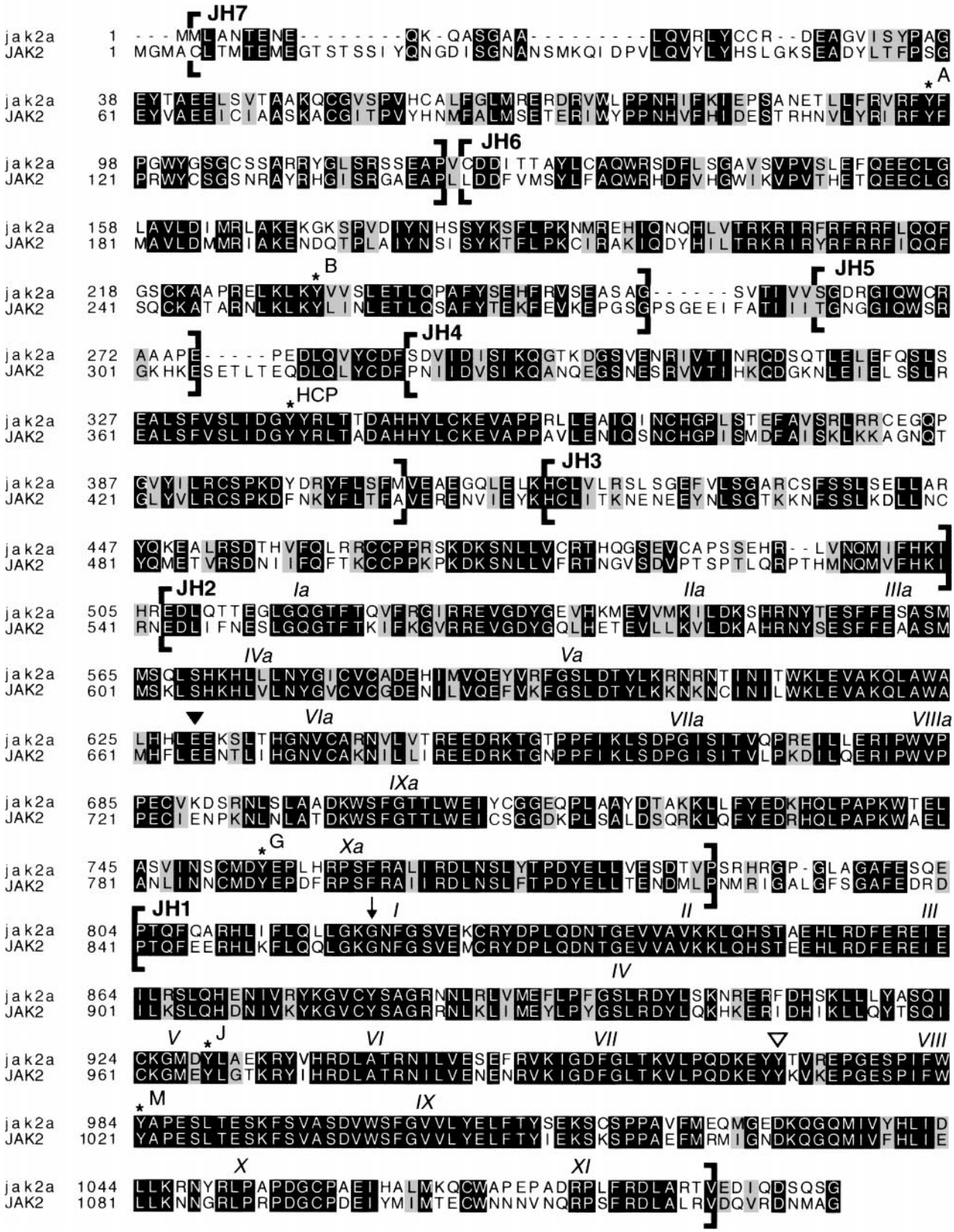
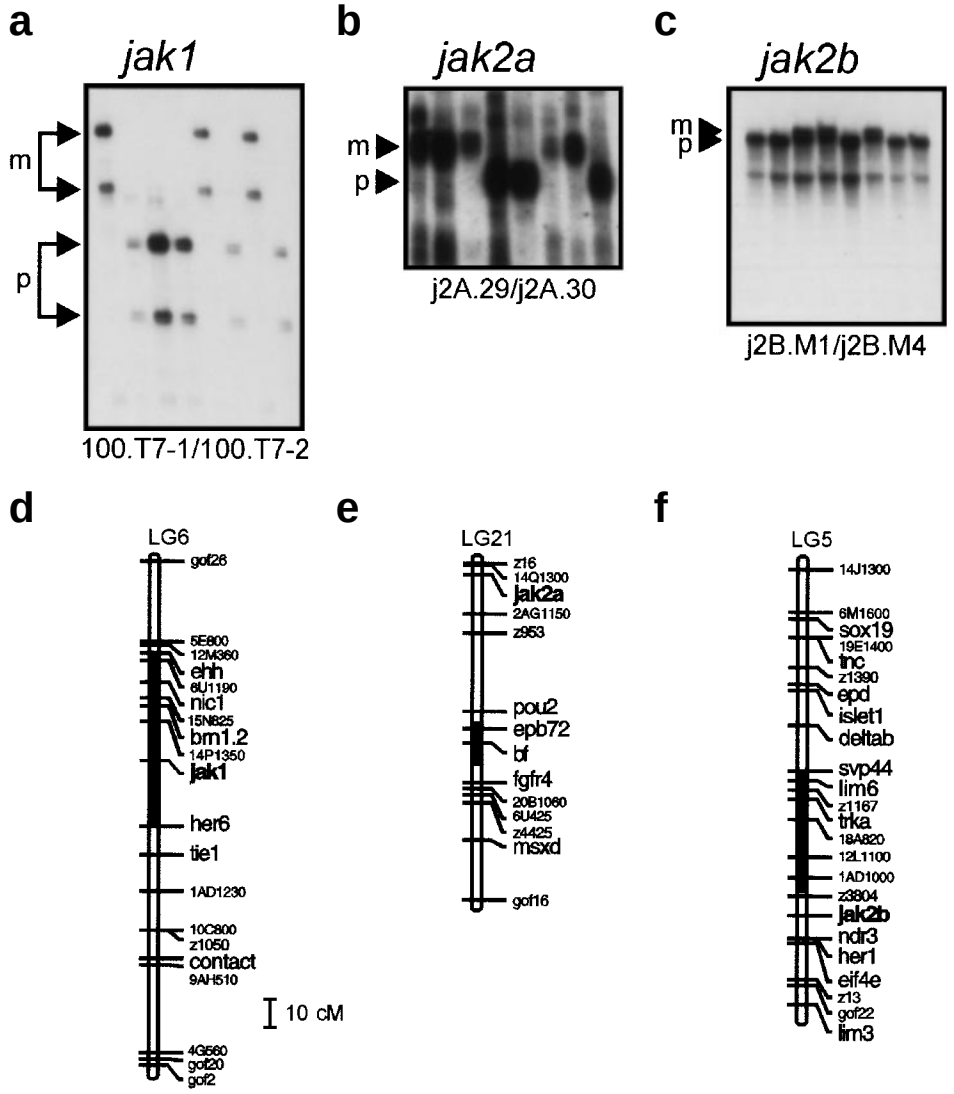


Fig 1 (Cont'd).

than is jak2a (75% identity). However, when nucleotide similarity is assessed across these domains, the zebrafish genes are more similar to each other (72% identity) than either is to any of the mammalian genes (*jak2a v* mammalian, 67% identity; *jak2b v* mammalian, 70% identity). Furthermore, there are 27 amino acid positions at which the zebrafish jak2 proteins are identical to each other but different from the mammalian sequence, suggesting the existence of a fish-specific substitution. A striking feature of the *jak2* paralogs is their extensive divergence, suggesting that the time since paralog duplication is of similar magnitude to that since divergence of the zebrafish and mammalian lineages. This is reflected in the short distances between the nodes leading to the divergence of the zebrafish and mammalian JAK2 sequences in Fig 1c.

Genetic mapping of the jak *genes.* Knowledge of the genetic position of a gene is important in assessing potential



g

LG 5	jak2b		tnc	deltab	trka	her1	lim3
LG 21	jak2a					epb72	bf
Hsa 9	JAK2	NOTCH1	TNC		NTRK2	LHX3	EPB72
Hsa 6		NOTCH4	TNX		NTRK4		BF
Mmu 17		Notch4	Tnx	Dll1	Ntrk1		H2-Bf

h

LG 6	jak1				her6		nic1	tie1 contact
Hsa 1	JAK1	NOTCH2	TNR		NTRK1			TIE1
Mmu 4	Jak1		Tnc		Hes3			Tie1
Mmu 2		Notch1				Lhx3	Epb7.2	Acra Gdf5

Fig 2.

linkage to a particular mutation and the identification of candidate genes from the collection of hematopoietic mutants. To generate polymorphic markers for each of the *jak* genes for genetic mapping, genomic DNA or cDNA sequence was used to design PCR primers for use in single-stranded conformational polymorphism (SSCP) and simple sequence-length polymorphism (SSLP) assays.

The C32xSJD mapping panel[53] was typed with a polymorphic marker for each gene. Using the segregation of the 100.T7-1/100.T7-2 SSLP polymorphism derived from physically linked genomic DNA in 72 meioses (Fig 2a), *jak1* was mapped to linkage group 6 (LG6) between 14P.1350.c and *her6* (Fig 2d). *jak2a* was mapped to distal LG21, 1.9 cM proximal to z16/14Q.1300.c (Fig 2e), on the basis of the segregation of the 3′ UTR-derived j2A.29/j2A.03 SSCP polymorphism in 81 meioses (Fig 2b). Segregation of an SSCP polymorphism derived from the 3′ UTR of *jak 2B* (j2B.M1/j2B.M4) in 48 meioses (Fig 2c) was used to place this gene on LG5 approximately equidistant between the z3804 and *her1*/z4299 markers (Fig 2f). The chromosome segments on which the *jak* genes are found were aligned with their counterparts from mouse and human, showing extensive conservation of synteny (Fig 2g and h).

Expression of jak2a *in the developing zebrafish.* Analysis of the developmental expression pattern of *jak2a* indicates that it may play a role in hematopoiesis. *jak2a* transcripts were first detected at a low level throughout the embryo at 10 hpf (Fig 3a), persisting until 14 hpf. During this period, the intensity of signal increased in the anterior part of the axis, eventually being strongest in the eyes. By 14 hpf (Fig 3b and c), cells of the medial lateral plate mesoderm expressed *jak2a* in a pattern consistent with the sites of earliest hematopoietic activity.[65] Cells in this region have earlier expressed *scl*[66] and *gata1* and *gata2* at 11 hpf.[67] Costaining of *jak2a* with *gata1* at 14 hpf and thereafter showed that both genes were expressed in identical regions of the lateral plate mesoderm (data not shown). This suggests that the first cells of the primitive wave of erythropoiesis express *jak2a* and that this expression is a later event in the commitment to this lineage than *gata1* expression. Cells in this region maintained *jak2a* expression as they moved from a lateral position to the midline and differentiated in the intermediate cell mass (ICM; Fig 3d), consistent with *jak2a* expression in proliferating proerythroblasts. By 24 hpf, high level staining was restricted to cells of the anterior ICM, although a low level of expression was detected in the brain and eyes (Fig 3e). The distribution of *jak2a* transcript at this stage differs from the hematopoietic expression of the vascular and stem cell marker *scl* in 2 important respects. As shown in Fig 3f, with white arrowheads, cells in a set of bilateral stripes located more rostrally than the ICM are *scl*-positive and thought to be persistent hematopoietic progenitor cells[66]; however, these cells did not express *jak2a*. Furthermore, although both *scl* and *jak2a* transcripts were detected at high levels in the anterior ICM, only *scl* expression is detected in the posterior ICM (solid arrowhead, Fig 3f). In both of these aspects, the expression of *jak2a* resembles that of *gata1*.[67] Because *scl* is expressed in both vascular and hematopoietic precursors,[66] we wished to establish unambiguously the identity of the cells that expressed *jak2a* message. Sectioning of embryos immediately postcirculation showed that *jak2a* expression was confined to cells contained within the vasculature with a large, rounded morphology (Fig 4). These cells also express *gata1* and hemoglobin (data not shown, see Detrich et al[67]) and the embryonic *globin* genes (see Fig 6, below), consistent with an erythroblast identity.

The primitive wave of hematopoiesis consists mainly of erythrocytes. *jak2a* transcripts were detected in maturing erythrocytes until 36 hpf (Fig 3g). Thereafter, *jak2a* expression in circulating cells decreased rapidly until 2.5 days postfertilization (dpf), at which time *jak2a* was not present in circulating blood or in any suspected hematopoietic site (Fig 3h). During the next 4 days of larval development, *jak2a* remained undetectable in blood cells, although low-level expression persisted in the dorsal midbrain, eyes, elements of the jaw, and fin buds (Fig 3h and i). At 8 days, *jak2a* expression was detected in the pronephros and in blood cells found lodged in the ventral tail veins (Fig 3j). Expression in the site of adult hematopoiesis, the pronephros,[65] and in circulating erythrocytes indicates that definitive erythropoiesis gives rise to cells that express *jak2a*.

Fig 2. Genetic mapping of the *jak1*, *jak2a*, and *jak2b* genes of zebrafish. (a) Segregation of the 100.T7-1/100.T7-2 SSLP *jak1* polymorphism in the C32xSJD cross. The C32xSJD mapping cross was typed for a polymorphic marker derived from genomic DNA associated with the *jak1* gene. PCR products amplified from genomic DNA of the haploid embryos of the C32xSJD mapping panel using the 100.T7-1/100.T7-2 primer pair were analyzed for length differences by denaturing polyacrylamide gel electrophoresis and 8 representative lanes are shown. The size variants of the products were assigned to the maternal (M) or paternal (P) genome at random, and segregation in the panel was scored. (b) Segregation of the j2A.29/j2A.30 SSLP *jak2a* polymorphism in the C32xSJD cross. PCR products amplified from genomic DNA of the haploid embryos of the C32xSJD mapping panel using the j2A.29/j2A.30 primer pair derived from the *jak2a* cDNA 3′ UTR were analyzed for length differences as described above and 8 representative lanes are shown. The size variants of the products were assigned to the maternal (M) or paternal (P) genome at random, and segregation in the panel was scored. (c) Segregation of the j2B.M1/j2B.M4 SSCP *jak2b* polymorphism in the C32xSJD cross. PCR products from genomic DNA of the haploid embryos of the C32xSJD mapping panel using the j2B.M1/j2B.M4 primer pair derived from the *jak2b* cDNA 3′ UTR were analyzed for sequence differences by nondenaturing polyacrylamide gel electrophoresis and 8 representative lanes are shown. The size variants of the products were assigned to the maternal (M) or paternal (P) genome at random, and segregation in the panel was scored. (d) Genetic map position of the *jak1* gene. Analysis of the segregation of the *jak1*-associated marker in 72 meiotic events of the C32xSJD mapping panel using the MapMaker and mapmanager programs[59,60] and further manual refinement places the zebrafish *jak1* locus on LG6. (e) Genetic map position of the *jak2a* gene. Analysis of the segregation of a *jak2a*-associated marker in 81 meiotic events of the C32xSJD mapping panel as described above places the zebrafish *jak2a* locus on LG21. (f) Genetic map position of the *jak2b* gene. Analysis of the segregation of a *jak2b*-associated marker in 48 meiotic events as described above places the zebrafish *jak2b* locus on LG5. (g and h) Synteny of the *JAK* family loci between the zebrafish, mouse, and human. A schematic diagram of the syntenic relationship between segments of the chromosomes of zebrafish (LG5, 6, and 21), human (Hsa 1, 9, and 6), and mouse (Mmu 2, 4, and 17) that contain members of the *JAK* gene family. The syntenic segments containing *JAK2* homologs are indicated in (a) and those containing *JAK1* homologs in (b). Note that genes have been illustrated in the same relative positions on syntenic chromosomes; however, in situ, local gene order may vary between chromosomes. The diagram is not to scale.

Although *rag1* expression in the thymus[68,69] was clearly detected by 5 dpf, *jak2a* was not expressed in the thymus (data not shown).

Thus, the timing and localization of *jak2a* expression in hematopoietic cells suggests that it may be involved in the transduction of signals into committed erythroblasts of both primitive and definitive lineages. Expression of *jak2a* in the brain and eyes suggests that intracellular signaling in these locations may also use the jak2a protein; however, the precise location of jak2a transcript in these structures was not determined.

Expression of jak2b *in the developing zebrafish.* Northern blot analysis using total RNA indicated that *jak2b* was expressed at a very low level during embryogenesis (data not shown). Whole mount in situ hybridization demonstrated that expression at 24 hpf was restricted to the lens and the nephritic duct (Fig 5a and b), persisting until 48 hpf (Fig 5c). The rostral extent of staining of *jak2b* in the nephritic duct (Fig 5b) is equivalent to that seen at 24 hpf with a probe to the *ret* gene.[70] Low-level expression of *jak2b* was seen in the fin buds in embryos at 2.5 dpf, coincident with *jak2a* expression, but by 3.5 dpf, no *jak2b* transcript was detectable by this method (data not shown). At 5 dpf, low-level *jak2b* expression was seen in the gill arches, elements of the jaw, and the anterior and posterior lateral line, persisting until 8 dpf (Fig 5d). Thus, based on thedistribution of transcript, *jak2b* might play a role in signaling during embryonic lens and nephritic duct development and in signaling in the larval lateral line and gills, but not in the development of the hematopoietic system.

Analysis of jak2a *expression in the zebrafish hematopoietic mutants.* Mutations that disrupt hematopoiesis have been identified in zebrafish.[36,37,47,67] The majority of these mutations were discovered by screening for the presence and color of circulating erythroblasts; hence, the majority represents genes required in erythropoiesis. Because the screens were performed at developmental stages up to 5 dpf, it seems likely that the target of the screen was erythroblasts of the primitive cohort.[37] Examination of mutant phenotypes allows mutant genes to be categorized into a scheme of erythroblast development as outlined by Orkin and Zon.[71] Embryos from selected mutant lines were examined for perturbation of *jak2a* expression by in situ hybridization at various time points before and/or after the onset of a visible phenotype. A summary of the results of this investigation is presented in Table 1, and the results are described below in detail.

Expression of jak2a *in a Hemangioblast mutant,* cloche. Homozygous *cloche* (*clo*) animals fail to produce blood or vasculature and die as embryos.[47,66,72,73] Consistent with a general failure in *clo* mutants to produce cells of the hematopoietic lineage, *jak2a* expression in the hematopoietic lateral plate mesoderm was not initiated at 13 hpf in approximately one quarter of the embryos from a given clutch and neither was it detected in hematopoietic tissue at any stage examined thereafter (Table 1), although *jak2a* central nervous system (CNS) expression appears normal. The expression of *jak2a* in the *clo* mutant background was compared with the expression of other lineage and stage specific markers at 24 hpf (Fig 6). Clutches of embryos from heterozygous incrosses were examined before the onset of circulation by in situ hybridization for expression of the stem cell marker *scl*,[66] the immature erythroblast marker *gata1*,[67] *jak2a*, and the embryonic α and β globins *αe1 globin* and *βe3 globin*, markers of primitive erythrocytic differentiation (Brownlie et al, manuscript in preparation). Three quarters of the embryos in a given clutch showed strong signal in the ICM from all gene probes (Fig 6a, c, e, g, and i). One quarter of embryos in a given clutch displayed a distinctive phenotype involving the dramatic reduction of signal from the ICM (Fig 6b, d, f, h, and j). In approximately 50% of the embryos that displayed a loss of expression in the ICM, persistent expression of *scl*, *gata1*, *αe1 globin*, and *βe3 globin* could be seen in 5 to 10 cells in the caudal part of the anterior ICM that appear to escape the $clo^{-/-}$ hematopoietic block (Fig 6b, d, h, and j; see Stainier et al[47]). However, ICM expression of *jak2a* was not observed in any embryo that also lacked anterior ICM expression ($n = 53$, 4 independent experiments).

Expression of jak2a *in an early onset hematopoietic mutant,* spadetail. In contrast to *clo*, embryos homozygous for the *spadetail* (*spt*) mutation specify and differentiate vasculature correctly.[73] However, the hematopoietic program is severely affected, with few, if any primitive erythroblasts reaching maturity. Clutches of embryos from *spt* heterozygous parents were examined for *jak2a* expression throughout the first 24 hours of development (Fig 7). Despite the expression of the stem cell marker *scl* in *spt* mutants in the lateral plate mesoderm at 14 somites (Fig 7d), and in contrast to wild-type embryos at this stage (Fig 7a), *jak2a* transcripts could not be detected in the *spt* homozygotes in regions of hematopoietic activity (Fig 7c). As *spt* homozygotes developed, *scl* expression in the lateral plate decreased dramatically, indicating a failure to maintain a population of early stem cells (Fig 7h). Occasionally in *spt* homozygotes, isolated *scl*, *gata1*, and embryonic *globin*-positive cells were visible in the ICM, as shown by arrowheads in Fig 7h (and data not shown, see Thompson et al[73]), indicating the emergence of a more mature primitive erythroid cell. However, *jak2a* message does not accumulate in the corresponding locations or in any hematopoietic tissue at the stages examined (Fig 7g). Combined with the data from *clo* mutants, this result indicates that, within the boundaries of the sensitivity of the technique, the expression of erythroid markers in the escaper cells of the caudal part of the anterior ICM is not accompanied by *jak2a* expression.

Expression of jak2a *in late onset hematopoietic mutant zebrafish.* Expression of *jak2a* was examined by in situ hybridization in zebrafish embryos carrying mutations in the *frascati* (*frs*), *chablis* (*cha*), *retsina* (*ret*), *weißherbst* (*weh*), *cabernet* (*cab*), *sauternes* (*sau*), *chardonnay* (*cdy*), and *chianti* (*cia*) genes, which display a late phenotypic onset.[36] In all mutants examined, *jak2a* expression was found in the cells of the ICM at 24 hpf and in circulating erythroblasts until 48 hpf, as seen in wild-type clutches (Table 1). In summary, *jak2a* expression is perturbed only in those mutants (*clo*, *spt*) that perturb erythropoiesis at early stages in development, supporting the hypothesis that *jak2a* is expressed in immature primitive erythroblasts in the zebrafish.

Linkage of the jak2a *gene to the hematopoietic mutants.* As described above, *jak2a* was mapped to the distal tip of LG21 (Fig 2b and e). Comparison of the map position of *jak2a* with those of the mutants with known positions on the zebrafish

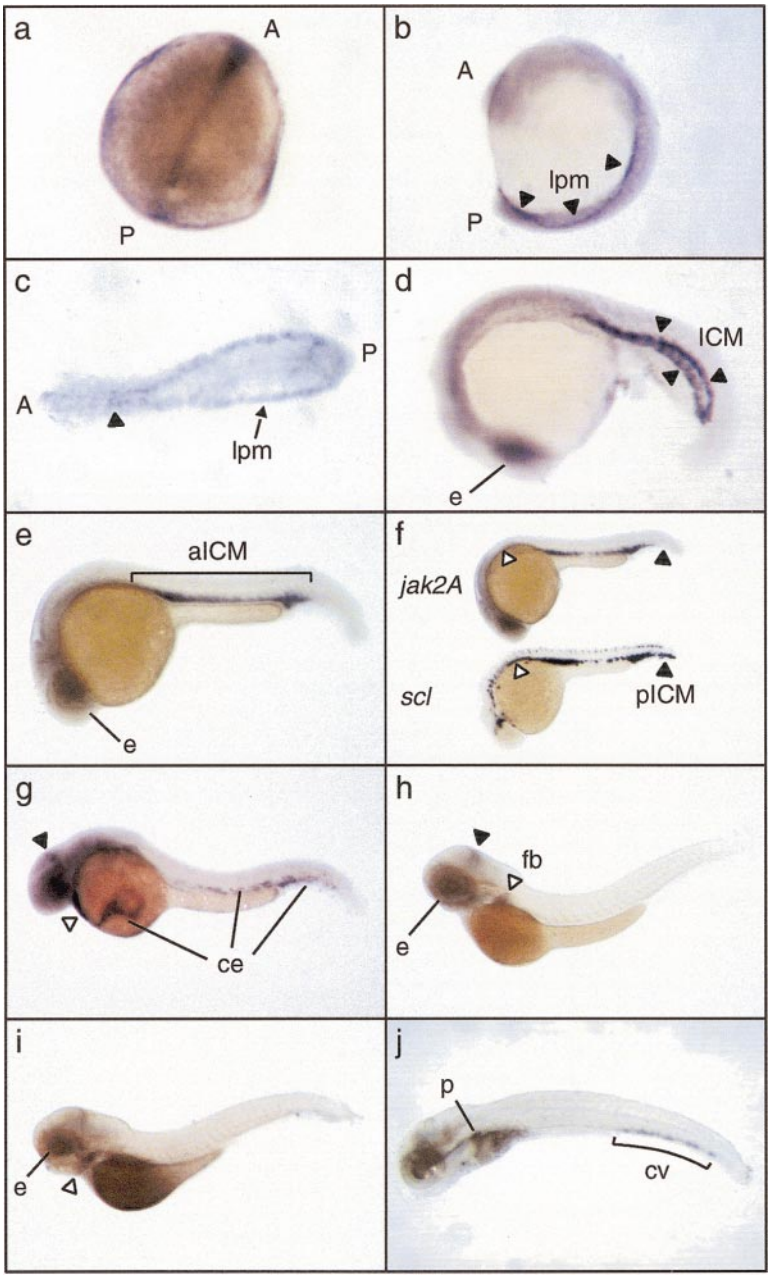


Fig 3. ***jak2a*** **expression in the developing zebrafish. The expression of** ***jak2a*** **in embryos at various developmental stages was examined by whole mount in situ hybridization. (a) 10 hpf embryo, dorsal view with anterior to the top.** ***jak2a*** **riboprobe gives a widespread, low-level signal. Elevated expression is apparent in the dorsal axis, but this corresponds to the thickest region of the embryo and does not reflect an increase in the density of transcript. (b) 14 hpf embryo, lateral view with anterior to the top and left. Arrowheads indicate the** ***jak2a*****-riboprobe labeled line of cells in the medial lateral plate mesoderm that gives rise to primitive blood lineages. (c) 14 hpf dorsal view of dissection of dorsal and posterior axial structures. Anterior (A) is to the left and posterior (P) is to the right.** ***jak2a*** **transcript is evident in a narrow ribbon of cells at the medial edge of the lateral plate mesoderm (arrow) that extend to a distinct anterior limit (arrowhead). (d) 20 hpf embryos, lateral view.** ***jak2a*** **riboprobe signal is detected at high level in the medially converging cells of the ICM (arrowheads) and in the eye. A lower level of signal is seen in the remainder of the anterior CNS, but not in the trunk or posterior body. (e) 24 hpf embryo, lateral view showing the labeling of the mature ICM (bracket) and in the eye and anterior CNS at a lower level by** ***jak2a*** **probe. (f) 24 hpf embryos, lateral view showing a comparison of** ***jak2a*** **with** ***scl*** **staining. Both** ***jak2a*** **and** ***scl*** **probes label the anterior ICM (see [e] for reference); however,** ***scl*** **is also detected in a dorso-anterior pair of bilateral stripes (open arrowhead) and in the posterior ICM (solid arrowhead), whereas** ***jak2a*** **is not. Expression of** ***scl*** **is also seen in cells of the CNS. (g) 36 hpf embryo, lateral view showing detection of** ***jak2a*** **message in circulating primitive erythrocytes (ce), cells can be detected in the axial vessels of the trunk and tail, on the yolk sac, and in the heart (open arrowhead). Elevated signal is also seen in the eyes and in the midbrain (solid arrowhead). (h) 2.5 dpf embryo, lateral view showing detection of** ***jak2a*** **transcript in the finbud (open arrowhead), the midbrain (solid arrowhead), and the eye. Note that, in contrast to (g), there is no** ***jak2a*** **signal from circulating blood. (i) 3.5 dpf larva, lateral view showing** ***jak2a*** **message restricted to the eye and elements of the pharyngeal arches (arrowhead). (j) 8 dpf larva, lateral view.** ***jak2a*** **transcript can be detected in the pronephros (arrow) and in circulating cells lodged in the vasculature of the tail (bracket). A, anterior; aICM, anterior intermediate cell mass; ce, circulating erythroblasts; cv, caudal vascular plexus; e, eye; fb, fin bud; ICM, intermediate cell mass; lpm, lateral plate mesoderm; P, posterior; p, pronephros; pICM, posterior intermediate cell mass.**

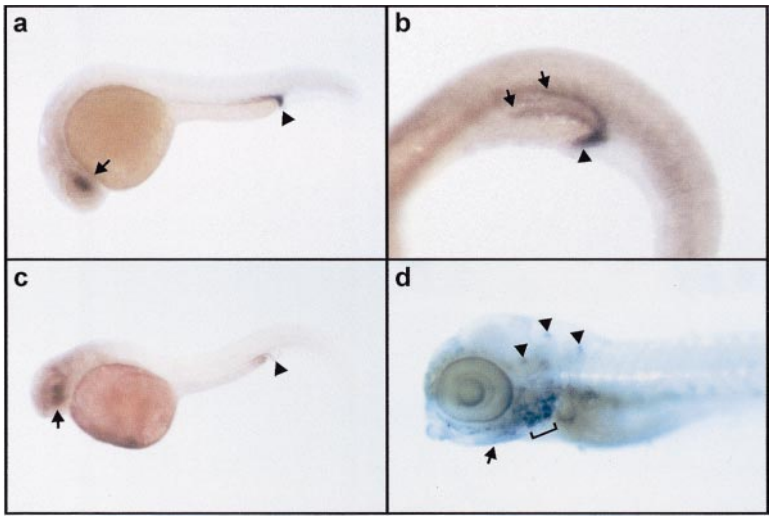


Fig 5. ***jak2b*** **expression in the developing zebrafish. The expression of** ***jak2b*** **in embryos at various developmental stages was examined by whole mount in situ hybridization. All embryos and larvae are shown with anterior to the left and dorsal to the top of the frame. (a) 24 hpf embryo, lateral view showing detection of** ***jak2b*** **message restricted to the lens of the eye (arrow) and in the nephritic ducts (arrowhead). (b) 24 hpf embryo, ventro-lateral view at higher magnification, showing the region of the nephritic duct stained by the** ***jak2b*** **riboprobe. The bilaterally symmetric nephritic ducts are visible (arrows) as is the distal tip of the ducts at the proctodeum (arrowhead). (c) 48 hpf embryo, lateral view showing expression of** ***jak2b*** **persisting in the lens of the eye (arrow) and decreasing from earlier levels in the nephritic ducts (arrowhead). (d) 8 dpf larva, lateral view at higher magnification, indicating detection of a low level of** ***jak2b*** **message in elements of the jaw (arrow), in the developing gills (bracket), and in the anterior lateral line (arrowheads). Expression of** ***jak2b*** **in the posterior lateral line is evident, but is not shown in this figure.**

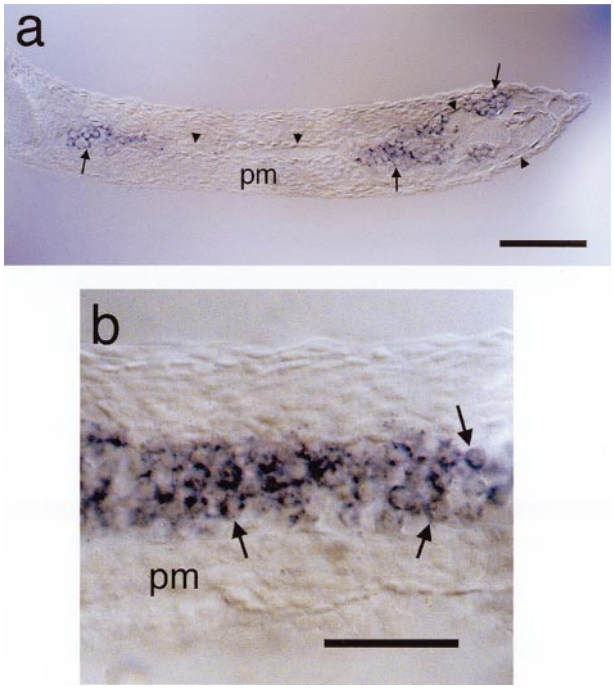


Fig 4. Expression of *jak2a* in circulating erythroblasts. The expression of *jak2a* in circulating cells of the primitive wave of hematopoiesis was examined in thin sections of animals after the onset of circulation (26 hpf) after whole mount in situ hybridization. Preparations are oriented with anterior to the left. (a) Transverse section of trunk and tail at the level of the dorsal aorta. Arrowheads demarcate the extent of the vasculature, indicating the anterior dorsal aorta and the posterior vascular sinus. *jak2a*-positive cells are confined within the vasculature (arrows). (b) Higher magnification of transverse section of the caudal vein showing large, rounded *jak2a*-positive cells (arrows) within the vasculature. pm, paraxial mesoderm. Scale bars: for (a), 100 μm; for (b), 50 μm.

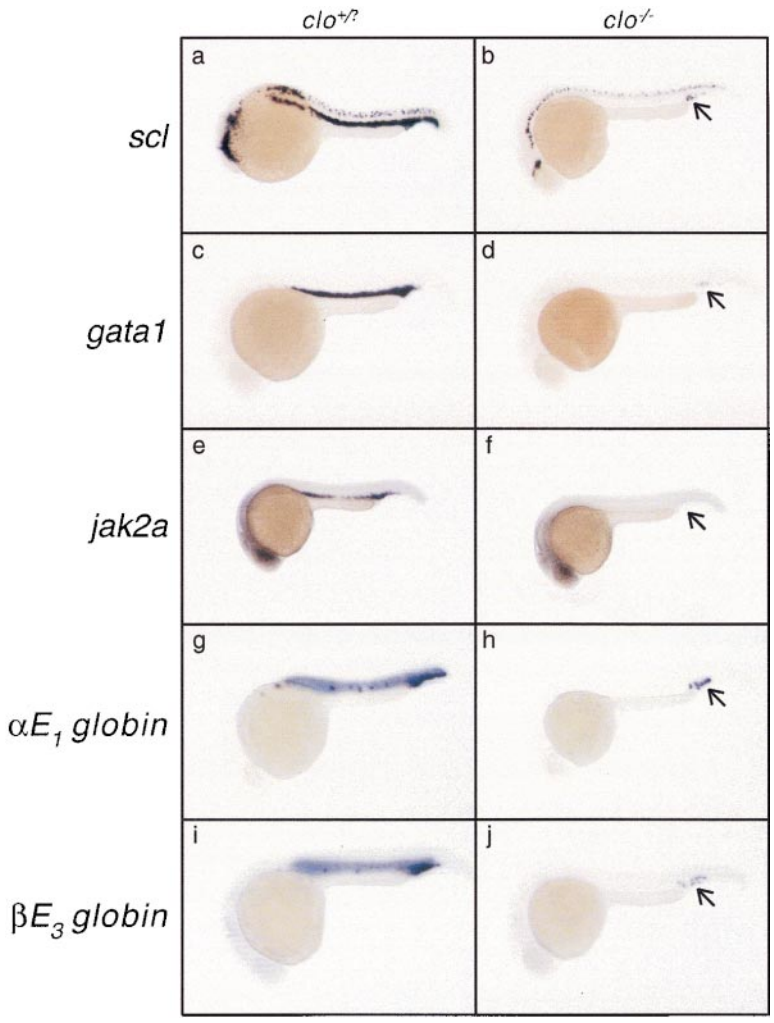


Fig 6. Comparison of *scl*, *gata1*, *jak2a*, αe_1 *globin*, and βe_3 *globin* expression in *cloche* mutant embryos. Clutches of embryos derived from heterozygous *clo* parents were raised for 24 hours and assayed for the expression of various hematopoietic and erythroid marker genes: *scl* (a and b); *gata1* (c and d); *jak2a* (e and f); $\alpha e1$ *globin* (g and h); and $\beta e3$ *globin* (i and j). Embryos are displayed in a lateral position with anterior to the left and dorsal to the top of each panel. Approximately three quarters of the embryos in a given clutch had the wild-type expression pattern of the gene in question (a, c, e, g, and i); note the prominent staining of the ICM. Approximately one quarter of the embryos in a given clutch showed a near or total absence of all hematopoietic marker gene expression in the ICM (b, d, f, h, and j); presumably, these are *clo* homozygotes. In approximately one half of mutant embryos, from 5 to 10 cells in the ICM express the *scl*, *gata1*, $\alpha e1$ *globin*, and $\beta e3$ *globin* marker genes (arrow in b, d, h, and j). No *jak2a* expression was observed in this area in any mutant embryo (f), ie, an embryo also lacking *jak2a* expression in the rostral part of the anterior ICM.

Table 1. Expression of *jak2a* in Hematopoietic Mutants

Mutant*	Allele†	Age of Onset‡	Stages Examined	*jak2a* Expression§
cloche (clo)	*m39*	12 hpf	13, 24, 48 hpf	Absent
spadetail (spt)	*b104*	12 hpf	14, 19, 24 hpf	Absent
frascati (frs)	*tm130d, tq223*	2-4 dpf	24, 36, 48 hpf, 3.5 dpf	Normal
chablis (cha)	*tu245/tu242e*	2-3 dpf	24, 36 hpf	Normal
retsina (ret)	*tr217*	3-4 dpf	18, 24, 36, 48 hpf	Normal
cabernet (cab)	*tl236*	4 dpf	24, 28 hpf	Normal
sauternes (sau)	*ty121*	2 dpf	24, 26, 32, 36 hpf	Normal
wießherbst (weh)	*th238*	2 dpf	24 hpf	Normal
chardonnay (cdy)	*te216*	2 dpf	24, 36 hpf	Normal
chianti (cia)	*tu25f*	3 dpf	24, 36, 48 hpf	Normal

*The hematopoietic phenotype of the mutants examined here are described in Ransom et al,[36] with the exception of *sau*,[38] *clo*,[47,66,72,73] and *spt*.[73]

†The strongest allele was chosen for analysis of *weh* and *ret*, whereas the weaker allele of *sau* was used. The *cha* alleles *tu245* and *tu242e* are believed to be clonal, and the *frs* alleles *tm130d* and *tq223* were tested both as homozygotes and trans-heterozygotes without observable differences.

‡The time in development when a clutch can be unambiguously scored as containing homozygous mutant embryos, taken from Ransom et al[36] with the exception of *clo* and *spt* (see * above).

§Clutches of embryos from mutant incrosses were scored for disruption of *jak2a* expression by comparison with wild-type embryos of the same developmental stages. Absent indicates that there was no *jak2a* expression in any hematopoietic tissue at the stages examined. Normal indicates that there was no variation within a clutch or when compared with wild-type clutches.

genetic map showed that *jak2a* was not linked to *cha*, *cdy*, *chi*, *clo*, *frs*, *gre*, *mon*, *pin*, *ris*, *ret*, *sau*, *spt*, or *weh* (data not shown). Thus, *jak2a* is not a candidate gene for any of these mutations. Linkage to additional, currently unmapped hematopoietic mutants *cab* and *mot* was tested by typing genomic DNA from mutant embryos with the *jak2a*-associated marker used to map the *jak2a* gene, as described above. The *jak2a* polymorphism did not segregate with either the cab^{tl236} or mot^{tm303c} phenotype (data not shown), indicating that *jak2a* is not linked to these mutations.

DISCUSSION

Recent studies in *Drosophila* and mouse have shown a role for members of the *JAK* gene family in the control of growth

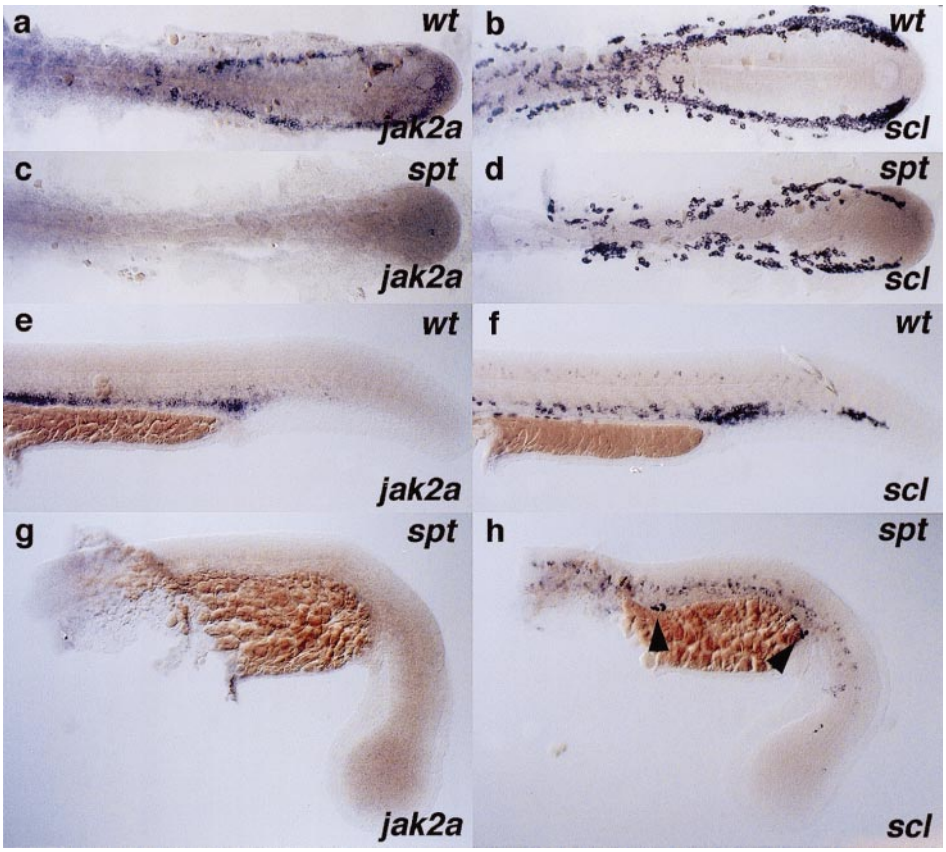


Fig 7. Expression of *jak2a* is perturbed in embryos with mutation in the *spadetail* gene. Clutches of embryos derived from heterozygous *spt* parents were raised for 14 and 24 hours and assayed for the expression of stem cell and erythroid marker genes *scl* (b, d, f, and h) and *jak2a* (a, c, e, and g), respectively. The *spt* mutant can be unambiguously scored at the developmental stages presented here by the loss of trunk somites; embryos shown in (c), (d), (g), and (h) are *spt* homozygotes. Note the loss of *jak2a* staining at all stages in the *spt* mutant embryos. Embryos in (a) through (d) are dissected and flat mounted, with the anterior to the left; all other embryos (e) through (h) are displayed in a lateral position with anterior to the left and dorsal to the top of each panel.

and differentiation of multiple blood cell lineages and in leukemogenesis. The *Jak2* gene is required in mice for successful erythropoiesis and *JAK2* is implicated in ALL in human patients. We show here that the zebrafish has 2 *jak2* genes that are expressed in the developing embryo and larva; however, only 1, *jak2a*, is expressed in the erythropoietic system. Our results in wild-type and mutant embryos suggest that *jak2a* may play an early role in primitive erythropoiesis in the zebrafish and, thus, is likely to represent the functional homolog of the mouse *Jak2* gene with respect to hematopoiesis.

Hematological implications of zebrafish jak2a *expression.* The timing of *jak2a* expression in wild-type embryos suggests that the presence of *jak2a* message defines an intermediate stage in the lineage of the primitive erythrocyte that occurs between the commitment of progenitors and the expression of the end-differentiated phenotype. Furthermore, the transience of *jak2a* expression indicates that any signaling into primitive erythroblasts via receptors that use jak2a occurs in a window of time as the erythroblasts mature from 14 hpf to approximately 2 dpf. Expression of *jak2a* in the developing erythrocytes of wild-type and mutant zebrafish has strong implications for the involvement of jak2a in cytokine signaling in hematopoiesis.

Analysis of *jak2a* expression in clo^{m39} and spt^{b104} homozygotes showed that *jak2a* transcription is not initiated at the normal time, and neither is it present in hematopoietic tissue at subsequent stages. This result is consistent with *jak2a* expression in cells of the hematopoietic lineage and allows inferences about *jak2a* function to be made. In some *clo* mutant embryos, a restricted number of primitive erythrocytes (5 to 10) form in a remnant tail vasculature; these cells are heme-reactive, indicating that a terminally differentiated state has been reached. Likewise, in *spt* mutants, isolated mature primitive erythroblasts can be detected. In contrast to wild-type erythroblasts, *jak2a* is not coexpressed with *gata1* or the embryonic *globins* in these cells (Figs 6 and 7), being completely absent from the ICM in every embryo examined, indicating that *jak2a* expression is not required for the completion of differentiation to a globin/heme-expressing stage in these cells. It may instead be required for an aspect of survival or proliferation, as is the case in the mouse.[12,13,22,23,74] Because *jak2b* is not expressed in erythropoietic tissue, cells without jak2a are likely to be without JAK2 function. Thus, the surviving primitive erythrocytes of *clo* and *spt* mutant embryos would not be expected to be receptive to cytokine or other signals that require jak2. Because it is presently unclear whether these cells represent a normal stage of erythropoiesis that has been unmasked by the absence of *clo* or *spt* or whether these cells are a peculiarity of *clo* and *spt* mutant embryos, we are unable to make strong conclusions about the requirement for jak2a function in the differentiation of wild-type erythrocytes.

Examination of *jak2a* expression in embryos from late onset hematopoietic mutants showed that the genetic deficiencies in these fish did not disrupt erythropoietic development before the putative cytokine-receptive stage defined by expression of *jak2a*. This result is not surprising, because their cell morphology, by analogy to the mouse, suggests a mature post–EPO-dependent stage.[71] The correlation of a failure to generate or maintain blood cell number with absence or loss of *jak2a* expression in mutants is consistent with a potential role for this gene in the proliferation or survival of polychromatic erythroblasts.

It is of interest to compare these results with recent findings obtained from gene targeting experiments in the mouse. Mice lacking functional *Jak2* exhibit significant defects in primitive and definitive erythropoiesis, although their development is otherwise overtly normal.[22,23] This is consistent with the high-level expression of *jak2a* seen in the erythroid cells of the zebrafish embryo and larva. The erythropenic phenotype of the $Jak2^{-/-}$ mice is more severe than mice carrying an *Epo* or *EpoR* null mutation,[75,76] with fewer circulating yolk sac-derived primitive erythrocytes and an earlier block in the progression of definitive erythropoiesis. Committed erythroblasts are present in these animals, but they do not expand and differentiate. Hemoglobinization of definitive erythroid cells is almost abolished in $Jak2^{-/-}$ fetal liver cells,[23] but the expression of embryonic globins specific for the primitive cohort appears less affected.[22] This phenotype is consistent with the expression and regulation of *jak2a* in wild-type and mutant zebrafish presented here; thus, the *jak2a* gene of zebrafish likely represents the functional homolog of the mouse *Jak2* gene with respect to its role in hematopoiesis.

Evolutionary relationships among the jak *genes.* The presence of 4 *JAK* genes per mammalian genome fits well with current theories about tetraploidization events early in the vertebrate lineage that suggest 2 successive duplications giving rise to 4 copies of an ancestral chromosome complement.[77] Genes of the *JAK* family in zebrafish map to separate chromosomes, indicating that tandem duplication is not the cause of the extra *jak2* genes in the zebrafish. Instead, they map to regions in which syntenies are conserved compared with their homologs in mouse and human, a region known as the Katsanis paralogy group.[78] This finding extends the observation that large portions of the chromosomes of early vertebrates remain intact, with disturbance mainly from local rearrangement,[59,79] and indicates that the cause of the initial *JAK2* duplication seems to have been a large-scale event, possibly involving 1 or more chromosomes (Fig 2g and h). If the paralogous duplication took place before the lineage of ray fin and lobe fin fish (ie, tetrapods) diverged, there must have existed a second *JAK2* paralog in the genome of both lineages. In this case, there may still be a second *JAK2* in existing tetrapod genomes. However, examination of mammalian *JAK2* cDNA sequences and ESTs in the databases indicates that all JAK2 proteins from different mammals reported are more than 95% identical to each other and that all cDNAs or ESTs from any given species are, in fact, from the same gene (data not shown). Consideration of *JAK1*, *JAK3*, and *TYK2* database entries in the same manner indicates that all listed sequences, ignoring splice variants, are orthologous or identical. In conclusion, mounting evidence of the existence of higher numbers of gene family members in zebrafish and other ray finned fish than in mammalian genomes[80,81] combined with the chromosomal localization data presented above favors the scenario in which duplicate *jak2* genes are an innovation specific to ray finned fish.

Comparison and implications of jak2 *expression patterns in the zebrafish.* The *jak* genes of the zebrafish are expressed at a high level in restricted groups of cells in the developing embryo and larva. Of particular interest is the divergence in expression

patterns of the *jak2a* and *jak2b* genes. The only site of coexpression during development was in the lens of the eye at 18 to 36 hpf (Figs 3e and g and 5a and c). Thus, the regulatory regions of the genes have diverged profoundly in activity, whereas the coding sequence has maintained a high conservation in sequence identity, consistent with the generation of genetic novelty by the alteration of gene expression patterns without radical changes to the biochemical activity of the protein product.[82]

Widespread expression of mammalian *JAK* mRNA detected in cell lines and adult tissues by Northern blotting[3,10,37,83-85] and the propensity of cytokine receptors to use multiple JAK proteins in signaling[1] suggested a near ubiquitous expression of these intracellular signaling components. In this view, developmental timing and positional cues would be supplied by the restricted expression of both extracellular signaling molecules and their cognate receptors. However, it is clear from this study that the expression of the appropriate signal transduction components could equally serve these timing and positional functions. Potentially, a closer examination of *JAK* expression in mammals may show a similar distribution and restriction of transcripts.

Prospects for genetic analysis of vertebrate JAK *function using the* jak2a *gene in zebrafish.* The ability to screen a vertebrate genome for mutations that modify *JAK* function, a task that would not be practical in the mouse, requires the identification or production of a mutation in a *JAK* gene. Linkage analysis presented above indicates that *jak2a* is not a candidate gene for the 17 hematopoietic mutants examined. The data on the timing of *jak2a* expression in wild-type and mutant zebrafish suggest that any role for *jak2* in erythropoiesis is confined to stages after the onset of *gata1* expression, implying possible *jak2a* function in cells equivalent to progenitor or proerythroblasts. This, along with the phenotype of a *Jak2*-deficient mouse, suggests that animals with a *jak2a* mutation would initially express markers for hematopoietic stem cells (HSCs) and progenitor cells such as *scl*, *lmo2*,[73] and *gata2* in an equivalent manner to wild-type.

Other hematopoietic mutants have not been tested for linkage with *jak2a*, namely, *bloodless*, *vlt*, *vmp*, *tbr*, *stb*, *paw*, and *clb*.[37,67] Of these, *bloodless*, *vlt*, and *vmp* show a hematopoietic phenotype that appears to be too early for the expected role of *jak2a*, whereas *tbr*, *stb*, *paw*, and *clb* appear to act too late. However, because these phenotypes are incompletely characterized, and it is not known whether any of these mutations is a complete loss of function in the gene in question, they cannot be ruled out as presenting potential *jak2a* mutant phenotypes. Because calculations presented at the conclusion of the large-scale screens indicate that approximately 50% saturation was approached,[86] a mutation in the *jak2a* gene may not have been isolated to date. Of course, it is formally possible that *jak2a* does not perform an essential function in the development of the erythrocyte lineage in the zebrafish.

An alternative strategy for the generation of a *JAK* phenotype would be to use 1 of the leukemogenic *JAK* alleles known from mammals and flies[29-33] to induce a neoplastic state in the zebrafish hematopoietic system. Screening the zebrafish genome for enhancer and suppressor loci of such a phenotype should yield information on genes controlling the initiation and progression of vertebrate hematopoietic neoplasia.

Given the parallels between mouse *Jak2* and zebrafish *jak2a*, it is interesting to speculate on the consequences of the potential subfunctionalization[87] of *jak2* in the zebrafish, a scenario for gene duplication in which complementary functions (eg, expression domains) can be lost by 2 gene duplicates, making both essential to survival. The expression of *jak2b* is not consistent with any role for this paralog in erythropoiesis; thus, *jak2a* appears likely to be the functional homolog of the mammalian *JAK2*. Mice that can be rescued from their requirement for *Jak2* function in erythropoiesis by reconstituting with wild-type hematopoietic stem cells may yield interesting additional phenotypes. One prediction of the studies presented here is that lens and kidneys of the rescued mice may show defects. To generalize from this case, the presence of extra gene copies in the zebrafish would not necessarily prove a hindrance to the analysis of gene function; rather, it may allow access to restricted or late onset phenotypes that might not be observable in the mouse.

In conclusion, the findings of this study underscore the potential use of the zebrafish to model the cytokine functions of mammals. Furthermore, the ability to search the genome of the zebrafish for loci that modify the severity of a hematopoietic phenotype would be invaluable in the analysis of the complex signaling events underlying the regulation of blood growth.

ACKNOWLEDGMENT

The authors thank Jana Stickland for her invaluable help with figures. Thanks are extended also to Cuong Do and to the members of the Growth Regulation and Cytokine Biology Laboratories for many discussions. Many thanks to the members of the Zon lab fish collective for providing mutant embryos and for their support and encouragement. Thanks to Ashley Bruce, Graham Lieschke, and Jensen Hjorth for constructive criticism of the manuscript and to Robert Ho, in whose lab this work was completed.

REFERENCES

1. Wilks AF, Harpur AG: Intracellular Signal Transduction: The JAK-STAT Pathway. Austin, TX, Landes, 1996

2. Darnell JE Jr: STATs and gene regulation. Science 277:1630, 1997

3. Harpur AG, Andres AC, Ziemiecki A, Aston RR, Wilks AF: JAK2, a third member of the JAK family of protein tyrosine kinases. Oncogene 7:1347, 1992

4. Tanner JW, Chen W, Young RL, Longmore GD, Shaw AS: The conserved box 1 motif of cytokine receptors is required for association with JAK kinases. J Biol Chem 270:6523, 1995

5. Frank SJ, Yi W, Zhao Y, Goldsmith JF, Gilliland G, Jiang J, Sakai I, Kraft AS: Regions of the JAK2 tyrosine kinase required for coupling to the growth hormone receptor. J Biol Chem 270:14776, 1995

6. Zhao Y, Wagner F, Frank SJ, Kraft AS: The amino-terminal portion of the JAK2 protein kinase is necessary for binding and phosphorylation of the granulocyte-macrophage colony-stimulating factor receptor beta c chain. J Biol Chem 270:13814, 1995

7. Chen M, Cheng A, Chen YQ, Hymel A, Hanson EP, Kimmel L, Minami Y, Taniguchi T, Changelian PS, O'Shea JJ: The amino terminus of JAK3 is necessary and sufficient for binding to the common gamma chain and confers the ability to transmit interleukin 2-mediated signals. Proc Natl Acad Sci USA 94:6910, 1997

8. Kohlhuber F, Rogers NC, Watling D, Feng J, Guschin D, Briscoe J, Witthuhn BA, Kotenko SV, Pestka S, Stark GR, Ihle JN, Kerr IM: A

JAK1/JAK2 chimera can sustain alpha and gamma interferon responses. Mol Cell Biol 17:695, 1997

9. Yan H, Piazza F, Krishnan K, Pine R, Krolewski JJ: Definition of the interferon-alpha receptor-binding domain on the TYK2 kinase. J Biol Chem 273:4046, 1998

10. Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A: Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol 11:2057, 1991

11. Velazquez L, Mogensen KE, Barbieri G, Fellous M, Uze G, Pellegrini S: Distinct domains of the protein tyrosine kinase tyk2 required for binding of interferon-alpha/beta and for signal transduction. J Biol Chem 270:3327, 1995

12. Gauzzi MC, Barbieri G, Richter MF, Uze G, Ling L, Fellous M, Pellegrini S: The amino-terminal region of Tyk2 sustains the level of interferon alpha receptor 1, a component of the interferon alpha/beta receptor. Proc Natl Acad Sci USA 94:11839, 1997

13. Zhuang H, Niu Z, He TC, Patel SV, Wojchowski DM: Erythropoietin-dependent inhibition of apoptosis is supported by carboxyl-truncated receptor forms and blocked by dominant-negative forms of Jak2. J Biol Chem 270:14500, 1995

14. Watanabe S, Itoh T, Arai K: JAK2 is essential for activation of c-fos and c-myc promoters and cell proliferation through the human granulocyte-macrophage colony-stimulating factor receptor in BA/F3 cells. J Biol Chem 271:12681, 1996

15. Shimoda K, Feng J, Murakami H, Nagata S, Watling D, Rogers NC, Stark GR, Kerr IM, Ihle JN: Jak1 plays an essential role for receptor phosphorylation and Stat activation in response to granulocyte colony-stimulating factor. Blood 90:597, 1997

16. Guschin D, Rogers N, Briscoe J, Witthuhn B, Watling D, Horn F, Pellegrini S, Yasukawa K, Heinrich P, Stark GR, Ihle JN, Kerr IM: A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. EMBO J 14:1421, 1995

17. Briscoe J, Rogers NC, Witthuhn BA, Watling D, Harpur AG, Wilks AF, Stark GR, Ihle JN, Kerr IM: Kinase-negative mutants of JAK1 can sustain interferon-gamma-inducible gene expression but not an antiviral state. EMBO J 15:799, 1996

18. Kawahara A, Minami Y, Miyazaki T, Ihle JN, Taniguchi T: Critical role of the interleukin 2 (IL-2) receptor gamma-chain-associated Jak3 in the IL-2-induced c-fos and c-myc, but not bcl-2, gene induction. Proc Natl Acad Sci USA 92:8724, 1995

19. Muller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbieri G, Witthuhn BA, Schindler C, Pellegrini S, Wilks AF, Ihle JN, Stark GR, Kerr IM: The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. Nature 366:129, 1993

20. Watling D, Guschin D, Muller M, Silvennoinen O, Witthuhn BA, Quelle FW, Rogers NC, Schindler C, Stark GR, Ihle JN, Kerr IM: Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-gamma signal transduction pathway. Nature 366:166, 1993

21. Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, King KL, Sheehan KC, Yin L, Pennica D, Johnson EM Jr, Schreiber RD: Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell 93:373, 1998

22. Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM, Grosveld G, Ihle JN: Jak2 is essential for signaling through a variety of cytokine receptors. Cell 93:385, 1998

23. Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K: Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell 93:397, 1998

24. Nosaka T, van Deursen JM, Tripp RA, Thierfelder WE, Witthuhn BA, McMickle AP, Doherty PC, Grosveld GC, Ihle JN: Defective lymphoid development in mice lacking Jak3. Science 270:800, 1995

25. Thomis DC, Gurniak CB, Tivol E, Sharpe AH, Berg LJ: Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science 270:794, 1995

26. Macchi P, Villa A, Gillani S, Sacco MG, Frattini A, Porta F, Ugazio AG, Johnston JA, Candotti F, O'Shea JJ, Vezzoni P, Notarangelo LD: Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 377:65, 1995

27. Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, Migone TS, Noguchi M, Markert ML, Buckley RH, O'Shea JJ, Leonard WJ: Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science 270:797, 1995

28. Hanratty WP, Dearolf CR: The Drosophila tumorous-lethal hematopoietic oncogene is a dominant mutation in the hopscotch locus. Mol Gen Genet 238:33, 1993

29. Harrison DA, Binari R, Nahreini TS, Gilman M, Perrimon N: Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. EMBO J 14:2857, 1995

30. Luo H, Hanratty WP, Dearolf CR: An amino acid substitution in the *Drosophila hop*$^{Tum-l}$ Jak kinase causes leukemia-like hematopoietic defects. EMBO J 14:1412, 1995

31. Luo H, Rose P, Barber D, Hanratty WP, Lee S, Roberts TM, D'Andrea AD, Dearolf CR: Mutation in the Jak kinase JH2 domain hyperactivates *Drosophila* and mammalian Jak-Stat pathways. Mol Cell Biol 17:1562, 1997

32. Lacronique V, Boureux A, Valle VD, Poirel H, Quang CT, Mauchauffe M, Berthou C, Lessard M, Berger R, Ghysdael J, Bernard OA: A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science 278:1309, 1997

33. Schwaller J, Frantsve J, Aster J, Williams IR, Tomasson MH, Ross TS, Peeters P, Van Rompaey L, Van Etten RA, Ilaria R Jr, Marynen P, Gilliland DG: Transformation of hematopoietic cell lines to growth-factor independence and induction of a fatal myelo- and lymphoproliferative disease in mice by retrovirally transduced TEL/JAK2 fusion genes. EMBO J 17:5321, 1998

34. Rowley A, Hunt T, Page M, Mainwaring G: Fish, in Rowley A, Ratcliffe N (eds): Vertebrate Blood Cells. Cambridge, MA, 1988, p 19

35. Glomski CA, Tamburlin J, Chainani M: The phylogenetic odyssey of the erythrocyte. III. Fish, the lower vertebrate experience. Histol Histopathol 7:501, 1992

36. Ransom DG, Haffter P, Odenthal J, Brownlie A, Vogelsang E, Kelsh RN, Brand M, van Eeden FJ, Furutani Seiki M, Granato M, Hammerschmidt M, Heisenberg CP, Jiang YJ, Kane DA, Mullins MC, Nusslein Volhard C: Characterization of zebrafish mutants with defects in embryonic hematopoiesis. Development 123:311, 1996

37. Weinstein BM, Schier AF, Abdelilah S, Malicki J, Solnica-Krezel L, Stemple DL, Stainier DY, Zwartkruis F, Driever W, Fishman MC: Hematopoietic mutations in the zebrafish. Development 123:303, 1996

38. Brownlie A, Donovan A, Pratt SJ, Paw BH, Oates AC, Brugnara C, Witkowska E, Sassa S, Zon LI: Positional cloning of the zebrafish *sauternes* gene: A model for congential sideroblastic anemia. Nat Genet 20:244, 1998

38a. Oates AC, Wollberg P, Pratt SJ, Paw BH, Johnson SL, Ho RK, Postlethwait JH, Zon LI, Wilks AF: Zebrafish *stat3* is expressed in restricted tissues during embryogenesis and *stat1* rescues cytokine signaling in a *STAT1*-deficient human cell line. Dev Dyn 215:352, 1999

39. Wilks AF: Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci USA 86:1603, 1989

40. Hanks SK, Quinn AM: Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. Methods Enzymol 200:38, 1991

41. Kawamura M, McVicar DW, Johnston JA, Blake TB, Chen YQ,

Lal BK, Lloyd AR, Kelvin DJ, Staples JE, Ortaldo JR, O'Shea JJ: Molecular cloning of L-JAK, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. Proc Natl Acad Sci USA 91:6374, 1994

42. Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski JJ: tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. Oncogene 5:1329, 1990

43. Binari R, Perrimon N: Stripe-specific regulation of pair-rule genes by *hopscotch*, a putative Jak family tyrosine kinase in *Drosophila*. Genes Dev 8:300, 1994

44. Knoth K, Roberds S, Poteet C, Tamkun M: Highly degenerate, inosine-containing primers specifically amplify rare cDNA using the polymerase chain reaction. Nucleic Acids Res 16:10932, 1988

45. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual (ed 2). Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1989

46. Westerfield M: The Zebrafish Book. Eugene, OR, University of Oregon, 1995

47. Stainier DY, Weinstein BM, Detrich HW 3rd, Zon LI, Fishman MC: *Cloche*, an early acting zebrafish gene, is required by both the endothelial and hematopoietic lineages. Development 121:3141, 1995

48. Ho RK, Kane DA: Cell-autonomous action of zebrafish *spt-1* mutation in specific mesodermal precursors. Nature 348:728, 1990

49. Altschul SF, Gish W: Local alignment statistics. Methods Enzymol 266:460, 1996

50. Higgins DG, Thompson JD, Gibson TJ: Using CLUSTAL for multiple sequence alignments. Methods Enzymol 266:383, 1996

51. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF: Stages of embryonic development of the zebrafish. Dev Dyn 203:253, 1995

52. Schulte-Merker S, Ho RK, Herrmann BG, Nusslein-Volhard C: The protein product of the zebrafish homologue of the mouse *T* gene is expressed in nuclei of the germ ring and the notochord of the early embryo. Development 116:1021, 1992

53. Johnson SL, Gates MA, Johnson M, Talbot WS, Horne S, Baik K, Rude S, Wong JR, Postlethwait JH: Centromere-linkage analysis and consolidation of the zebrafish genetic map. Genetics 142:1277, 1996

54. Tautz D: Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463, 1989

55. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T: Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci USA 86:2766, 1989

56. Goff DJ, Galvin K, Katz H, Westerfield M, Lander ES, Tabin CJ: Identification of polymorphic simple sequence repeats in the genome of the zebrafish. Genomics 14:200, 1992

57. Johnson SL, Midson CN, Ballinger EW, Postlethwait JH: Identification of RAPD primers that reveal extensive polymorphisms between laboratory strains of zebrafish. Genomics 19:152, 1994

58. Knapik EW, Goodman A, Atkinson OS, Roberts CT, Shiozawa M, Sim CU, Weksler Zangen S, Trolliet MR, Futrell C, Innes BA, Koike G, McLaughlin MG, Pierre L, Simon JS, Vilallonga E, Roy M, Chiang PW, Fishman MC, Driever W, Jacob HJ: A reference cross DNA panel for zebrafish (*Danio rerio*) anchored with simple sequence length polymorphisms. Development 123:451, 1996

59. Postlethwait JH, Yan YL, Gates MA, Horne S, Amores A, Brownlie A, Donovan A, Egan ES, Force A, Gong Z, Goutel C, Fritz A, Kelsh R, Knapik E, Liao E, Paw B, Ransom D, Singer A, Thomson M, Abduljabbar TS, Yelick P, Beier D, Joly JS, Larhammar D, Talbot WS: Vertebrate genome evolution and the zebrafish gene map. Nat Genet 18:345, 1998

60. Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L: MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174, 1987

61. Manly K, Cudmore R, Kohler G: Map Manager at mcbio.med.buffalo.edu/mapmgr.html. Buffalo, NY, Roswell Park Cancer Institute, 1996

62. Gurniak CB, Thomis DC, Berg LJ: Genomic structure and promoter region of the murine Janus-family tyrosine kinase, *Jak3*. DNA Cell Biol 16:85, 1997

63. Darnell JE Jr, Kerr IM, Stark GR: Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264:1415, 1994

64. Schaefer TS, Sanders LK, Nathans D: Cooperative transcriptional activity of Jun and Stat3 beta, a short form of Stat3. Proc Natl Acad Sci USA 92:9097, 1995

65. Al-Adhami MA, Kunz YW: Ontogenesis of haematopoietic sites in *Brachydanio rerio* (Hamilton-Buchanan) (Teleostei). Cell Growth Differ 19:171, 1977

66. Liao EC, Paw BH, Oates AC, Pratt SJ, Postlethwait JH, Zon LI: SCL/Tal-1 transcription factor acts downstream of *cloche* to specify hematopoietic and vascular progenitors in zebrafish. Genes Dev 12:621, 1998

67. Detrich HW 3rd, Kieran MW, Chan FY, Barone LM, Yee K, Rundstadler JA, Pratt S, Ransom D, Zon LI: Intraembryonic hematopoietic cell migration during vertebrate development Proc Natl Acad Sci USA 92:10713, 1995

68. Willett CE, Cherry JJ, Steiner LA: Characterization and expression of the recombination activating genes (*rag1* and *rag2*) of zebrafish. Immunogenetics 45:394, 1997

69. Willett CE, Zapata AG, Hopkins N, Steiner LA: Expression of zebrafish *rag* genes during early development identifies the thymus. Dev Biol 182:331, 1997

70. Marcos-Gutierrez CV, Wilson SW, Holder N, Pachnis V: The zebrafish homologue of the *ret* receptor and its pattern of expression during embryogenesis. Oncogene 14:879, 1997

71. Orkin SH, Zon LI: Genetics of erythropoiesis: Induced mutations in mice and zebrafish. Annu Rev Genet 31:33, 1997

72. Liao W, Bisgrove BW, Sawyer H, Hug B, Bell B, Peters K, Grunwald DJ, Stainier DY: The zebrafish gene *cloche* acts upstream of a *flk-1* homologue to regulate endothelial cell differentiation. Development 124:381, 1997

73. Thompson MA, Ransom DG, Pratt SJ, MacLennan H, Kieran MW, Detrich HW 3rd, Vail B, Huber TL, Paw B, Brownlie AJ, Oates AC, Fritz A, Gates MA, Amores A, Bahary N, Talbot WS, Her H, Beier DR, Postlethwait JH, Zon LI: The *cloche* and *spadetail* genes differentially affect hematopoiesis and vasculogenesis. Dev Biol 197:248, 1998

74. Quelle FW, Wang J, Feng J, Wang D, Cleveland JL, Ihle JN, Zambetti GP: Cytokine rescue of p53-dependent apoptosis and cell cycle arrest is mediated by distinct Jak kinase signaling pathways. Genes Dev 12:1099, 1998

75. Wu H, Liu X, Jaenisch R, Lodish HF: Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. Cell 83:59, 1995

76. Lin CS, Lim SK, D'Agati V, Costantini F: Differential effects of an erythropoietin receptor gene disruption on primitive and definitive erythropoiesis. Genes Dev 10:154, 1996

77. Ruddle FH, Bentley KL, Murtha MT, Risch N: Gene loss and gain in the evolution of the vertebrates. Dev Suppl 120:155, 1994

78. Katsanis N, Fitzgibbon J, Fisher EMC: Paralogy mapping: Identification of a region in the human *MHC* triplicated onto human chromosomes 1 and 9 allows the prediction and isolation of novel *PBX* and *NOTCH* loci. Genomics 35:101, 1996

79. Postlethwait JH, Talbot WS: Zebrafish genomics: From mutants to genes. Trends Genet 13:183, 1997

80. Wittbrodt J, Meyer A, Schartl M: More genes in fish? Bioessays 20:511, 1998

81. Amores A, Force A, Ekker M, Yan Y-L, Amemiya C, Fritz A, Ho RK, Joly L, Langeland J, Prince V, Wang Y-L, Westerfield M, Postlethwait JH: Genome duplications in vertebrate evolution: Evidence from zebrafish *hox* clusters. Science 282:1711, 1998

82. Ohno S: Evolution by Gene Duplication. Heidelberg, Germany, Springer Verlag, 1970

83. Partanen J, Makela TP, Alitalo R, Lehvaslaiho H, Alitalo K: Putative tyrosine kinases expressed in K-562 human leukemia cells. Proc Natl Acad Sci USA 87:8913, 1990

84. Howard OM, Dean M, Young H, Ramsburg M, Turpin JA, Michiel DF, Kelvin DJ, Lee L, Farrar WL: Characterization of a class 3 tyrosine kinase. Oncogene 7:89, 1992

85. Siyanova EY, Serfas MS, Mazo IA, Tyner AL: Tyrosine kinase gene expression in the mouse small intestine. Oncogene 9:2053, 1994

86. Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, van Eeden FJ, Jiang YJ, Heisenberg CP, Kelsh RN, Furutani-Seiki M, Vogelsang E, Beuchle D, Schach U, Fabian C, Nusslein-Volhard C: The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. Development 123:1, 1996

87. Force A, Lynch M, Pickett FB, Amores A, Postlethwait JH: The preservation of duplicate genes by complementary, degenerative mutations. Genetics 151:1531, 1999

88. Dalal I, Arpaia E, Dadi H, Kulkarni S, Squire J, Roifman CM: Cloning and characterization of the human homolog of mouse *Jak2*. Blood 91:844, 1998

89. Feng J, Witthuhn BA, Matsuda T, Kohlhuber F, Kerr IM, Ihle JN: Activation of Jak2 catalytic activity requires phosphorylation of Y1007 in the kinase activation loop. Mol Cell Biol 17:2497, 1997

90. Ito Y, Mikawa S, Kobayashi E, Wada Y, Minezawa M: Direct Genbank submission. Genebank no. AB006011, 1997

91. Duhe RJ, Rui H, Greenwood JD, Garvey K, Farrar WL: Cloning of the gene encoding rat JAK2, a protein tyrosine kinase. Gene 158:281, 1995