Genetic Evidence for an Evolutionarily Conserved Role of IL-7 Signaling in T Cell Development of Zebrafish

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In mammals, the cytokine IL-7 is a key regulator of various aspects of lymphocyte differentiation and homeostasis. Because of the difficulty of identifying cytokine homologs in lower vertebrates and the paucity of assay systems and reagents, the degree of functional conservation of cytokine signaling pathways, particularly those pertaining to lymphocyte development, is unclear. In this article, we report on the analysis and characterization of three zebrafish mutants with severely impaired thymopoiesis. The identification of affected genes by positional cloning revealed components of the IL-7 signaling pathway. A presumptive null allele of the zebrafish homolog of the IL-7 receptor (il7r) chain causes substantially reduced cellularity of the thymus but spares B cell development in the kidney. Likewise, nonsense mutations in the zebrafish homologs of janus kinases Jak1 and Jak3 preferentially affect T cell development. The functional interactions of the cytokine receptor components were examined in the three groups of fish hetero- or homozygous for either il7r and jak1, il7r and jak3, or jak1 and jak3 mutations. The differential effects on T cell development arising from the different genotypes could be explained on the basis of the known structure of the mammalian IL-7R complex. Because IL-7 signaling appears to be a universal requirement for T cell development in vertebrates, the mutants described in this article represent alternative animal models of human immunodeficiency syndromes amenable to large-scale genetic and chemical screens. The Journal of Immunology, 2011, 186: 000–000.

The cytokine IL-7 plays several important roles during lymphocyte development, survival, and homeostatic proliferation (1). Like other related cytokines (IL-2, IL-4, IL-9, IL-15, and IL-21), IL-7 is a member of the so-called common γ-chain (γc) family (2). It is produced by many different stromal cell types, including epithelial cells of the thymus and the intestine (3–5). The receptor for IL-7 consists of a heterodimer, the IL-7Rα–chain, and the γc; the IL-7Rα–chain is also part of the receptor for the thymic stromal lymphopoietin (TSLP) cytokine, and γc is also a component of other cytokine receptors (2). In the receptor for TSLP, a cytokine that is related to IL-7, the IL-7Rα–chain pairs with the TSLP receptor (TSLPR) instead with the γc (6, 7). The IL-7R shares many of its downstream signaling components with other cytokine receptors; in the receptor–proximal complex, the IL-7Rα–chain interacts with Jak1, whereas Jak3 binds to the γc (2). By contrast, the TSLPR requires Jak2 instead of Jak3 for activation of STAT proteins (8).

In mice, the phenotypes of null mutations of all four core components of the IL-7r complex, IL-7rα (9), γc (10), Jak1 (11), and Jak3 (12, 13) and of its ligand IL-7 (14) have been established. In humans, immunodeficient patients carrying mutations in IL-7rα (15–17), γc (18–20), and Jak3 (21–23) have been reported. Interestingly, the phenotypes are similar between humans and mice but not always identical (2), pointing to interspecific differences of cytokine requirements in the development and function of different hematopoietic lineages. For instance, IL7r deficiency leads to the absence of T cells in both humans and mice, whereas B cells are present in humans but absent in mice (9, 15–17).

Whether cytokine signaling is required during lymphocyte development also in lower vertebrates is currently unknown. This is due to the lack of informative mutants, incomplete information concerning the complexity of the cytokine gene families, and the paucity of suitable in vitro assay systems. With regard to IL-7 signaling, genes encoding homologs of IL-7rα, TSLPR, and γc chains (24) as well as those encoding the receptor–proximal JAKs have been detected in fish (25). By contrast, it is often difficult to make an unambiguous assignment of presumptive γc cytokines by homology search in genome and expressed sequence tag databases available for lower vertebrates (26), because the primary amino acid sequences of ILs appear to be poorly conserved. Hence, despite its central importance in the immune system of mammals, the role, if any, of IL-7 signaling in lymphocyte development and function of lower vertebrates remains unclear.

In this article, we describe a genetic approach to examine the role of IL-7 signaling in lower vertebrates. This strategy made use of a large collection of zebrafish mutants with thymus abnormalities that were identified in forward genetic screens (27, 28). Three mutations, affecting key components of the IL-7 signaling pathway, Il-7rα, Jak3, and Jak1, were identified by positional cloning. Collectively, our findings provide genetic evidence for an evolutionarily conserved role of IL-7 signaling in T cell development.

Department of Developmental Immunology, Max Planck Institute of Immunobiology and Epigenetics, D-79108 Freiburg, Germany.

1Current address: Wallinger and Partners, Munich, Germany.

2Current address: Vertebrate Development Laboratory, Cancer Research United Kingdom, London Research Institute, London, United Kingdom.

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Address correspondence and reprint requests to Dr. Thomas Boehm, Department of Developmental Immunology, Max Planck Institute of Immunobiology and Epigenetics, Stuebeweg 51, D-79108 Freiburg, Germany. E-mail address: boehm@immunbio.mpg.de

The online version of this article contains supplemental material.

Abbreviations used in this article: γc, common γ-chain; 5 dpf, 5 days postfertilization; gh, growth hormone; TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor.

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IL-7 SIGNALING IN ZEBRAFISH

Materials and Methods

Animals

Details of the forward genetic screens to identify genes regulating thymopoiesis have been described previously (27, 28); the three mutants described in this article belong to the Tübingen arm of our screen. The *ikaros* (tg*GFP*) line was described previously (29). Briefly, in the original round of mutant identification, 20–30 larvae of each F3 clutch were screened via *rag1* in situ hybridization at 120 h postfertilization to detect alterations in thymus development. A clutch was considered positive when 20–30% of the larvae showed a similar alteration in the *rag1* expression pattern or intensity. To confirm and recover mutations, F2 pairs producing putative mutants among their F3 offspring were crossed out, and the resulting new F3 families underwent the same inbreeding and screening procedure. Using molecular probes, all mutants were subsequently analyzed for potential abnormalities of hematopoietic cells, development of pharyngeal endoderm and ectoderm, and structures derived from neural crest at various time points during the first 5 d of embryonic development (27, 28).

Linkage analysis

The genomic localizations of the three zebrafish mutations analyzed in this paper were determined using the Tübingen marker set for genome scans (version 4) on F2 Tübingen × Wik crosses of the mutant carriers. Primer sequences are available from the MGH Web site (http://zebrafish.mgh.harvard.edu). For fine-scale mapping, new markers were generated; they are listed in Supplemental Table I. Before detailed phenotypic analysis, fish were outcrossed to wild-type fish for several generations to eliminate potentially confounding background mutations.

**Morphants**

Morphants were generated by injection of anti-sense oligonucleotides (Gene Tools, Philomath, OR) encompassing the sequences of the initiation codons (Table I) at 100 μM as described previously (29).

**In situ hybridization analysis**

Procedures for RNA in situ hybridization were described previously (28, 30). Probes for *rag1*, *gh*, *foxn1*, and *igu* have been described previously (28). The probe for *ifih7* corresponds to nt 389–1467 in GenBank accession number NM_001113507.1; the probe for *jak3* corresponds to nt 29–590 in GenBank accession number XM_002663087; the probe for *jak1* corresponds to nt 1284–2068 in GenBank accession number NM_131073.1. All GenBank sequences can be accessed at http://www.ncbi.nlm.nih.gov/nucldet/

**TCR rearrangement assays**

These assays were performed as described previously (29).

**Results**

Figure 1. Identification and characterization of HY022, IP045, and HX157 zebrafish mutants. A, Whole-mount RNA in situ hybridization of wild-type and homozygous mutant embryos at 5 dpf with probes for *rag1* and *gh*. *rag1* labels differentiating thymocytes in the bilateral thymus anlage (arrowheads). *gh* labels growth hormone-producing cells in the hypophysis (arrow); this signal serves as an internal control for hybridization and the tissue specificity of the mutant phenotype. Original magnification ×30. B, Ratio of *rag1* and *gh* RNA in situ hybridization signals as a measure of thymopoietic activity (left panel) of wild-type fish (Wt) and HY022, IP045, and HX157 homozygous mutants. The *gh* signal (expressed in arbitrary units [A.U.]) is similar in all four genotypes (right panel). Mean values ± SEM are shown; the number of embryos analyzed at 5 dpf is indicated at the top. Hybridization signals were quantified as the areas of two-dimensional projections of the three-dimensional hybridization signal. C, Representative frames of the thymus region (outlined with dotted lines) of time-lapse analyses using an *ikaros:eGFP* reporter line taken at 5 dpf. Each green dot represents an *ikaros*-expressing lymphocyte progenitor; the number of hematopoietic cells is proportional to *rag1*-expressing thymocytes. Original magnification ×200.

Live microscopy of fish

The procedures for live imaging were described previously (29, 30).

**Statistical analysis**

Details for statistical procedures used are given in the figure legends.

An F3 forward genetic screen of fish mutagenized with N-ethyl N-nitrosourea was conducted to identify recessive mutations that result in abnormal thymopoiesis of zebrafish embryos (27, 28, 30, 31). In this paper, we report on a group of three mutants (HY022, IP045, and HX157) affecting IL-7 signaling. The mutants were identified by whole-mount RNA in situ hybridization at day 5 postfertilization (5 dpf) using a *rag1* probe to detect differentiating lymphocytes in the thymus. Although the thymus is clearly evident in wild-type fish, the number of *rag1*-expressing cells is reduced in all three mutants but not completely abolished (Fig. 1A). To provide a measure of thymopoietic activity, the signal intensities of the *rag1* probe in wild-type and mutant fish were normalized using the signals emanating from a growth hormone (*gh*) gene probe that marks the developing hypophysis. It became clear that IP045 mutants suffered the most severe reduction in thymopoiesis (Fig. 1B). To examine the phenotypes further, we have used an *ikaros:eGFP* transgene; the fluorescent protein is expressed in hematopoietic progenitors cells, including thymocyte precursors, and can thus be used to visualize embryonic hematopoiesis and thymus colonization in real-time (29). High-resolution time-lapse analysis confirmed the results obtained with *rag1*-specific RNA in situ hybridization. The severe impairment of thymopoiesis is reflected in the reduced number of *ikaros*-expressing cells; likewise, the differences among the three mutants are also evident (Fig. 1C). In conclusion, the above-mentioned analyses suggested that thymopoiesis is at least quantitatively impaired in all three mutants, albeit to different extents.

**Positional cloning of affected genes**

To establish the molecular nature of the affected genes in the three mutants, we employed the positional cloning technique. Linkage mapping localized these mutations to chromosomes 21 (HY022), 8...
To determine the nature of these mutations, we sequenced the coding exons and flanking intron-exon boundaries of the zebrafish homologs of the IL-7R genes implicated in the HY022, IP045, and HX157 mutants (Supplemental Table I). In HY022 mutants, the critical interval spanned 0.5 cM and the mutation was found to be 0.1 cM away from the closest informative marker (HY022-126); in IP045 mutants, the critical interval comprised 2.9 cM and the closest marker (IP045-108) was less than 0.16 cM away from the mutation; in HX157 mutants, the critical interval was determined to be 1.2 cM and the closest marker (HX157-415) was <0.064 cM away from the mutation. In all three cases, genetically defined candidate genes were sequenced in their entirety, including exon/intron junctions, and compared with wild-type sequences derived from recombinant clones spanning the critical regions. In this way, deleterious mutations in zebrafish homologs of three components of the presumptive IL-7 receptor complex were identified.

In the HY022 mutant, the insertion of an extra thymidine residue was found in exon 4 of the gene encoding the presumptive zebrafish homolog of the IL-7Rα (iIL7r) chain (allele designation iIL7r<sup>21380</sup>) (Fig. 2B); because of this frameshift, the mutant protein is predicted to contain only about one-third of the extracellular domain (Fig. 2C, top panel). On the basis of the known structure of human IL-7R/IL-7Rα complex (32), the truncated receptor protein, if produced at all, is probably unable to interact with the cytokine. Hence, we conclude that this mutation corresponds to a null allele. The results of two additional experiments suggested that the phenotype in HY022 fish was indeed caused by the mutation in the iIL7r gene. First, all 71 mutant fish (identified according to their low thymopoietic index) were found to be homozygous for the mutation, indicating close linkage (<0.7 cM); this conclusion is supported by the fact that all fish (n = 172) with normal phenotype were found to be either wild-type or heterozygous for the mutation. Second, gene-specific morphants were found to exhibit significantly lower <i>rags1</i>/<i>ish</i> ratios (compare panels in Fig. 1B), indicating that the knockdown of <i>iIL7r</i> phenocopies the impaired thymopoiesis of the <i>iil7r</i> mutant (Table I).

In the IP045 mutant, a cytidine to thymidine transition was found in the first residue of codon 336 of the zebrafish homolog of the Jak3 gene (allele designation <i>jak3<sup>25078</sup></i>; Fig. 2B); the Q336X nonsense mutation is predicted to truncate the protein before the tyrosine kinase domains (Fig. 2C, middle panel). The mutant protein is predicted to retain the domains required for binding to the γc (33); hence, if present, in functionally significant amounts, the truncated version may function in a dominant-negative manner, possibly competing with the wild-type protein for γc interaction sites. The results of two additional experiments suggested that the phenotype in IP045 fish was indeed caused by the mutation in the <i>jak3</i> gene. First, all 82 mutant fish (identified according to their low thymopoietic index) were found to be homozygous for the mutation, indicating close linkage (<0.6 cM); this conclusion is supported by the fact that all fish (n = 209) with normal phenotype were found to be either wild-type or

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**FIGURE 2.** Positional cloning of genes affected in HY022, IP045, and HX157 zebrafish mutants. A, Chromosomal locations of mutant loci. Informative markers (see Supplemental Table I for details) are depicted above a schematic of the chromosomal regions of interest; below, the recombination fractions at these sites and the locations of the affected genes (asterisks) are indicated. The relevant bacterial artificial chromosome (BAC) clones, whose sequences were used for comparisons, are also indicated. B, Representative sequence chromatograms of mutated sites (highlighted in red underneath) in iIL7r, jak3, and jak1 genes. The conceptual translations are given in a single-letter code; X denotes a stop codon. The insertion in iIL7r occurs between nt 669 and 676 in GenBank accession number NM_001113507.1; the mutation in jak3 occurs at nt 1006 in GenBank accession number XM_002663087; the mutation in jak1 occurs at nt 1973 in GenBank accession number NM_131073.1. C, Schematics of the proteins encoded by the mutated genes. The wild-type (WT) structures and relevant functional domains are depicted above the predicted structures of the mutant proteins; FNIII, fibronectin type III domain; TM, transmembrane domain; Box1, a domain mediating the interaction with Jak proteins; FERM, domain mediating binding to Box1 domain of cytokine receptors; pseudokinase, a regulatory domain; kinase, a catalytic domain. In the mutant iIL7r protein, the frameshift mutation is predicted to append five unrelated amino acids to the N-terminal portion of the protein (indicated by black rectangle). For the jak proteins, the nonsense mutations are indicated by the letter X.
heterozygous for the mutation. Second, gene-specific morphants were found to exhibit significantly lower rag1/gh ratios (compare panels in Fig. 1B), indicating that the knockdown of jak3 phenocopies the impaired thymopoiesis of the jak3 mutant (Table I).

In HX157 mutants, a cytidine to thymidine transition was detected in the first nucleotide of codon 588 of the zebrafish homolog of the jak1 gene (allele designation jak1<sup>e22598</sup>, Fig. 2B); the R580X missense mutation is situated at the beginning of the tyrosine kinase domains (Fig. 2C, bottom panel). As with the mutant form of jak3, the mutant jak1 protein retains the receptor binding domain (34) and may act in a dominant-negative fashion, in this case competing with wild-type jak1 for interaction with the il7 receptor chain if present in functionally significant amounts. The results of two additional experiments suggested that the phenotype in HX157 fish was indeed caused by the mutation in the jak1 gene. First, all 233 mutant fish (identified according to their low thymopoietic index) were found to be homozygous for the mutation, indicating close linkage (<0.2 cM); this conclusion is supported by the fact that all fish (n = 176) with normal phenotype were found to be either wild-type or heterozygous for the mutation. Second, gene-specific morphants were found to exhibit significantly lower rag1/gh ratios (compare panels in Fig. 1B), indicating that the knockdown of jak1 phenocopies the impaired thymopoiesis of the jak1 mutant (Table I).

Characterization of the il7r mutant

As shown in Fig. 1, the number of ikaros-expressing hematopoietic cells in the thymus appears to be severely reduced in fish homozygous for the frameshift mutation in il7r. The number of rag1-expressing cells is also reduced; nonetheless, this shows that il7r is not required for rag expression in developing thymocytes. Adult homozygous mutants are often smaller than their heterozygous or wild-type siblings, and tend to die earlier (Supplemental Table II). In surviving fish, the thymus is small and hypocellular compared to unaffected, although il7r-expressing cells are of similar numbers to the wild-type organ (Fig. 3). Expression of foxn1 by RT-PCR. The expression of foxn1, a characteristic marker for thymic epithelial cells (29, 35), is unaffected, although foxn1-positive cells are less dispersed in the thymic rudiment as a result of the reduced number of hematopoietic cells (Fig. 3C). By contrast, the cellularity of the head kidney (where myelopoiesis and B cell development occur) is similar in all genotypes (Fig. 3D); histological analysis revealed that cells of all hematopoietic cell lineages were present in similar distribution. Using RNA in situ hybridization, the numbers of rag1- and igu-expressing cells were found to be identical (Fig. 3E, 3F). Collectively, these results suggest that within the hematopoietic compartment, the il7r mutation primarily affects T cell development. At present, the reason for the reduced life expectancy of il7r homozygous mutants is unknown; we note, however, that il7r is expressed in the brain of zebrafish embryos (Supplemental Fig. 1) and was previously shown to have a role in the developing brain of rodents (36).

Characterization of jak mutants

The aforementioned analyses indicated that thymopoiesis is severely impaired in jak3 and jak1 mutants. Yet, the mutants are viable and fertile and do not exhibit a shortened lifespan (Supplemental Table II). Second, gene-specific morphants were found to exhibit significantly lower rag1/gh ratios (compare panels in Fig. 1B), indicating that the knockdown of jak3 phenocopies the impaired thymopoiesis of the jak3 mutant (Table I).

Genetic interactions

The abovementioned results support the notion that in zebrafish the functional roles in T cell development of il7r, jak3, and jak1 are similar to their homologs in mammals. However, although the structure of the mammalian IL-7R/ligand complex was revealed by detailed genetic, cell biological, biochemical, and structural studies in humans (36), the lack of suitable Abs and in vitro assays precludes similar experiments in the zebrafish model. To circumvent these limitations, we used the genetic interaction method to examine the functions of il7r, jak3, and jak1 in more detail. To this end, we generated all three pairwise double mutants to examine whether the observed phenotypes would have been predicted under the assumption that the known structure/function relationships of mammalian components (2, 32) also hold for zebrafish. At 5 dpf, we determined the thymopoietic index (calculated as the ratio of rag1 versus gh signals; compare panels in Fig. 1B) to be able to quantitatively assess the effect of the compound genotypes on thymopoiesis (Fig. 5). Genetic interaction is presumed to be present when the phenotype of a double-mutant organism deviates from the expected neutral phenotype. In this study, we use the product definition of genetic interaction, because this model is best suited to identify functional relationships (37). To this end, we calculated the expected phenotypes (i.e., rag1/gh ratios) for the three double-mutant pairs and compared them to the observed values. As shown in Fig. 6B, we found that the phenotypes of all double mutants are less severe than expected under the neutrality function, strongly suggesting the presence of genetic interaction. This observation supports the notion that il7r, jak1, and jak3 are in a common pathway that regulates early T cell development and suggest that the overall composition and function of the IL-7R complex (Fig. 6A) in fish is similar to that in mammals.

Discussion

Our results support the notion that in zebrafish the functional roles in T cell development of il7r, jak3, and jak1 are similar to their homologs in mammals. The phenotypes observed in double mutants are significantly different from the neutral expectation...
and thus suggest the presence of genetic interaction among the three genes studied in this paper. In genetic interaction analysis, a so-called synthetic interaction is said to occur when a double mutant has a more extreme phenotype than expected from single-mutant phenotypes. By contrast, when the phenotype is less severe than expected, an alleviating interaction is present. This situation often occurs when the gene products function in the

FIGURE 3. Phenotype of the il7r mutant at 7 wk of age. A, Histological sections of the thymus (H&E stain) indicating a substantially reduced cellularity of the mutant tissue. Note also that the thymus is substantially smaller in the mutant. Scale bars, 200 μm in upper panels, 50 μm in lower panels. B, RNA in situ hybridization of thymus tissue sections with a rag1-specific probe to detect developing thymocytes (bluish color). Scale bars, 200 μm. In the wild-type thymus, the hybridization occurs in the outer (cortical) region of the thymus; note that in the mutant, the regular organization of the cortex/medulla regions is disrupted, as indicated by the centrally located rag1 signal. C, RNA in situ hybridization of thymus tissue sections with a foxn1-specific probe to detect the epithelial microenvironment. Note that the Foxn1-expressing cells in the mutant are located closer to each other owing to the lack of hematopoietic cells. Scale bars, 200 μm. D, Histological sections of the head kidney (H&E stain) indicating comparable cellularity of mutant tissue. Scale bars, 200 μm in upper panels, 50 μm in lower panels. E, RNA in situ hybridization of head kidney tissue sections with a rag1-specific probe to detect developing lymphocytes. In the kidney, rag1-expressing cells are B cell progenitors. Scale bars, 200 μm. F, RNA in situ hybridization of head kidney tissue sections with an Igμ gene (igm)-specific probe to detect developing and mature B cells. The number and distribution of igm-positive cells is not changed in il7r mutants. Scale bars, 200 μm.

FIGURE 4. Reduced thymopoiesis in jak mutants. A, Histological sections of the thymus (hematoxylin/eosin stain) indicating a substantially reduced cellularity of the mutant tissues. Scale bars, 100 μm. B, RNA in situ hybridization of thymus tissue sections with a rag1-specific probe to detect developing thymocytes in the thymic rudiment (outlined with dashed lines). Scale bars, 200 μm.

FIGURE 5. Phenotypes of fish with compound genotypes at 5 dpf. A, Thymopoietic indices (rag/gh) (compare Fig. 1) of il7r (denoted 7)- and jak3 (denoted 3)-single and il7r/jak3-double mutants. The double heterozygotes are highlighted in gray. B, Thymopoietic indices (rag/gh) of il7r- and jak1 (denoted 1)-single and il7r/jak1-double mutants. The double heterozygotes are highlighted in gray. C, Thymopoietic indices (rag/gh) of jak3- and jak1-single and jak3/jak1-double mutants. The double heterozygotes are highlighted in gray. Note that the survival rate of double mutants is greatly reduced. Mean values ± SEM are shown; the number of embryos analyzed for each genotype is indicated at the top. *p < 0.05, **p < 0.01 (Student t test, two-tailed).
same pathway (37). Our analysis provides clear evidence for the latter type of genetic interaction and is thus compatible with the hypothesis that the known structure/function relationships of mammalian components (2, 32) also hold for zebrafish. Because of a lack of suitable reagents and assay systems, we have not been able to directly evaluate the nature of the mutant proteins. The predicted truncated il-7ra–chain encodes only part of the extracellular domain that appears unlikely to be capable of interacting with its ligand hence, based on structural grounds, the il7r allele identified in this paper must be considered to be a null allele. We interpret the lower thymopoietic index in fish heterozygous for the il7r allele identified in this paper as a sign of haploinsufficiency, in- treatment of the extracellular domain that appears unlikely to be capable of binding sites on il-7r, they could act as dominant-negative forms, competing with wild-

FIGURE 6. Evolutionary conserved IL-7 signaling complex. A, Schematic of the IL-7R complex. For detailed references, see text. In human patients with immunodeficiency syndromes, mutations in IL7R, JAK3, and IL2RG (encoding the γc) have been described previously; in mice, the genes encoding all five components of the ligand/core receptor complex (IL-7, IL-7ra, γc, Jak1, and JAK3) have been inactivated by targeted mutations in embryonic stem cells. B, Genetic interaction analysis at 5 dpf. The observed ragl/gh ratios of double-mutants (red bars; means ± SD) are compared with the calculated values (means ± SD) expected under the product neutrality function (black bars); the levels of significance P for rejection of the null hypothesis were calculated using the Welch test and corrected for multiple testing using the Bonferroni procedure. All values were normalized to the wild-type value, which was set to 1.

tebrate evolution. For the first time, to our knowledge, they clearly demonstrate that IL-7 signaling is not only required for tetrapods, but also for teleost fish. Although all efforts to identify il-7 in zebrafish so far have proven unsuccessful, our results provide the strongest evidence yet for its existence. Nonetheless, the identification of an IL-7 homolog and the characterization of appropriate mutant fish would be required to definitively prove an essential role of the IL-7 signaling axis in fish.

Moreover, the three zebrafish mutants described here make a significant contribution to the list of animal models of human immunodeficiency syndromes caused by IL7R (15–17) and JAK3 (21–23) mutations. Because of the exceptional experimental amenity of the zebrafish, we envisage that our mutants could be used for large-scale chemical and genetic screens to determine not only if and how the ill effects caused by the malfunction of IL-7R components could be ameliorated but also to interfere with their normal function (38).

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

References

Supplemental information

Legends to Figures

**Supplemental Fig. 1.** Expression patterns of *il7r*, *jak3*, and *jak1* genes in zebrafish embryos at 5 dpf. Shown are whole-mount RNA *in situ* hybridizations using anti-sense (left panels) and sense probes (right panels). The location of the thymus is indicated by arrows. Note that *il7r* and *jak1* are expressed widely, whereas the expression of *jak3* is confined to the thymus.

**Supplemental Fig. 2.** The development of the hypophysis is unaffected in *il7r*, *jak3* and *jak1* single mutants. Shown are the signal intensities of the *gh* probes in embryos of the indicated genotypes (*il7r* is denoted as 7, *jak3* is denoted as 3, and *jak1* is denoted as 1) in arbitrary units (A.U.); c.f. Fig. 1 and 4. A, Genotypes obtained from *il7r* and *jak3* intercrosses. B, Genotypes obtained from *il7a* and *jak1* intercrosses. C, Genotypes obtained from *jak3* and *jak1* intercrosses. Note that the survival rate of double mutants is greatly reduced. It is unclear why the signal area for *gh* is enlarged in some genotypes; this may be related to the role of *il7r* and *jak1* in the central nervous system; c.f. Supplemental Fig. 1.

In each panel, the numbers of embryos are indicated at the top; means ± s.e.m. are shown. Single asterisks denote statistical differences at the P<0.05 level, two asterisks denote significance at the P<0.01 level from wild-type values (Student’s t-test; two-tailed).

Supplemental Table 1. Markers used in this study
Supplemental Table 2. Distribution of genotypes
Table S1. Markers used in this study

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Table S2. Genotype distributions

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