Development 123, 255-262
Printed in Great Britain © The Company of Biologists Limited 1996
DEV3362

Genetic analysis of fin formation in the zebrafish, *Danio rerio*

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SUMMARY

In the zebrafish, *Danio rerio,* a caudal and pectoral fin fold develop during embryogenesis. At larval stages the caudal fin fold is replaced by four different fins, the unpaired anal, dorsal and tail fins. In addition the paired pelvic fins are formed. We have identified a total of 118 mutations affecting larval fin formation. Mutations in 11 genes lead to abnormal morphology or degeneration of both caudal and pectoral fin folds. Most mutants survive to adulthood and form a surprisingly normal complement of adult fins.

Mutations in nine genes result in an increased or reduced size of the pectoral fins. Interestingly, in mutants of one of these genes, *dackel* (*dak*), pectoral fin buds form initially, but later the fin epithelium fails to expand. Expression of *sonic hedgehog* mRNA in the posterior mesenchyme of the pectoral fin bud is initiated in *dak* embryos, but not maintained.

Mutations in five other genes affect adult fin but not larval fin development. Two mutants, *longfin* (*lof*) and *another longfin* (*alf*) have generally longer fins. *Stein und bein* (*sub*) has reduced dorsal and pelvic fins, whereas *finless* (*fls*) and *wanda* (*wan*) mutants affect all adult fins. Finally, mutations in four genes causing defects in embryonic skin formation will be briefly reported.

Key words: embryonic fin fold, pectoral fins, *sonic hedgehog*, adult fins, skin, zebrafish

INTRODUCTION

In the zebrafish, as in other fishes, larval fins differ from the fins present in the adult by both number and morphology. Larvae possess two pectoral fins (Fig. 1C) and a caudal fin fold (Fig. 1A). Pectoral fins develop from a fin bud that is homologous to the tetrapod limb bud in structure and gene expression (Krauss et al., 1993; Sordino et al., 1995). In contrast to the tetrapod limb bud, proliferation of the fin bud stops soon after its formation and the pectoral fin folds arise from an apical ectodermal ridge like structure.

The caudal fin fold, consisting of a dorsal part, a ventral part posterior to the anus, and an anterior ventral part below the yolk tube (Fig. 1A), develops from a structure very similar to the apical ectodermal ridge of the pectoral fin bud, at approximately 22 hours post fertilization (hpf). This apical ectodermal ridge-like structure is 6-9 cells wide and consists of a layer of epiderm covered by periderm. Fin epithelium is formed by folding of this primordium onto itself (Dane and Tucker, 1985).

At the hatching period (Kimmel et al., 1995), the pectoral and the caudal fin folds each consist of two layers of epiderm covered by periderm. In the space between the two layers of epiderm collageneous fibers, the so-called actinotrichia are present (Figs 1D, 2A). The epiderm is probably responsible for the formation of these fibers (Bouvet, 1974). Additional extracellular fibers are present, which cross the subepidermal space, and might be important for the maintenance of the folded structure (Dane and Tucker, 1985). While the pectoral fins are maintained throughout development, the single caudal fin fold is replaced by three distinct adult fins, the unpaired anal, tail and dorsal fins, during the first four weeks of post embryonic development (Fig. 1B). In the fin fold, at the positions where these fins will develop, mineralized segmented finrays are formed, the lepidotrichia. Lepidotrichia are part of the dermal skeleton of the fish, in contrast to the radial bones which are part of the endoskeleton (Fig. 1E,F). The larval fin fold between these definitive fins disappears. In addition, the paired pelvic fins, the homologues of the posterior tetrapod limbs, develop. The embryonic origin of the adult fin mesenchyme and the cells that form the lepidotrichia is not clear. It has been suggested that they derive from neural crest in the unpaired fins and from mesoderm in the paired fins (Smith et al., 1994).

In our screen we scored for larval fin and skin defects (Haffter et al., 1996). 118 mutants were identified in which fin development is affected in the embryo; in addition we isolated several dominant and recessive mutations affecting the development of the adult fin (Table 1). Ten mutants were identified that have defects in the formation of the embryonic skin. The screen for embryonic fin or skin mutants was performed between 24 and 48 hours of development using a 20-80× mag-

nification on a dissecting scope (for details see Materials and Methods). The aim of this study is to present a brief overview of all skin and fin mutants, isolated in our mutational screen. For this reason we have also included short descriptions of mutants that have additional phenotypes and are described elsewhere in more detail (for references, see Table 1).

MATERIALS AND METHODS

Fish maintenance

Fish maintenance and crosses were done as described in Mullins et al. (1994). For photography the following mutant alleles were used: *nel*^{tq207}, *rfl*^{tr240}, *pif*^{tm95b}, *fyd*^{tj2b} and *bla*^{ta90} homozygotes, *frf*^{tm317a}/*frf*^{tf5}, *lep*^{tq236}/*lep*^{tj222} transheterozygotes, *uki*^{tc256d}, *dre*^{tm146d}, *ika*^{tm127c}, *krm*^{tc227d}, *dak*^{tw25e}, *box*^{te242}, *smp*^{td11b}, *ddf*^{ta50a} *mes*^{tm105} homozygotes, *pgy*^{ty40}, *alf*^{dty86}, *lof*^{dt2} and *wan*^{dty127} heterozygotes.

Screening procedure for fin and skin mutants

Fish of the F_2 generation (families; for details, see Haffter et al., 1996a) were set up for spawning. These F_2 fish were visually inspected for fin and pigmentation morphology. Dominant mutations should affect 50% of the fish in a given F_2 family.

To identify zygotic recessive fin and skin mutations, embryos from individual F_2 sibling matings were inspected between 48 and 60 hpf. Using a DRC Zeiss dissecting microscope equipped with 0.8×, 2×, 4× and 8× magnification, the morphology of the caudal fin fold and the pectoral fin folds were examined in 12 embryos from each clutch (for details see Haffter et al., 1996).

In situ hybridization and skeletal staining

In situ hybridization was done as described by Hammerschmidt et al. (1996). Skeletal staining was done as described by van Eeden et al. (1996).

Photography

Photographs of live and stained embryos were taken with a Zeiss axiophot microscope, on Kodak ektachrome 64T or 160T slide film. Pictures were scanned using a Nikon coolscan slide scanner, and composite images were made using the Adobe Photoshop software package on a Macintosh computer.

RESULTS

Mutations affecting all larval fins

Mutations causing fin edema or fin degeneration were frequently identified in our screen. We kept such mutants if at least some mutant larvae had an air-filled swimbladder (43 out of an estimated 70). These 43 mutants can be grouped into six complementation groups, which can be subdivided into two phenotypic subgroups.

Mutants for the *pinfin* (*pif*), *nagel* (*nel*), *fransen* (*fra*) or *blasen* (*bla*) genes show bubbly fin folds at 24 hpf. Fluid accumulates in the fin folds, probably between the two epidermal layers. In contrast, mutants for *rafels* (*rfl*) and *frayed* (*fyd*) can be identified first at 48 hpf by the irregular fin fold edges and the presence of rounded-up cells in the fin folds. In the strongest alleles that we kept, the fin folds collapsed, and around 120 hpf all fin folds were severely reduced. An overview of the phenotypes is given in Fig. 2A-F. Mutants for all six genes are adult-viable and fertile. Mutant adults have a subtle phenotype: fin rays are present and are of normal length, but their number is variably reduced. The degree of reduction varies even between siblings homozygous for the same mutant allele. For example, in one severely affected *pif* mutant fish only 8 instead of the average 19 segmented lepidotrichia were present in the tail fin. In such cases the tail fin is bar-shaped instead of triangular, leaving the periodicity of the fin rays unchanged. In contrast one other homozygous *pif* mutant from the same family had a normal number of lepidotrichia in the tail fin.

Four complementation groups have been defined that lead to undulations of the fin epithelium. In *frilly fins* (*frf*) and *microwaved* (*med*) mutants the distance from the base of the fin fold to the border is slightly reduced. The fin epithelium has curtain-like undulations (Fig. 2G,H), especially at the tail tip. Homozygous mutants for *frf* and *med* are viable. *Med* mutants do not show an adult phenotype, whereas *frf* mutants do: fins of these fish are reduced in length; adults are 30-50% shorter and have a kink at the head-trunk boundary.

Mutations in *dreumes* (*dre*), *leprechaun* (*lep*) and *ukkie* (*uki*) have a subtle effect on the caudal fin fold, causing slight undulations of the fin epithelium. The larval pectoral fins are most strongly affected in these mutants and show a clear increase in

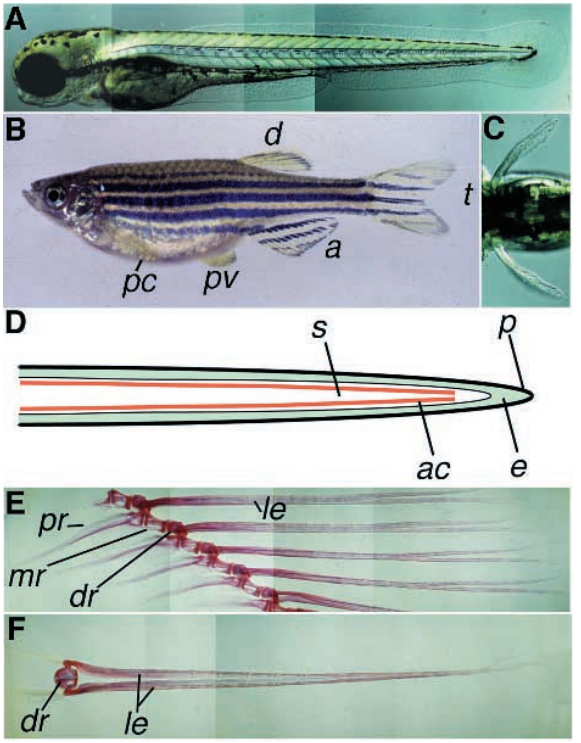

Fig. 1. Overview of wild-type fins in zebrafish. (A) Wild-type embryo at 84 hpf, caudal fin. (B) Adult female showing the five different types of fins. Going from anterior to posterior, the pectoral fins (pc) and the pelvic fins (pv), the dorsal fin (d), the anal fin (a) and the tail fin (t). (C) Dorsal view of an approximately 84 hpf wild-type embryo showing the pectoral fins. (D) Schematic drawing of a frontal section through an embryonic fin. Epiderm (e, green) is covered by periderm (p, black), in the subepidermal space (s) between the two epidermal layers actinotrichia (ac, red) are present in a double layer. Actinotrichia are also visible in Fig. 2A. (E) Skeletal staining of a part of the adult anal fin and the supporting skeleton. The endoskeletal radials consist of the proximal (pr), the medial (mr) and a distal (dr) part. The segmented lepidotrichia (le), belonging to the dermal skeleton are connected to the distal radial. (F) Frontal view of one adult anal fin segment showing the bilaterally arranged lepidotrichia connected to the distal radial (dr).

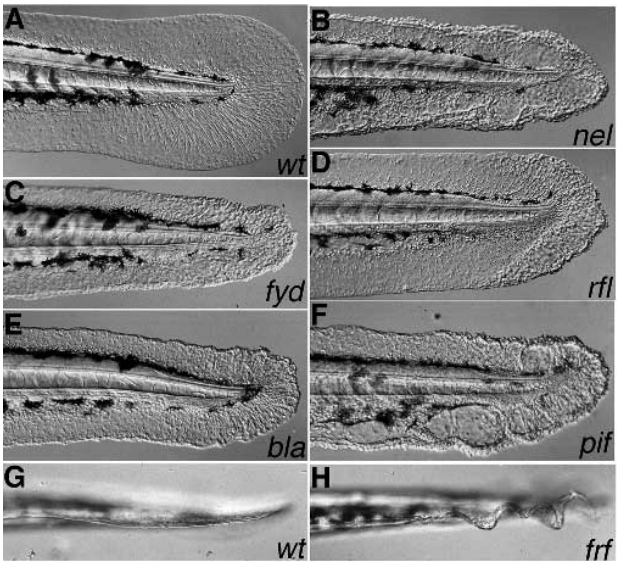

Fig. 2. Mutants in which the entire fin fold is affected. (A) Wild-type tail fin lateral view, 48 hpf. Note the thin lines that are running from the base to the tip of the fin, which represent the actinotrichia. *Nagel* (B), *frayed* (C), *rafels* (D), *blasen* (E) and *pinfin* (F) mutants, respectively. In *nel* and *pif* mutants the bubbly appearance is still visible; later the fin fold will degenerate. (G) Wild-type tail fin, ventral view. (H) *frilly fins* mutant tail fin, ventral view. The undulations in the fin fold are most pronounced at the tip of the tail.

size (Fig. 3A,B), which at 120 hpf leads to an unusual fold in the dorsal part of the pectoral fins. These mutants also have a smaller pupil (Heisenberg et al., 1996) and an abnormally shaped ear in the embryo (Whitfield et al., 1996). Homozygous larvae for all three genes survive to adulthood, but remain small in size. In homozygous *uki* adults the number of lepidotrichia was found to be slightly increased (1-6 per fin, except the anal fin). Adult *dre* and *lep* have not been analyzed in detail. In both *uki* and *dre*, but not in *lep*, adults the anal fin is absent (Fig. 4A,B); it is not clear how this phenotype is related to the larval fin phenotype.

Three further genes have been identified that are required for all larval fins. A mutation in *tutu* causes deletions of the fin folds. These deletions are variable in size, and sometimes holes are present in the fin epithelium. *Tutu* mutants are semiviable and have variably reduced fins. The embryonic phenotype of *u-boot* (*ubo*) mutants is similar to *rafels*; the fin folds are reduced, slightly thicker and have irregular borders. Mutants for this gene also lack the horizontal myoseptum (van Eeden et al., 1996) and have a reduced number of chromatophores (Kelsh et al., 1996). The fin folds of *moonshine* (*mon*) mutants also have irregular edges. In addition this mutation leads to defects in blood formation and to an abnormal iridophore pigmentation of the larvae (Kelsh et al., 1996; Ransom et al., 1996). A phenotypic comparison of all fin mutants is given in Table 2.

Mutations affecting the larval pectoral fins

Mutations in the *ikarus* (*ika*), *sonic-you* (*syu*) and *chameleon* (*con*) genes cause a variable reduction in the size of the pectoral fins. In all three mutants the degree of reduction frequently varies even between the left and the right fin fold in one embryo (Fig. 3C-E). *Syu* and *con* embryos in addition lack the horizontal myoseptum and have neural tube defects (Brand et al., 1996; van Eeden et al., 1996). *Ika* mutants do not have any other obvious defects and survive to adulthood. In *ika* adults the pectoral fin phenotype is also variable, ranging from absence to apparently normal pectoral fins (Fig. 4C,D,E). All other fins, including the paired pelvic fins, are normal in homozygotes.

Mutations in two genes, *dackel* and *boxer*, lead to a complex but very similar phenotype in the embryo. Mutants for the *dackel* gene (*dak*) (Fig. 3F) show a stronger phenotype in all aspects. Homozygotes form pectoral fin buds, but the pectoral fin does not develop further. As a result the pectoral fin fold is absent at 120 hpf. In addition to the fin phenotype, *dak* mutants have defects in the projection of retinal axons (Karlstrom et al., 1996; Trowe et al., 1996), in the formation of branchial arches (Schilling et al., 1996) and in ear morphology (Whitfield et al., 1996). Whole-mount in situ analysis with *sonic hedgehog* (*shh*) (Krauss et al., 1993), which is expressed in the posterior part of the fin bud, shows that *shh*

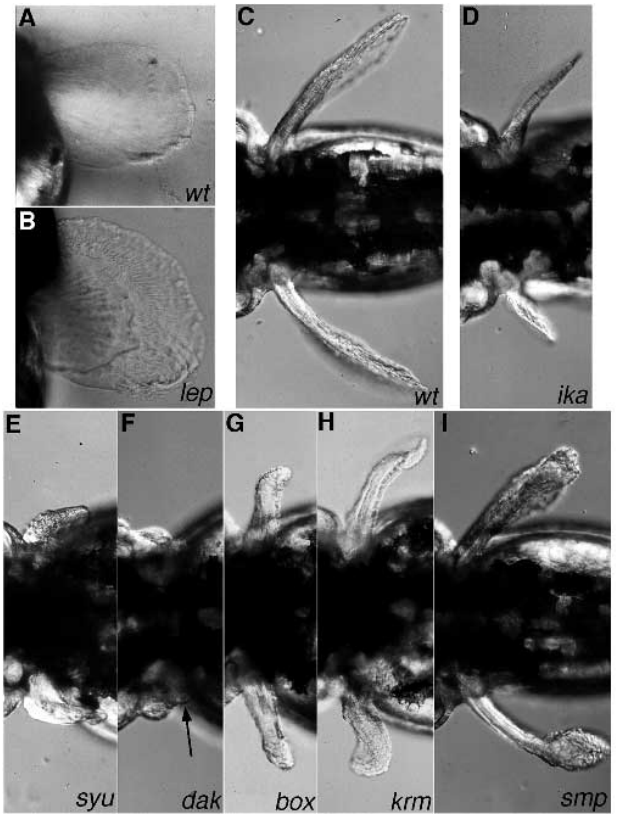

Fig. 3. Comparison of 3.5-day-old embryos with mutations affecting the pectoral fins. (A,B) Frontal views, (C-I) dorsal views. Wild type (A) and *leprechaun* (B) mutant embryos showing the increase in pectoral fin size. (C) Wild-type pectoral fins. Both mutations in *ikarus* (D) and *sonic you* (E) lead to a variable reduction in the size of the pectoral fins; in D an *ika* mutant with a clear difference in size between left and right pectoral fin is shown. (F) In *dackel* mutants pectoral fins are almost invisible at this stage (arrow). (G) *Boxer* mutants have reduced pectoral fins. The distal parts of the pectoral fins seem to be more affected than the proximal part and show signs of degeneration. This is not observed in *ika* and *syu* mutants. (H) Mutant *krom* embryo showing variably bent pectoral fins. (I) *stomp* mutant embryo with a degenerating pectoral fin fold.

expression is initiated but not maintained in *dak* mutants (Fig. 5). *Sonic hedgehog* encodes a secreted protein, which has been identified as a likely mediator of polarizing activity within the vertebrate limb bud (for a review, see Tabin, 1995). Mutations in the second gene, called *boxer* (*box*) (Fig. 3G), cause similar but somewhat weaker phenotypes. In this mutant the proximal part of the pectoral fins appears normal but the distal fin fold is absent, and rounded up cells are present on the tip of the

Table 1. Fin mutants in zebrafish

Gene	Alleles	Phenotype (viability)	Other references
Group 1: mutations affecting all fins			
nagel (*nag*)	*ta84, tb22, tl28b, tm42b, tm51, tm68, tn3, tp41, tr3, ty58, tc8, tu27, tn210, tm147c, tg254b, te335, tj258, tt231, tq207, tq274, tw234b, tl246a, ty124b*	Fin degeneration (v)	
rafels (*rfl*)	*tb233b, tp66, tc228, tm235b, tq266c, tr240, tc280b, tg308b, te217, tz245, te370b*	Fin degeneration (v)	
fransen (*fra*)	*tc17, tk219a, tm55*	Fin degeneration (v)	
pinfin (*pif*)	*tm95b, tj16, te262d, tr274*	Fin degeneration (v)	
frayed (*fyd*)	*tj2b*	Fin degeneration (v)	
blasen (*bla*)	*ta90*	Fin degeneration (v)	
Unresolved	*tz296*	Fin degeneration (v)	
frilly fins (*frf*)	*tf5, th42, tp34, ty69, tp207, tm317a, tz252*	Undulating fin abnormal body shape in adult (v)	
microwaved (*med*)	*tt281*	Undulating fin (v)	
tutu (*tut*)	*tg30*	Variable fin deletions (v)	
u-boot (*ubo*)	*tp39*	Fin degeneration, no horizontal myoseptum (l)	a
moonshine (*mon*)	*te345, ty57, tc239b, tz276, tu244b, tb222b, tc246b, tg234*	Fin degeneration, iridophore pigmentation, blood formation (l)	b,c
Group 2: mutations affecting mainly pectoral fins			
ikarus (*ika*)	*tm127c*	Small pectoral fins (v)	
sonic-you (*syu*)	*tq252*	Small pectoral fins, no horizontal myoseptum (l)	a
chameleon (*con*)	*tf18b, ty60, tm15a, th6*	Small pectoral fins, no horizontal myoseptum, thin neural tube (l)	a,d
krom (*krm*)	*tc227d*	Curly pectoral fins, paired fins reduced in adult (v)	
dackel (*dak*)	*tw25e, to273b, tf205, to79c*	Small pectoral fins, retinotectal projection, retarded ear, abnormal jaw (l)	e,f,g,h
boxer (*box*)	*tm4, tm70g, tg308c, te242, tm317c,tw24z, tp67z, to232*	Small pectoral fins, retinotectal projection, retarded ear, abnormal jaw (l)	e,f,g,h
Unresolved	*to79c*	Small pectoral fins, retinotectal projection, retarded ear, abnormal jaw (l)	e,g
stomp (*sto*)	*td11b*	Pectoral fin necrosis (v)	
dreumes (*dre*)	*tm146d*	Ear defects, small pupil, enlarged pectoral fins small adult, no anal fin (v)	f,i
leprechaun (*lep*)	*tq236, tj222*	Ear defects, small pupil, enlarged pectoral fins, small adult (v)	f,i
ukkie (uki)	*tc256d*	Ear defects, small pupil, enlarged pectoral fins (v)	f,i
Group 3: mutations affecting the ventral tail fin			
piggytail (*pgy*)	*dty40, tc227a, ta206, dti216, tx223, tm124*	Dorsalized, dominant phenotype: ventral tail fin deletion (d,l)	j
mini fin (mfn)	*ty130a, tc263, tf211, tt203, tv9, tf215a, tn217, tb241a*	Dorsalized, ventral tail fin deletion (l)	j
lost-a-fin (*laf*)	*tm110b*	Dorsalized, ventral tail fin deletion (l)	
swirl (*swr*)	*dta72, tc300a*	Dorsalized, dominant phenotype: ventral tail fin deletion (d,l)	j
mercedes (*mes*)	*tz209, tm305*	Ventralized, ventral tail fin duplication (v)	k
dino (*din*)	*tm84, tt250*	Ventralized, ventral tail fin mutliplication (l)	k
Group 4: mutations with reduced arches and smaller pectoral fins			
hammerhead (*ham*)	*to16, te296c*	Jaw defects, smaller pectoral fins (l)	l
pekinese (*pek*)	*td14*	Jaw defects, smaller pectoral fins (l)	l
head on (*hen*)	*tq251f, tt209, tu248*	Jaw defects, smaller pectoral fins (l)	l
jellyfish (*jef*)	*tw37*	Jaw defects, smaller pectoral fins (l)	l
schmerle (*she*)	*tg203e, th210*	Jaw defects, smaller pectoral fins (l)	l
Unresolved	*ty118a, td14, ty22e, to219, tv42b, ti23, tx239*	Jaw defects, smaller pectoral fins	l
Group 5: mutations affecting the adult fins only			
stein und bein (*sub*)	*tq289*	Reduced number of otoliths, reduced pelvic fins (v)	m
finless (*fls*)	*te370f*	No adult fins (v)	m
wanda (*wan*)	*dty127*	Reduced fins, abnormal pigmentation and body shape (d,?)	m
longfin (*lof*)	*dt2*	Long fins (d,v)	m
another longfin (*alf*)	*dty86*	Long fins (d,?)	m

v, homozygous viable; l, homozygous lethal; d, dominant mutation; ?, homozygous viability not tested or not known.

References: a, van Eeden et al., 1996; b, Kelsh et al., 1996; c, Ransom et al., 1996; d, Brand et al., 1996; e, Trowe et al., 1996; f, Whitfield et al., 1996; g, Shilling et al., 1996; h, Karlstrom et al., 1996; i, Heisenberg et al., 1996; j, Mullins et al., 1996; k, Hammerschmidt et al., 1996; l, Piotrowski et al., 1996; m, Haffter et al., unpublished results.

Table 2. Phenotypic comparison of fin mutants

Mutant	Structure							
	Initial fin bud	Larval pectoral fin	Caudal fin fold	Adult pectoral fin	Adult pelvic fin	Dorsal fin	Anal fin	Tail fin
nagel	wt	o,d	o,d	lr	p,na	lr	lr	lr
rafels	wt	d	d	lr	p,na	lr	lr	lr
fransen	wt	o,d	o,d	lr	p,na	lr	lr	lr
pinfin	wt	o,d	o,d	lr	p,na	lr	lr	lr
frayed	wt	d	d	lr	p,na	lr	lr	lr
blasen	wt	o,d	o,d	lr	p,na	lr	lr	lr
frilly fins	wt	w	w	r	r	r	r	r
microwaved	wt	w	w	wt	wt	wt	wt	wt
tutu	wt	vd	vd	p,na	p,na	r	r	r
u-boot	wt	ir	ir	–	–	–	–	–
moonshine	wt	ir	ir	–	–	–	–	–
ikarus	wt	s	wt	s	wt	wt	wt	wt
sonic-you	wt	s	wt	–	–	–	–	–
chameleon	wt	s	wt	–	–	–	–	–
krom	wt	c	wt	vd	vd	wt	wt	wt
dackel	wt	a	wt	–	–	–	–	–
boxer	wt	r	wt	–	–	–	–	–
stomp	wt	d	wt	wt	wt	wt	wt	wt
dreumes	wt	e,w	e,w	p,na	p,na	p,na	a	p,na
leprechaun	wt	e,w	e,w	p,na	p,na	p,na	p,na	p,na
ukkie	wt	e,w	e,w	p,li	p,li	p,li	a	p,li
piggytail	wt	na	vd	wt	wt	wt	wt	vd
lost-a-fin	wt	na	vd	–	–	–	–	–
swirl	wt	na	vd	wt	wt	wt	wt	vd
mercedes	wt	wt	du	wt	wt	wt	wt/du	wt/du
dino	wt	na	du	–	–	–	–	–
hammerhead	wt	s	wt	–	–	–	–	–
head on	wt	s	wt	–	–	–	–	–
jellyfish	wt	s	wt	–	–	–	–	–
schmerle	wt	s	wt	–	–	–	–	–
stein und bein	wt	wt	wt	wt	vd	vd	wt	wt
finless	wt	wt	wt	a	a	a	a	a
wanda	wt	wt	wt	vd,lr	vd,lr	vd,lr	vd,lr	vd,lr
longfin	wt	wt	wt	lo	lo	lo	lo	lo
another longfin	wt	wt	wt	lo	lo	lo	lo	lo

a, absent; c, curly; d, degeneration; du, duplication; e, enlarged; ir, irregular edges; li, number of lepidotrichia increased; lo, longer fins; lr, number of lepidotrichia reduced; na, not analyzed; o, oedema; p, present; r, reduced; s, small; vd, variable deletions; w, wavy; wt, wild type; –, not determinable, lethal mutation.

fin. Both mutations are lethal, probably due to lack of an air-filled swimbladder.

krom mutants have curled pectoral fins at 72 hpf (Fig. 3H). In most homozygous adult *krom* fish the part of both pectoral and pelvic fins that contains the lepidotrichia is absent. The three unpaired fins appear unaffected. Sometimes this adult phenotype was not fully penetrant; in such cases single pectoral or pelvic fins were present unilaterally.

The *stomp* (*sto*) gene is defined by a single allele. The mutant phenotype of this allele is variable in expressivity and only partially penetrant (Fig. 3I). In *smp* mutant embryos the pectoral fin epithelium shows weak degeneration, whereas the epithelium of the caudal fin fold is normal. No obvious phenotype is visible in *smp* homozygous adult fish.

14 additional mutants have been identified where outgrowth of both the pectoral fins and the branchial arches is reduced. Homozygous mutants do not have any tissue in front of their eyes. Complementation analysis in this phenotypic group has defined at least five genes: *hammerhead* (*ham*), *pekinese* (*pek*), *head on* (*hen*), *jellyfish* (*jef*) and *shallow chin* (*son*), and six mutations are unresolved. These genes are probably required for proper cartilage differentiation (Piotrowski et al., 1996).

Dorsalizing and ventralizing mutations affect the ventral tail fin

In our screen, two groups of mutations were isolated that affect the basic body plan of the embryo. They display dorsalization or ventralization phenotypes. The ventral tail fin is very sensitive to such mutations, and is therefore thought to be the ventralmost stucture of the embryo. These mutants are described in more detail elsewhere (Mullins et al., 1996; Hammerschmidt et al., 1996).

The dorsalizing mutants *swirl* (*swr*), *somitabun* (*sbn*), *piggytail* (*pgy*), *minifin* (*mfn*) and *lost-a-fin* (*laf*) show deletions of the ventral tail fin. For instance, heterozygous *pgy* mothers cause a dominant phenotype in heterozygous progeny: these embryos do not have the posterior part of ventral tail fin (Fig. 6A,B). Some heterozygotes survive to adulthood; they are often shorter than wild-type fish and lack the tail fin and sometimes part of the anal fin (Mullins et al., 1996).

Mutations in the ventralizing *mercedes* (*mes*) and *dino* (*dio*) genes result in a duplication or multiplication of the ventral tail fin, respectively (Fig. 6C,D) (Hammerschmidt et al., 1996). *Mes* homozygous embryos survive to adulthood and have partially duplicated tail and anal fins.

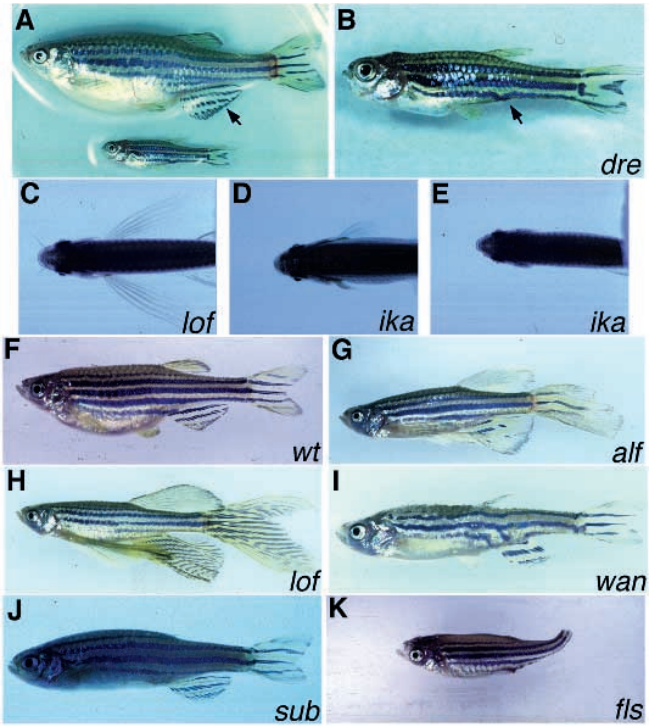

Fig. 4. Adult fin phenotype of several of the mutants identified in our screen. (A) Comparison of *dre* mutant embryo (bottom) with a wild-type sibling (top). (B) Higher magnification of the *dre* mutant, showing absence of the anal fin (arrow). *lof*/+; +/+ (C) and two *lof*/+;*ika*/*ika* fish (D,E), exemplifying the variablility of the *ika* phenotype. (F) Wild-type adult. Both *long fin* (H) and *another long fin* (G) heterozygous fish have an increased length of the fins. (I) *wanda* heterozyogous fish showing strong reduction of the dorsal fin, abnormal pigment pattern and abnormal body shape. (J) Severe example of *stein und bein* mutant fish. Both pelvic fins are absent and the dorsal fin is severely reduced. In less severe cases the dorsal fin is normal and only one of the two pelvic fins is missing. (K) *finless* mutant fish; all fins are missing.

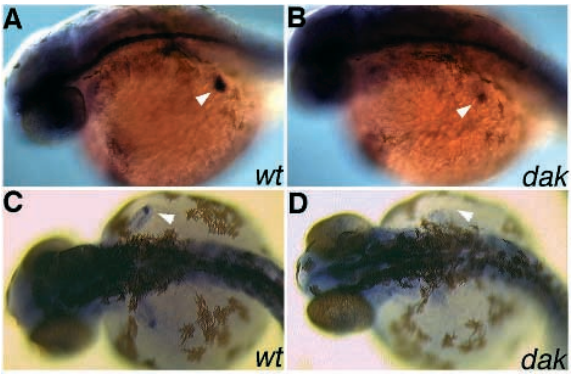

Fig. 5. Whole-mount in situ hybridization with *sonic hedgehog*. Wild type (A) and *dackel* mutant embryo (B) at 28 hpf. Mutant *dak* embryos have reduced staining in the pectoral fin buds (arrowheads); no differences were detected in other regions that express this gene (data not shown). Wild type (C) and *dak* mutant embryo (D) at 32 hpf. In contrast to wild type, no *shh* expression above background is detectable in the pectoral fins of *dak* mutants at this age (arrowheads).

Mutations affecting the adult fins

Three dominant mutations affecting fin development between larval and adult stages have been identified. *Another long fin* (*alf*, Fig. 4G) was found to cause formation of longer fins, similar to the previously described dominant *long fin* (*lof*) mutation (Fig. 4H) (Tresnake, 1981). In *lof* and *alf* heterozygotes fin rays are longer, but *alf* heterozygotes show in addition irregular segmentation and irregular bifurcation. A dominant mutation in *wanda* (*wan*, Fig. 4I), causes severe reduction of the fins in heterozygous fish. Distal fin rays, if present, appear unsegmented. In addition the fish have an abnormal body shape and an abnormal pigment pattern (Haffter, unpublished observations).

Homozygous adults for the *stein und bein* (*sub*) gene lack the pelvic fins in approximately 75% of the cases, and some of these fish lack the unpaired dorsal fin as well (Fig. 4J). This mutation was originally identified by the variable absence of otoliths in homozygous embryos. At present it is unclear if the two phenotypes are caused by a single mutation or by two independent, linked mutations. We have not identified any crossover events in 25 meioses. In total we isolated viable mutations in three genes, which result in defects in the paired fins of the adult. *ikarus* affects the adult pectoral fins, *stein und bein* the pelvic fins and *krom* affects both.

Homozygous larvae for the *finless* (*fls*, Fig. 4K) gene were only recently identified among the progeny of another mutant stock. In *fls* embryos no obvious fin defects are visible, while *fls* adults lack all fins.

DISCUSSION

Zebrafish fin fold differentiation and specification

Although the analysis of the fin mutants that we have isolated is in an early phase, comparison of the different types of mutants enables some conclusions to be drawn about the process that leads to the formation of fins. We did not find any mutants that affect only differentiation of the caudal fin fold without also affecting differentiation of the larval pectoral fins, indicating that

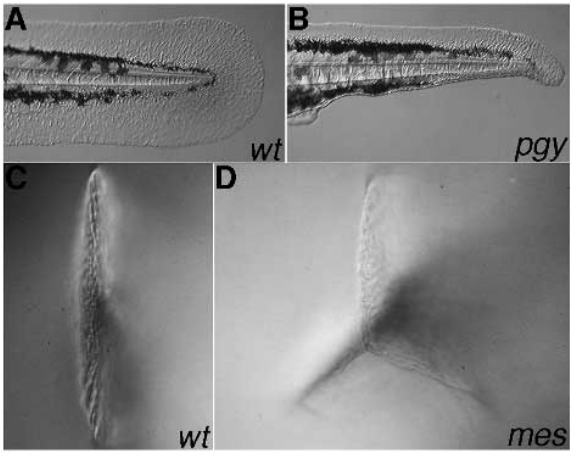

Fig. 6. Tail fin phenotype of weakly dorsalized and ventralized mutants. Wild type (A) and *piggytail* heterozygous embryo (B), side view. The ventral posterior part of the tail fin is missing. Wild type (C) and homozygous *mercedes* mutant embryo (D), viewed from posterior. On the ventral side of the *mercedes* tail two fins are visible, giving the tail fin the shape of a star with three arms.

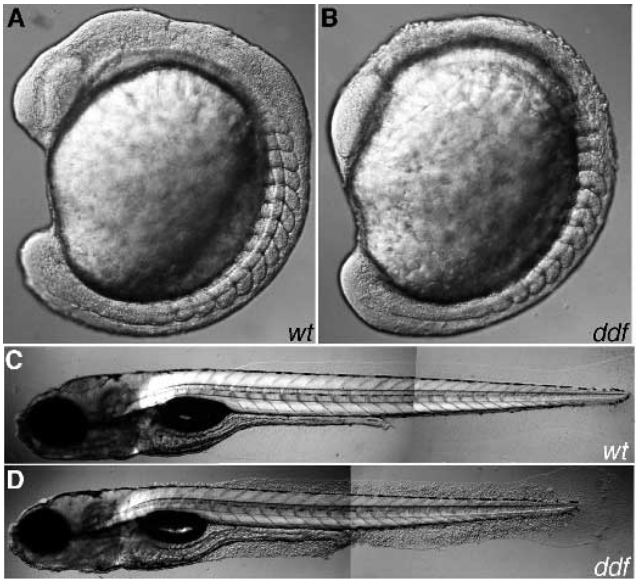

Fig. 7. Phenotype of *ddf* mutant embryos. Wild type (A) and *ddf* mutant (B) at the 15-somite stage. Rounded-up cells are predominantly present on the head and the prospective hindbrain. The head is apposed more closely to the yolk compared to wild type. (C,D) 5-day-old wild type and *ddf* mutant, respectively. Rounded-up cells are now easily visible in the fins. The angle between head and trunk appears normal at this stage.

the process that leads to the formation of the folded fin epithelium is similar in the tail fin and the pectoral fin.

Most of the fin mutations that lead to a degeneration of all fin folds of the embryo, often cause surprisingly mild phenotypes in the fins of the adult. Thus, proper differentiation of the larval fin fold is not a prerequisite to forming adult fins. It appears that a different set of genes function during larval stages to replace the embryonic fin folds. In the larval pectoral fin a set of additional genes is required, defined by the mutations that specifically affect pectoral fins.

The *finless* mutation is an example of the reverse scenario: no effects are seen in the larval fin fold, whereas adults lack all fins. This substantiates the idea that the differentiation processes forming the larval fin fold and adult fins are largely independent.

In contrast, in dorsalizing or ventralizing mutants, such as *swr*, *pgy* or *mes*, the deletion or duplication of the larval caudal ventral fin fold is a consequence of an abnormal specification process. In dorsalizing mutants, the absence of specification is not compensated in the adult and these fish lack the ventral tail fin. Thus, at least in the ventral part of the tail, adult fins require the same tissue specification as the larval fin fold.

Mutations affecting the pectoral fins

Dackel mutants show initiation of *sonic hedgehog* (*shh*) expression in the finbud, but fail to maintain it. *shh* is thought to be required for specifying the anteroposterior axis in the vertebrate limb (Riddle et al., 1993). How the phenotype that we observe in *dak* mutant embryos relates to the failure to maintain *shh* expression is not clear, since expression of *shh* is dependent on the expression of other patterning genes in the fin bud (Yang and Niswander, 1995). Thus, reduced expression of *ssh* in the later stages might reflect defects in other patterning genes required for proper *ssh* expression. In mouse there is evidence that the genes required for the patterning along the three axes of the tetrapod limb depend upon each other. For example, targeted disruption of the *wnt-7a* gene leads, in addition to dorsoventral patterning defects, to a reduction of the expression of *shh* and *fgf-4* (Parr and McMahon, 1995). *shh* might play a role in anteroposterior patterning, and *fgf-4* might be required for proximodistal patterning of the limb bud.

The *boxer* phenotype is similar to, but weaker than the *dackel* phenotype in all aspects. In *boxer* mutants only the most distal part of the larval pectoral fin seems to be affected, while the phenotypes of *ika*, *syu* and *con* mutants are best described as an overall reduction of the size of these fins without obvious differential defects along the proximodistal axis. *shh* expression in the pectoral fin buds appears normal in *boxer* mutants at 28 hpf. Since the defect in *box* embryos is restricted to the more distal parts of the fins, *shh* expression might be affected during later stages, which we did not examine. Preliminary analysis of *syu* and *ika* mutants at 28 hpf did not reveal any obvious defects in *shh* expression.

Might one of the mutants presented here be the result of a mutation in the *shh* gene itself? *Chamaeleon* and *sonic you* are amongst the most likely candidates, since they also seem to be involved in the process of patterning the neural tube and somites (Brand et al., 1996; van Eeden et al., 1996). All these processes are thought to be mediated by *shh*. Testing these mutations for linkage to the *shh* gene will soon answer this question.

Conclusion

The fin mutants described in this study will be of great value for studying various aspects of fin development. Those lacking the full complement of adult fins can be used to study the transition from an larval fin fold the to mature fin. Mutations affecting the pectoral fins buds can contribute to a genetic dissection of the steps by which pattern emerges in the fin/limb buds.

Table 3. Mutations affecting embryonic skin

Gene	Alleles	Phenotype (viability)
dandruff (*ddf*)	*ta50a, tj6, ti251, tc289*	Rounded up cells on skin, head retraction (v)
goosepimples (*gsp*)	*tk34a, tk38, tt221*	Rounded up cells on skin, head retraction (l)
bouillabaisse (*bob*)	*tu255a*	Rounded up cells on skin, head retraction (l)
penner (*pen*)	*to6*	Rounded up cells on skin (l)
Unresolved	*tk63*	Rounded up cells on skin, head retraction (l)

v, viable; l, lethal.

APPENDIX

Mutations affecting the embryonic skin

Mutations in least four genes, *goosepimples* (*gsp*), *dandruff* (*ddf*), *bouillabaisse* (*bob*) and *penner* (*pen*), cause defects in the embryonic skin (Table 3). *Ddf*, *gsp* and *bob* mutant embryos have similar phenotypes, while the *pen* phenotype is more subtle.

Mutant *ddf* embryos can already be recognized at the 12-somite stage by their abnormal head shape and the presence of rounded-up cells in the head region (Fig. 7A,B). At the pharyngula stage, loose cells can be seen floating around in the chorion and the entire surface of the embryo is covered with rounded-up cells. In addition to this skin defect, the head-trunk angle in mutant *ddf* embryos is abnormal. During the pharyngula stage, the head of wild-type embryos is curved around the yolk, but becomes straightened within the next 48 hours. *Ddf* mutant embryos, in contrast, still have a curved body axis at 72 hpf. Eventually about half of the embryos recover and develop an air-filled swimbladder (Fig. 7C,D). Homozygous *ddf* adults do not have an obvious phenotype.

Goosepimples mutants have a similar phenotype and like *ddf*, form a swimbladder. However, these homozygotes die within 2 weeks. *bob* mutants start to show general degeneration at the early pharyngula period and are completely lysed at 60 hpf.

Penner (*pen*) mutants can first be recognized at 96 hpf by an accumulation of round cells, predominantly in a region below the branchial arches and at the base of the pectoral fins (data not shown). Homozygous *pen* mutants die during larval stages.

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(Accepted 23 January 1996)