Development 123, 399-413
Printed in Great Britain © The Company of Biologists Limited 1996
DEV3352

Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva

Michael Granato[1,*], Fredericus J. M. van Eeden[1], Ursula Schach[1], Torsten Trowe[2], Michael Brand[1,†], Makoto Furutani-Seiki[1], Pascal Haffter[1], Matthias Hammerschmidt[1,‡], Carl-Philipp Heisenberg[1], Yun-Jin Jiang[1], Donald A. Kane[1], Robert N. Kelsh[1,§], Mary C. Mullins[1,¶], Jörg Odenthal[1] and Christiane Nüsslein-Volhard[1]

[1]Max Planck Institut für Entwicklungsbiologie, Spemannstrasse 35, Abteilung Genetik, [2]Abteilung Physikalische Biologie, 72076 Tübingen, Germany

*Author for correspondence at present address: University of Pennsylvania, Department of Cell and Developmental Biology, 605 Stellar-Chance, Philadelphia, PA19104-6058, USA
[†]Institut für Neurobiologie, Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany
[‡]Harvard University, Biolabs, 16 Divinity Avenue, Cambridge, Massachusetts 02138, USA
[§]University of Oregon, Insitute of Neuroscience, Eugene, Oregon 97403, USA
[¶]University of Pennsylvania, Department of Cell and Developmental Biology, 605 Stellar-Chance, Philadelphia, PA 19104-6058, USA

SUMMARY

Zebrafish embryos and larvae have stage-specific patterns of motility or locomotion. Two embryonic structures accomplish this behavior: the central nervous system (CNS) and skeletal muscles. To identify genes that are functionally involved in mediating and controlling different patterns of embryonic and larval motility, we included a simple touch response test in our zebrafish large-scale genetic screen. In total we identified 166 mutants with specific defects in embryonic motility. These mutants fall into 14 phenotypically distinct groups comprising at least 48 genes. Here we describe the various phenotypic groups including mutants with no or reduced motility, mechanosensory defective mutants, 'spastic' mutants, circling mutants and motor circuit defective mutants.

In 63 mutants, defining 18 genes, striation of somitic muscles is reduced. Phenotypic analysis provides evidence that these 18 genes have distinct and consecutive functions during somitic muscle development. The genes *sloth* (*slo*) and *frozen* (*fro*) already act during myoblast differentiation, while 13 genes appear to function later, in the formation of myofibers and the organization of sarcomeres. Mutations in four other genes result in muscle-specific degeneration.

103 mutations, defining at least 30 genes, cause no obvious defects in muscle formation and may instead affect neuronal development. Analysis of the behavioral defects suggests that these genes participate in the diverse locomotion patterns observed, such as touch response, rhythmic tail movements, equilibrium control, or that they simply confer general motility to the animal. In some of these mutants specific defects in the developing nervous system are detected. Mutations in two genes, *nevermind* (*nev*) and *macho* (*mao*), affect axonal projection in the optic tectum, whereas axon formation and elongation of motorneurons are disrupted by mutations in the *diwanka* (*diw*) and the *unplugged* (*unp*) genes.

Key words: locomotion, muscle development, neuronal development, spinal cord, motorneurons, motor circuits, reciprocal inhibition, zebrafish

INTRODUCTION

Although locomotion and behavior are more characteristic for juvenile and adult stages of aquatic vertebrate development, simple patterns of motility and behavior can in fact be observed during embryonic and early larval stages. In zebrafish embryos, the first spontaneous muscle contractions occur at 18 hours postferilization (Felsenfeld et al., 1990). At about 24 hours, mechanical stimuli induce a wiggle reaction that is independent of where a stimulus is applied along the body axis. At 48 hours, the first signs of differentiated response reactions to tactile stimuli are recognizable. Although the embryo is resting most of the time, touching the tail tip induces a fast and straight movement away from the stimulus source. In contrast, mechanical stimuli near the head of the embryo induce a fast escape response, where the embryo turns 180° along its horizontal body axis. At 96 hours the larva is freely swimming, changes swimming directions spontaneously, and is able to direct its swimming towards targets. Thus embryonic and early larval motility and behavior of the zebrafish can be divided into different periods characterized by different patterns of locomotion, similar to the early swimming behavior described in *Xenopus* (van Mier, 1988, 1989).

Our knowledge about genes mediating and controlling embryonic locomotion of vertebrates comes from a large number of studies on skeletal muscle development and

neuronal development using different approaches. Myogenic regulatory genes were identified by their potential to activate endogenous muscle-specific genes when introduced in non-muscle cell types (reviewed by Olson and Klein, 1994). The functions of these myogenic factors in an embryonic context were explored by gene inactivation through homologous recombination in transgenic mice (reviewed by Buckingham, 1994; Olson and Klein, 1994; Weintraub, 1993). These results have enabled the assignation of distinct functions to the different myogenic factors during muscle formation (reviewed by Rudnicki and Jaenish, 1995). A further feature of muscle development is the differential expression of contractile protein isoforms, which correlates with the expression of myogenic regulatory factors (Buckingham et al., 1992; Ontell et al., 1993a,b).

Genes involved in patterning the vertebrate neural plate or defining neuronal identity have mainly been isolated by homology cloning from invertebrates. The potential roles of the vertebrate homologues have been examined by overexpression or by expression of dominant negative forms of the candidate molecules in *Xenopus* embryos, and by gene knockouts in mice (Chitnis et al., 1995; Hemmati-Brivanlou et al., 1994; Hemmati-Brivanlou and Melton, 1994; Joyner and Guillemot, 1994; Tsuchida et al., 1994). Neuronal guidance and target recognition as intrinsic properties of the nervous system have long been proposed, but only recently have biochemical approaches elucidated the nature of a group of proteins capable of influencing growth cone behavior (Kennedy et al., 1994; Messersmith et al., 1995; Serafini et al., 1994; Tessier-Lavigne, 1994; for reviews, see Dodd and Schuchardt, 1995 and Marx, 1995). In addition, a diverse collection of mouse mutants displaying behavioral defects has been studied, and in some cases the behavioral defect has been correlated with specific defects in the developing CNS (D'Arcangelo et al., 1995; Ghetti and Triarhou, 1992; Lalonde and Thifault, 1994; Norman et al., 1995; Ogawa et al., 1995). In zebrafish, striated muscle and CNS development have been described in detail and genetic screens have identified a number of genes with functions in muscle or CNS development (Felsenfeld et al., 1990; Grunwald et al., 1988; Kimmel et al., 1985, 1991; Kuwada, 1995; Papan and Campos-Ortega, 1994; Van Raamsdonk et al., 1974, 1978, 1982; Waterman, 1969; Westerfield et al., 1990).

So far no systematic approach towards the identification of components controlling and mediating embryonic locomotion in vertebrates has been reported. Large-scale screens in invertebrates have been extremely successful in identifying many components functionally involved in the various pathways of muscle or neuronal development. In *C. elegans*, a large collection of mutants affecting motility or simple behaviors of the larvae has been generated during past decades (Bargmann, 1993; Brenner, 1974; reviewed by Thomas, 1994). Phenotypic and molecular analyses of these mutants have revealed insights into muscle formation (Ahnn and Fire, 1994; Williams and Waterston, 1994), neuronal cell type specification and neuronal connectivity (for reviews, see Culotti, 1994; Duggan and Chalfie, 1995; Hedgecock, 1992). Similarly, in *Drosophila* a number of genetic screens have identified genes controlling neuromuscular specificity, neuronal pathfinding, PNS patterning and mechanosensory transduction (Kernan et al., 1994; Salzberg et al., 1994; Van Vector et al., 1993).

We sought genes mediating and controlling the different patterns of embryonic locomotion in the zebrafish, and therefore included a simple touch response test in our large-scale genetic screen. We identified 166 mutations causing reduced or abnormal locomotion behavior of the zebrafish embryo. These mutants fall into 14 different phenotypic groups, defining at least 48 genes. Four groups comprising 63 mutants have visible defects in trunk muscle formation or maintenance. These 63 mutants define at least 18 genes and phenotypic analysis suggests that they have specific functions in myoblast differentiation, myofibril organization or maintenance of muscle tissue. 103 motility mutants, defining at least 30 genes, do not have visible defects in trunk muscle development. An initial characterization of these mutants shows that they represent a diverse collection of behavioral mutants with no or reduced motility, mechanosensory defects, 'spastic' motility, circling behavior and motor circuit deficits. Some of these mutants have unique defects in axon formation of primary motorneurons or defects in retinotectal projection.

MATERIALS AND METHODS

Maintenance and breeding

Maintenance and breeding of fish has been described elsewhere (Mullins et al., 1994); identification and isolation of mutants, as well as complementation analysis, are described in an accompanying paper by Haffter et al. (1996). For complementation testing with existing motility mutants, *fub* and *nic* stocks were kindly provided by Dr Kimmel's laboratory in Eugene.

Screening for embryonic motility

Between 48 and 60 hours postfertilization, 12 larvae from every F_2 cross were subjected to a tactile stimulus. Using a needle, a gentle stimulus was applied at the tail of the larvae and its reaction observed. Wild-type larvae at this stage of development are not very active and are resting most of the time. Upon application of the tactile stimulus they swim away from the source of the stimulus. The same larvae were also visually inspected for motility after 5 days of development. The dish was stirred so that most of the larvae were swirled to the center of the dish. Wild-type larvae spontaneously start swimming away from the center. Motility mutants were anesthetized and the trunk muscle striation was examined using the incident light of the dissecting microscope. Somitic trunk muscles of wild-type larvae display a rainbow-colored birefringency. For further analysis of muscle mutants their axial skeletal muscle was examined using polarized light on a Zeiss Axiophot, as described by Felsenfeld et al. (1990).

Video-taping of embryos and larvae

Embryos and larvae were video-taped using a standard video camera and video recorder. Single frames were captured onto a connected computer and processed using Adobe Photoshop software.

Histological section of trunk muscle tissue

Staged embryos and larvae were anesthetized and fixed in 2.5% glutaraldehyde in PBS for 20 hours at 4°C, rinsed several times with PBS and embedded in Epon. Sections were taken from the lateralmost position of the first six somites and counterstained with toluidine blue to reveal cellular morphology.

Immunohistochemistry

Antibody labeling was performed as described by Bernhardt et al. (1990) and detected using a peroxidase kit (Vector), according to the manufacture's instructions. The znp-1 antibody stains axonal projec-

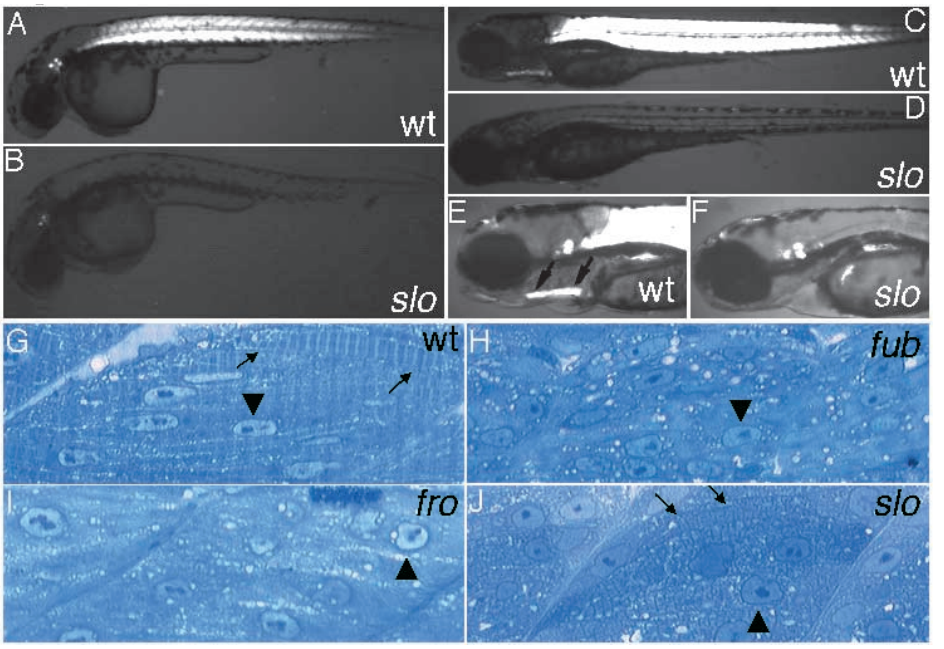

Fig. 1. Comparison of muscle differentiation between wild-type (A,C,E,G), *slo* (B,D,F,J), *fro* (I) and *fub* (H) embryos using polarized light (A-F) or trunk muscle sections (G-J). At 30 hours birefringency is reduced in *slo* embryos (B) compared to wild-type embryos (A). At later stages (96 hours) muscle birefringency is detectable in somitic muscle and in jaw muscle of wild-type larvae (C, E), while in *slo* mutants (D, F) birefringency is completely absent in somitic and jaw muscles. The arrows in E delineate the jaw muscles. (G) Sagital-lateral section through 36 hours wild-type somitic muscle tissue showing muscle fibers (arrows) and elongated nuclei (arrowhead). In *fub* mutants muscle fibers are absent; however, the nuclei are elongated (arrowhead) and resemble those of wild type (H). In contrast, nuclei in *fro* (I) and *slo* (J) are round (arrowhead). (J) Occasionally muscle fibers can be detected in *slo* somitic tissue (arrows).

tions of primary motorneurons and has previously been described (Trevarrow et al., 1990).

Dye labeling of retinotectal projections

Lipophilic tracer dyes were injected at specific position of the retina, as described by Baier et al. (1996).

RESULTS

Phenotypic groups and complementation analysis

Applying a fast and simple motility test, a total of 166 mutants with primarily locomotion defects were identified. More than 95% of these motility mutants are embryonic lethal; in only seven cases were homozygous viable alleles isolated (labeled with † in Tables 1-3). Mutants with visible defects such as abnormal brain morphology, brain degeneration, general degeneration or abnormal ear development, all defects that might be the primary cause of behavioral abnormalities, were discarded or described elsewhere (Table 4; Brand et al., 1996a; Furutani-Seiki et al., 1996; Jiang et al., 1996; Whitfield et al., 1996). Mutants with

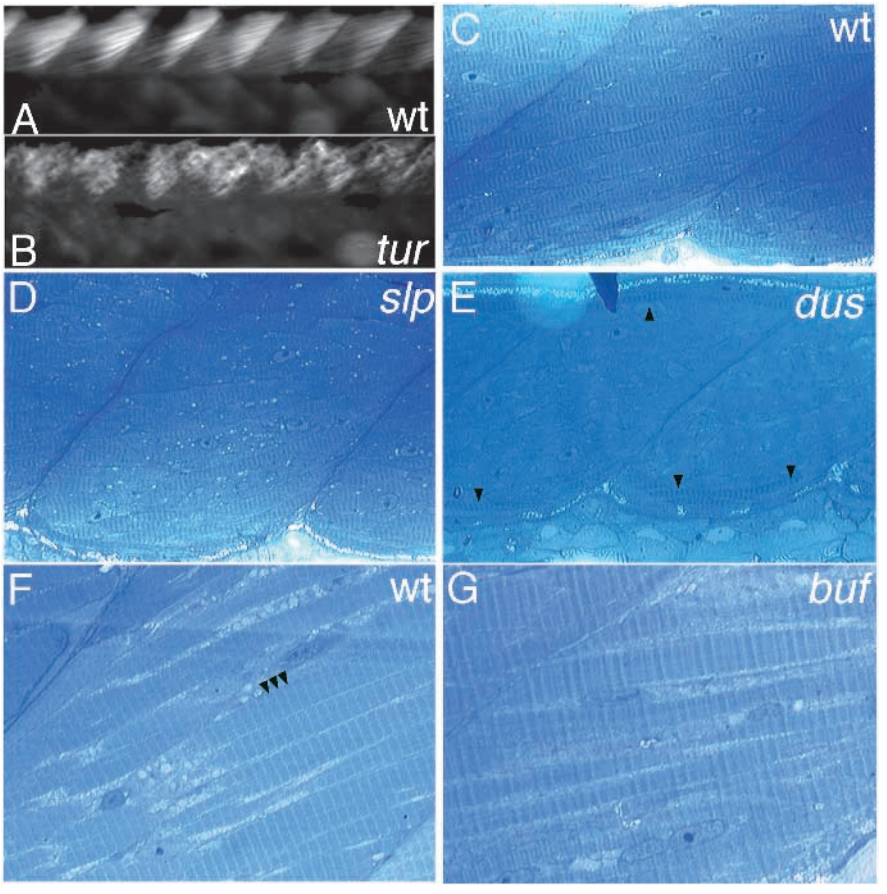

Fig. 2. Comparison of muscle birefingency between wild type and muscle mutants. Muscle fibers are affected in *tur* (B), *slp* (D), *dus* (E) and *buf* (G) mutants compared to wild type (A,C,F). Optical section through lateral myotomes in a 72-hour wild-type embryo (A) shows muscle fibers in the dorsal half of the somites. In *tur* mutants, similar to all mutants in the phenotypic groups A2 and A3, birefringency appears reduced or disorganized (B). In *slp* mutants about 20% fewer muscle fibers are visible (D) and in *dus* mutants (E) only residual fibers can be seen (arrowheads), compared to wild-type embryos at 60 hours (C). In wild-type muscle tissue (F) segmental organization of the sarcomeres is visible; the arrowheads indicate three individual units. In *buf* mutants sarcomeric organization is perturbed (G). Sarcomeres appear less evenly spaced and reduced in size.

behavioral defects at 48 to 60 hours were kept only if no signs of general degeneration were visible after 5 days of development. We identified new alleles of two previously described genes, *fibrils unbundled* (*fub*) and *nicotinic receptor* (*nic*) (Felsenfeld et al., 1991; Westerfield et al., 1990). The 166 motility mutants were subdivided into three major groups. Mutants in group A have no or reduced motility combined with a reduction of muscle striation, whereas those in groups B and C have no obvious muscle defects. In group B mutants, embryonic locomotion is abolished or reduced, while in group C mutants, motility is abnormal rather than reduced.

Motility mutants with defects in trunk muscle development (A1 to A4)

Among the 166 motility mutants, 63 mutants showed a reduced birefringency of somitic muscle fibers, suggesting defects in muscle development. These muscle mutants were further subdivided into four different phenotypic groups (A1 to A4) and complementation analysis was performed within the different groups. The results are shown in Table 1.

(A1) Immotile mutants, with no or reduced muscle striation

Mutations in three genes, *frozen* (*fro*), *sloth* (*slo*) and *fibrils unbundled* (*fub*), result in completely paralyzed embryos. The mutant phenotypes become visible by 20 hours, as spontaneous muscle contractions become apparent in wild-type embryos but not in mutant embryos. Mutant embryos remain completely immotile and fail to hatch from their chorions. Birefringency of somitic muscle tissue and later also of the jaw muscles is strongly reduced or completely absent in these mutants (Fig. 1A-F). The birefringency in wild-type muscles is caused by parallel thread-like myofibrils within muscle fibers. Loss of birefringency might therefore indicate absence of striated muscle tissue, lack of muscle fibers or loss of fiber organization. To distinguish between these possibilities we compared histological sections of trunk muscle from wild type to *slo*, *fro* and *fub* mutant embryos.

Analysis of these sections revealed that at earlier stages of development (36 hours) mutant *slo*, *fro* and *fub* embryos already lack organized muscle fibers (Fig. 1G-J). Furthermore, muscle cell nuclei of *slo* and *fro* mutant embryos are round and not elongated as in *fub* or wild-type myotomal cells (Fig. 1G-J). Mutations in the *fub* gene have previously been isolated and the gene is thought to act during early stages of myofibril organization, rather than in cellular differentiation of muscle cells (Felsenfeld et al., 1991). Staining of *slo* and *fro* mutant embryos with an RNA probe specific to *sna1*, which is normally expressed in the paraxial mesoderm, did not reveal any differences to control embryos (data not shown). Thus, the mesodermal cells that eventually give rise to the somitic muscle tissue are present in *fro* and *slo* mutant embryos, suggesting indispensable functions of both genes after specification of paraxial mesoderm but before myofiber formation.

(A2) Mutants with reduced motility, reduced striation and (A3) mutants with reduced motility, reduced striation and heart defects

Between 48 and 60 hours, wild-type embryos hatch from their chorions and react to tactile stimuli with a fast escape response. Mutant *turtle* (*tur*), *buzz-off* (*buf*), *faulpelz* (*fap*), *slow motion* (*slw*), *schnecke* (*sne*), *hermes* (*hem*), *duesentrieb* (*dus*) and *mach two* (*mah*) (all A2) and *slop* (*slp*), *jam* (*jam*) and *slinky* (*sky*) (all A3) embryos also hatch from their chorion, but only react with a low-speed escape response when stimulated. Comparison with wild-type embryos reveals a reduction of birefringency in these mutants, suggesting that defects in somitic muscle development are the primary cause for the reduced locomotion of the affected embryos. Optical sections of muscle tissue from these mutant embryos at 72 hours show a reduction in birefringency compared to wild types (Fig. 2A,B). To further characterize these mutants, we examined sections of mutant trunk muscle tissue under a compound light microscope. In most sections analyzed, muscle fiber arrangement appeared indistinguishable from wild-type-derived muscle tissue. However in sections of three mutants we found significant changes in

Table 1. Mutants with defects in locomotion and somitic muscle

Gene	Abbreviation	n	Alleles
Class A: reduced muscle striation			
A1: immotile, no or reduced striation			
sloth	*slo*	2	*tu44c, tm201*
frozen	*fro*	1	*to27c*
fibrils unbundeled	*fub*	2	*ta51b, tu44a*
A2: reduced motility, reduced muscle striation			
*turtle**	*tur*	22	*ta86a*§,‡, *tm158, ts39, tc236a, tc310*§, *te305b, tg307, th236b, th271, ti262b, tk247a, tl203, tm209c, tm243c, tm292a, tm314, to255a, tt225, tt272a, tu216a, tv214b, tz268*
*buzz-off**	*buf*	5	*te228*‡, *th234, ti209, ts215, ts246*
faulpelz	*fap*	2	*ta218, tc222*
slow motion	*slw*	1	*tl225b*
schnecke	*sne*	1	*tt253*
hermes	*hem*	2	*tt286b, tt227b*
duesentrieb	*dus*	1	*tq250*
mach two	*mah*	1	*tm127b*†
Unresolved		7	*tb222c, te252c, tg248c, tj254e, tm271b, tu254a, tt254b*
A3: reduced motility, reduced muscle striation, heart			
slop	*slp*	1	*tq235c*
jam	*jam*	1	*tr254a*
slinky	*sky*	1	*ts254*
A4: reduced striation, somite degeneration			
sapje	*sap*	2	*tm90c, tj7*
softy	*sof*	3	*te234, tz212, tm272a*
schwammerl	*sml*	2	*tt279, tg252*
runzel	*ruz*	1	*tk258a*
Unresolved		5	*tu238a, tf212b, teg15a, ta222a, tk209a*

*Different allele strengths observed; †viable allele; ‡strongest allele; §allele lost.

muscle fiber organization. In *slp* mutant embryos, the amount of trunk muscle fibers is reduced (Fig. 2D). About 80% muscle fibers are detectable in the somitic tissue compared to equivalent sections from wild-type muscle (Fig. 2C). *Slp* embryos, similar to *jam* and *sky* (group A3) embryos, share an additional phenotype. At around 48 hours the hearts of these mutants beat slower than in wild type; the heart ventricles are slightly smaller and blood accumulates on the yolk (for details of heart morphology and function, see Chen et al., 1996). At 72 hours the heart valves do not work efficiently, causing a reduced circulation. It is not clear whether the amount of cardiac muscle fibers is decreased to an extent similar to the somitic muscle fibers.

In sections of *dus* mutant embryos, reduction of muscle fibers is more pronounced. Most of the examined muscle tissue is deprived of muscle fibers and only a few fibers are visible (compare Fig. 2E with 2C). In contrast to *slp* and *dus*, in *buf* mutant embryos, muscle fiber organization rather than the total amount of trunk muscle fibers is affected. In wild-type embryos, the muscle fibers contain longitudinal striations visible with light microscopy. These longitudinal striations consist of parallel myofibrils. Each myofibril consists of repeated, even spaced segments, called sarcomeres. In mutant *buf* embryos sarcomeric segmentation is visible, but the segments appear reduced in size and irregularly spaced compared to those of wild-type muscle fibers (Fig. 2F,G).

(A4) Mutants with reduced striation and somite degeneration

Muscle fiber formation in mutant *sapje* (*sap*), *softy* (*sof*), *runzel* (*ruz*) and *schwammerl* (*sml*) embryos is initially indistinguishable from wild type. Eventually, mostly around 96 hours, mutant larvae swim more slowly and muscle birefringency is reduced (Fig. 3A-C). Lesions become obvious in somitic segments. In mutant *sap* or *sof* larvae these lesions are restricted to small somitic areas (Fig. 3E,F), while in *ruz* mutants they are spread over the entire somites (Fig. 3G). In all these mutants, degeneration is restricted to somitic muscle tissue; heart and jaw muscles are not obviously affected. Other late developing structures like the liver or the cartilaginous jaw appear normal in mutant larvae, indicating that

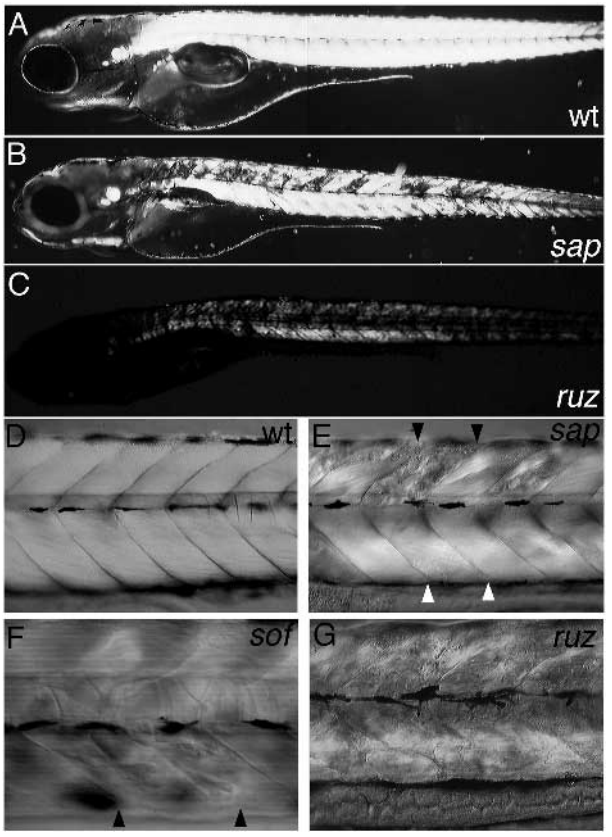

Fig. 3. Loss of birefringency and lesions in the somites become apparent in somite degeneration mutants. In *sap* (B) and *ruz* (C) mutants birefringency is decreased around 96 hours, compared to wild type (A), and lesions become visible in the somitic muscle tissue. (D) Lateral view of a wild-type larva around 96 hours; the somitic segments are separated by distinct boundries. (E,F) In *sap* and *sof* mutants degeneration affects a few individual somitic segments (black arrowheads indicate somite boundaries). The ventral part of the affected somite (E) appears normal (between white arrowheads). (G) In *ruz* mutants degeneration is affecting all somites.

Table 2. Mutants with reduced locomotion

Gene	Abbreviation	n	Alleles
Class B: reduced motility, normal striation			
B1: immotile			
sofa potato	*sop*	3	*tj19d, ts29, tf207c*
relaxed	*red*	1	*ts25*
nicotinic receptor	*nic*	1	*tk48d*
B2: immotile and heart affected			
heart attack	*hat*	1	*te313*
herzschlag	*hel*	1	*tg287*
Unresolved		1	*tk34b*
B3: recoverer			
unplugged	*unp*	1	*te314b*
Unresolved		1	*tf228a*
B4: twitch once			
*shocked**	*sho*	3	*ta51e, te301, ta229g*‡
twitch once	*two*	3	*th26, tm335, tq265b*
B5: reduced touch respond			
alligator	*ali*	1	*tm342*
fakir	*far*	1	*tm154*†
macho	*mao*	1	*tt261*
steifftier	*ste*	1	*tf220*
crocodile	*cro*	1	*tw212d*
schlaffi	*sla*	3	*ty112, th239, tg230*§
B6: reduced motility, normal striation			
slumber	*slm*	3	*tt208, tm221, tm132c*
Unresolved		23	*tf41b, tf47b, tp94g, tk64a, tm110a, tb204a, tc244d, tc246c, tc319b, tc226c, tg280d, th242f, tk219c, tl226b, tq221b, ts299a, tt215b, tu25a, tu227a, tw222a, ty204a, tz272a, tz309a*

*Different allele strengths observed; †viable allele; ‡strongest allele; §allele lost.

the phenotype is not due to a general degeneration process of the embryo.

Mutants with reduced (Group B) or abnormal (Group C) embryonic locomotion

In embryos of the remaining 103 motility mutants, we could not detect obvious defects in trunk muscle development. These mutants were subdivided into ten phenotypic groups according to their behavioral phenotype, and complementation analysis was performed within these groups (Table 2). Complementation is almost complete in eight of the ten groups, but in the remaining two groups (B6 and C4) the large number of mutants with similar phenotypes render efficient complementation analysis difficult. In contrast to the group of muscle mutants (A1 to A4), the behavioral phenotypes of these mutants are more diverse, resulting in more phenotypic groups. Mutants in six groups (B1 to B6) have a reduced motility, whereas in the remaining four groups (C1 to C4), embryonic locomotion is abnormal rather than reduced.

Mutants with reduced embryonic locomotion (B1 to B6)

(B1) Immotile mutants and (B2) immotile mutants with heart defects

Mutations in the five genes *sofa potato* (*sop*), *relaxed* (*red*), *nicotinic receptor* (*nic*), *heart attack* (*hat*) and *herzschlag* (*hel*), lead to completely immotile embryos by 24 hours. Mutant embryos do not react to touch and eventually fail to hatch from their chorion. When dechorionated manually, mutant *sop*, *led* and *nic* embryos still remain immotile but otherwise develop until 120 hours as do their wild-type siblings. A mutation in the *nic* gene, *b107*, has previously been isolated. Mutant embryos fail to accumulate functional nicotinic receptors at the neuromuscular junctions (Westerfield et al., 1990).

Mutations in *hat* and *hel* affect motility and in addition heart function. Embryos are completely immotile, similar to *sop*, *nic* and *red* mutants. At 24 hours a normal-looking heart is visible in mutant embryos, but it only beats with low frequency. Lack of a functional heart abolishes blood circulation, so that the affected embryos form edemas and die within 48 hours. *Still heart* (*sth*) *casanova* (*cas*) and *tw212e* embryos have similar phenotypes and were identified on the basis of their heart phenotype. These mutants are listed in Table 4 and are described elsewhere (Chen et al., 1996).

(B3) Recovering mutants

At 24 hours, mutant *unplugged* (*unp*) embryos are also almost immotile, but in contrast to *sop*, *led* and *nic* mutants, recover from their mutant phenotype. Embryonic motility improves and by the sixth day of development *unp* mutant embryos can swim short distances. Using an antibody that recognizes primary motorneurons, we find abnormal morphology and reduced outgrowth of ventrally projecting primary motorneurons (Eisen et al., 1986) in mutant *unp* embryos. In wild-type embryos a set of three primary motorneurons per somitic hemisegment projects axons to distinct muscle targets. Each of these primary motorneurons can be identified by its position within the spinal cord and its stereotypic axonal projection (Fig. 4A). By 28 hours one of the three primary motorneurons, CaP, has extended its axons towards the ventral part of the somite (Fig. 4B). In *unp* mutants, ventrally extending

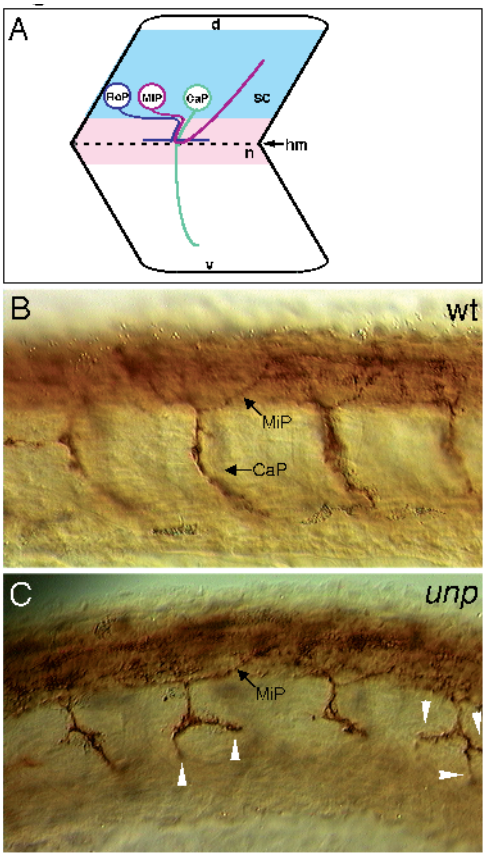

Fig. 4. Motorneuron development is affected in *unp* mutants. (A) The three primary motorneurons in the ventral half of the spinal cord (sc), CaP, MiP and RoP, occupy unique positions along the antero-posterior axis and extend stereotypic, axonal projections. (B) The antibody znp-1 stains the CaP axons in the ventral and the MiP axons in the dorsal somites of wild-type embryos. (C) In *unp* mutants MiP axons appear to elongate normally toward dorsal, while axons extending ventrally have abnormal morphology. They form multiple branches (arrowheads) and do not extend as far as in wild-type embryos. Whether these aberrant axons originate from CaP somatas is not clear. CaP, caudal primary motorneuron; MiP, middle primary motorneuron; RoP, rostral primary motorneuron; d, dorsal; v, ventral; sc, spinal cord; n, notochord; hm, horizontal myoseptum.

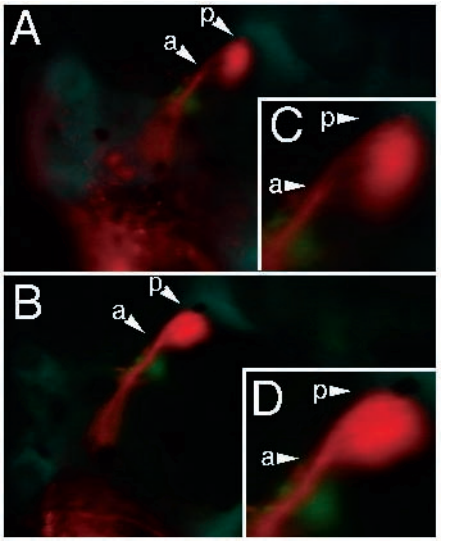

Fig. 5. Retinotecal projection phenotype in *mao* mutants. In wild-type larvae motorneuron-dye-labeled nasodorsal axons enter the optic tectum and defasciculate in the posterior half of the tectum (A,C). In *mao* mutants these axons defasciculate prematurely in the anterior half of the tectum (B,D). a, anterior tectum; p, posterior tectum.

axons have an abnormal morphology, with one or two neurites branching off the extending axon and projecting in different directions. Although the axons project ventrally, as expected for CaP axons, it is not clear if these axons originate from CaP somata, as the identity of these neurons was not independently determined. The middle primary motorneurons (MiP), which extend their axons toward the dorsal part of the somites, appear normal in *unp* embryos (Fig. 4C). To determine if mutations in the *unp* gene generally affect axonal morphology of spinal cord neurons, we labeled axonal projections of commissural interneurons and mechanosensory neurons (Rohon-Beard neurons) in the spinal cord of mutant *unp* embryos with specific antibodies. In both cases we found that the labeled projections in mutant embryos were indistinguishable from those in wild-type embryos (M.G., unpublished observations).

(B4) Twitch once mutants

Mutant *shocked* (*sho*) and *twitch once* (*two*) embryos react around 48 hours with just a single tail flip when a tactile stimulus is applied. Around 120 hours mutant larvae jump upon touch but do not swim. Weak vibrations only in the tail are visible and no alternating and rhythmic tail movements occur.

(B5) Mutants with reduced touch response

Six genes are indispensable for touch perception of the zebrafish embryo: *alligator* (*ali*), *fakir* (*far*), *macho* (*mao*), *steifftier* (*ste*), *crocodile* (*cro*) and *schlaffi* (*sla*). Mutant embryos in this phenotypic class are less sensitive to tactile stimuli than wild type and do not swim away when touched. If, however, mutant larvae are swirled, they start swimming, suggesting that touch perception rather than swimming capability is affected. Between 24 and 36 hours, mutant *far* embryos are insensitive to touch in the tail region but sensitive in the yolk region. Touch sensitivity improves over time and by around 120 hours, mutant *far* larvae are almost indistinguishable from their wild-type siblings. Homozygous larvae survive to adulthood and are fertile, and no behavioral phenotype is obvious in these adult fish. In contrast, mutant *mao* larvae do not survive to adulthood but die after 6 days of development. *Mao* embryos are almost insensitive to touch at 48 hours. Interestingly, we identified additional defects at later stages, affecting retinotectal axonal projection. In 120-hour wild-type larvae, nasodorsal axons terminate in a topographically defined field in the posterior half of the optic tectum (Fig. 5A,C; for details, see Baier et al., 1996; Trowe et al., 1996). In *mao* larvae the nasodorsal axons reach the optic tectum and start to defasciculate prematurely in more anterior positions of the optic tectum, resulting in an enlarged termination field (Fig. 5B,D). Temporoventral axons appear to project to the anterior part of the tectum, as in wild-type larvae.

In *ste* mutant embryos, consecutive tactile stimuli decrease the touch response of the embryo. After the first stimulus embryos jump but do not swim; after the next stimulus the only visible reaction of the mutant embryos is a slight vibration with their tail. Further stimuli do not result in any detectable activity. Wild-type embryos do not show obvious exhaustion at this rate of stimulation.

(B6) Mutants with reduced motility

We isolated 26 mutants with reduced motility, in which somitic muscle appeared normal. In most of these mutants the phenotype is obvious at around 24 to 60 hours. In some of these mutants the locomotion defect becomes less pronounced with age. None of the mutants is homozygous viable.

Mutants with abnormal embryonic motility (C1 to C4)

About one third (54/166) of the motility mutants react abnormally to tactile stimuli. These mutants were further subdivided according to their behavioral phenotypes (Table 3). Mutants in three groups have obvious defects in coordination of antagonistic motor systems in the trunk (C1 to C3), while mutants in a fourth group fail to maintain balance during swimming (C4).

(C1) 'Accordion' mutants

Mutations in any of the seven 'accordion' group genes *accordion* (*acc*), *ziehharmonika* (*zim*), *diwanka* (*diw*), *bandoneon* (*beo*), *quetschkommode* (*que*), *bajan* (*baj*) and *expander* (*exp*), cause 'accordion'-like movements. Around 24 to 28 hours, wild-type embryos show characteristic wiggling movements when touched (Fig. 6A-F). Applying tactile stimuli to mutant embryos induces a shortening of the body axis by about 5-10%, followed by an extension back to the normal body size (Fig. 6G-L). Eventually, this abnormal behavior increases the mechanical stress on the notochord, resulting in local lesions within it (Fig. 7). The behavioral phenotype of these mutant embryos suggests that somitic muscles on both

Table 3. Mutants with abnormal locomotion

Gene	Abbreviation	n	Alleles
Class C: abnormal motility			
C1: accordion			
*accordion**	*acc*	7	*tp72x, ty20, tc249a, ti284a*†, *tm286, tn218b, tq206*§
*ziehharmonika**	*zim*	3	*tf222a, tm205*§, *tm206*‡
*bandoneon**	*beo*	7	*ta86d, ta92*†, *tm115, tw38f*§, *tf242, tp221, tu230*‡
diwanka	*diw*	3	*ts286, tv205a, tz290*
quetschkommode	*que*	1	*ti274*
bajan	*baj*	1	*tf247*
expander	*exp*	1	*tu12*
Unresolved		1	*ta222b*
C2: twitch twice			
space cadet	*spc*	2	*ty85d, te226a*
spaced out	*spo*	2	*tm272b, ts239*†
*twitch twice**	*twi*	3	*tg224a, tw204*§,*tx209*
C3: crazy fish			
techno trousers	*tnt*	1	*tk57*
roller coaster	*roc*	1	*tc239d*
wavy	*way*	2	*ta51x, ta85c*
hertz	*her*	1	*ts299c*
C4: circlers			
*nevermind**	*nev*	2	*tr230b*§, *ta229f*
Unresolved		15	*tm90d, tc242b, tc256e, tc317e,tc320b, tc323d, te370e*†, *th263b, tj264a, tm246a, tm276d, tr202b, tr279a*†, *ty220d, tz300a,*

*Different allele strengths observed; †viable allele; ‡allele lost; §strongest allele.

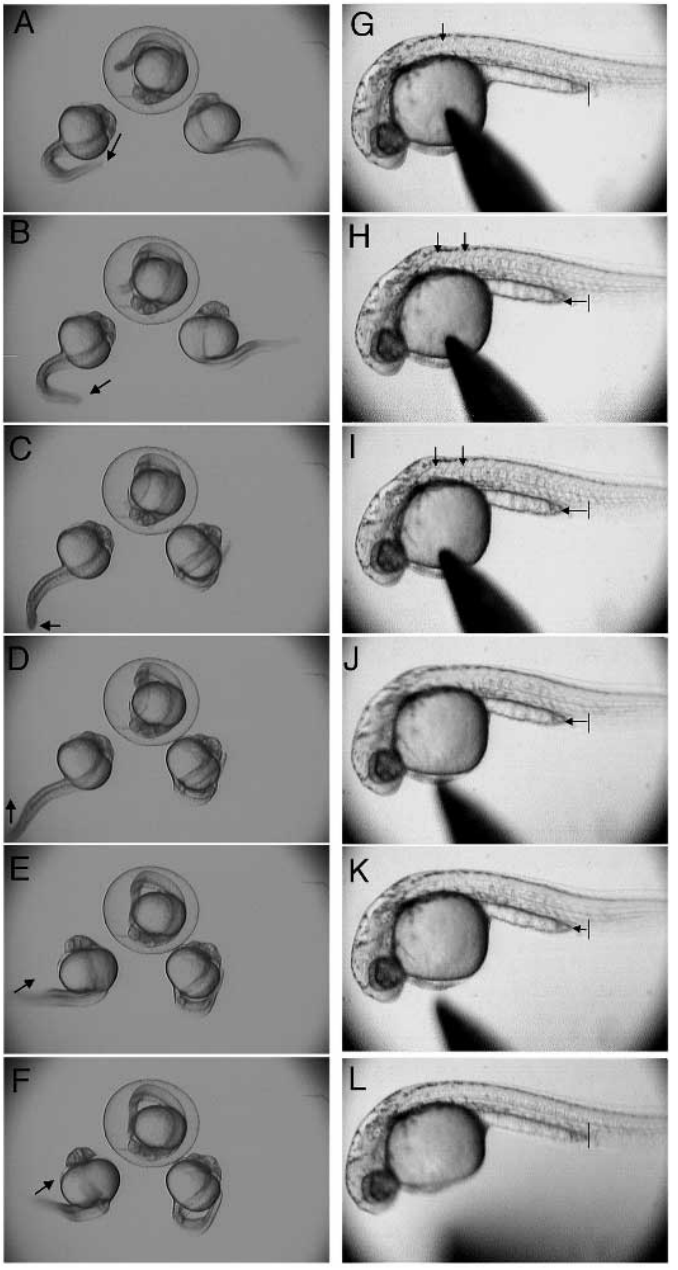

Fig. 6. Wild-type and 'accordion' behavior at around 30 hours. Wild-type embryos (A-F) and *beo* mutant embryos (G-L) were liberated from their chorions and their behavior monitored. Wild-type embryos move their tail from one side of the body to the other (A-F). In contrast, 'accordion' group mutants contract along their body axis (G-L). A line behind the yolk extension is added as a constant reference. The lengths of the horizontal arrows reflect the contraction along the antero-posterior axis of the embryo. The vertical arrows point to the region in the anterior notochord that becomes compressed during the contraction (H,I).

sides of the midline contract simultaneously. In wild-type embryos an inhibitory mechanism prevents motorneurons on one side from firing when muscles on the opposite side are contracting (Fetcho, 1992; Roberts, 1989). In *Xenopus* embryos and in goldfish, this reciprocal inhibition mechanism is thought to be mediated by commissural interneurons, which connect

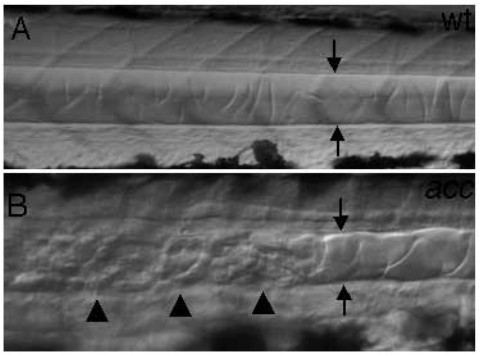

Fig. 7. Notochord lesions in 'accordion' group mutants. Lateral view on a 60-hour live wild type (A) and *acc* mutant (B). (A) Shows a wild-type notochord, demarcated by two arrows. In *acc* embryos (B), local lesions in the notochord become visible (arrowheads); next to this lesion the notochord appears unaffected (arrows).

the two motor systems on either side of the trunk (Fetcho, 1990; Roberts et al., 1988; Soffe, 1987). In *Xenopus* these neurons express the inhibitory neurotransmitter glycine (Roberts et al., 1988). To investigate if lack of reciprocal inhibition can induce an 'accordion' phenotype, we blocked glycine function in wild-type embryos with the alkaloid strychnine. Application of low doses (70 nM) of strychnine to 28-hour wild-type embryos evoked an 'accordion'-like behavior, indistinguishable from the mutant phenotype. This supports the idea that the behavioral phenotype in the seven 'accordion' group mutants results from an impaired mechanism of reciprocal inhibition. To see if morphological defects in the nervous system of the mutants account for the 'accordion' phenotype, we examined the gross neuroanatomy of the spinal cord using specific antibodies. In *diw* mutant embryos we detected severe defects in primary motorneuron development. Whereas in 28-hour wild-type embryos the znp-1 antibody stained axons of the primary motorneurons, CaP, MiP and RoP (Fig. 8A,C), in *diw* embryos antibody staining was only visible on a few ventrally projecting axons. These axons extend a reduced distance towards their ventral target, as compared to wild-type embryos (Fig. 8B,D). MiP or RoP axons are absent in mutant embryos, as judged by immunoreactivity with the znp-1 antibody. Thus, axonal outgrowth of primary motorneurons is affected by mutations in the *diw* gene. However, antibody stainings revealed that axonal projections of commissural interneurons, mechanosensory neurons (Rohon-Beard neurons) and Mauthner neurons in *diw* embryos are indistinguishable from their counterparts in wild-type embryos (M.G., unpublished observations). Because lack of axonal outgrowth is not generally observed in the neuronal cell types examined, but is restricted to primary motorneurons, we conclude that there is a specific function of the *diw* gene in motorneuron development. In none of the other 'accordion' group mutants were we able to identify similar defects in motorneuron development.

(C2) Twitch twice mutants

At around 96 hours, wild-type larvae become very active and start swimming freely. Swimming occurs by alternating and rhythmic left-right movements of the tail, similar to swimming movements of adult fish (Fig. 9A-F). Mutant *space cadet* (*spc*),

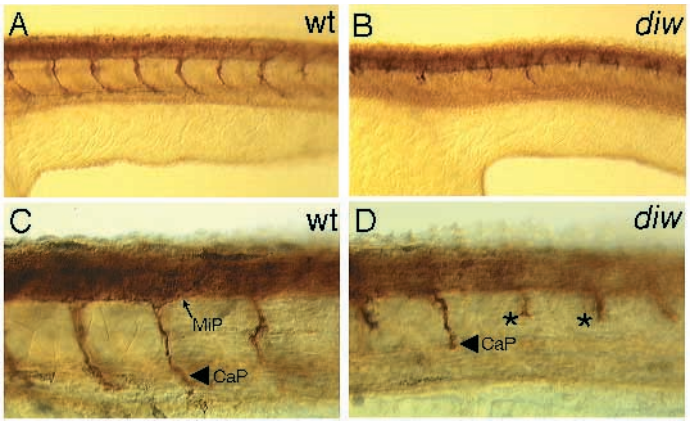

Fig. 8. Axonal outgrowth of primary motorneurons are affected in *diw* mutants. Antibody labeling with the znp-1 antibody stains axonal projections of primary motorneurons in 30-hour wild type (A,C) and residual axons in *diw* mutant embryos (B,D). (A) Lateral view of a wild-type embryo stained with znp-1. Per segment, one CaP axon is visible. In *diw* mutants these axons are shorter (B). (C) Higher magnification of the same wild-type embryo as in A. The MiP and the CaP axons are labeled. (D) Higher magnification of the same *diw* embryo as in B. Short, CaP-like axons are stained (asterisk). No axons resembling dorsally projecting MiP axons are labeled.

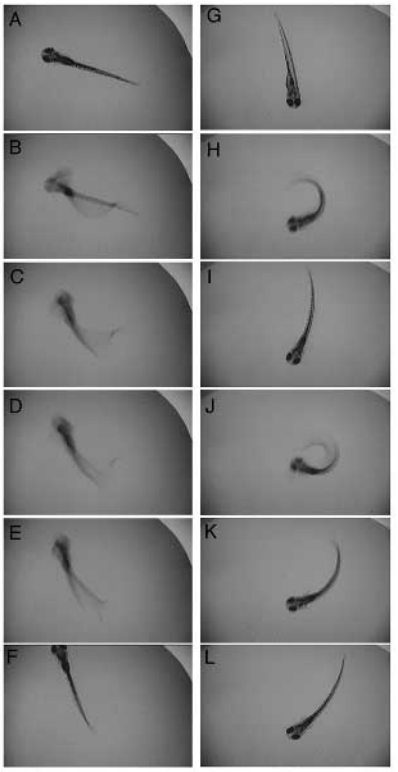

Fig. 9. Behavioral phenotype of the twitch twice group. Wild type (A-F) and *spc* mutant larvae (G-L) locomotion behavior were recorded at 120 hours. Single frame sequences illustrate alternating tail movements in wild-type larvae (A-F). Mutant *spc* larvae do not alternate their tail movements, but bend their tail consecutively towards the same side (G-L).

spaced out (*spo*) and *twitch twice* (*twi*) larvae are indistinguishable from their wild-type siblings until free swimming behavior starts. At about 96 hours, mutant larvae repeatedly bend their tail to the same side resulting in a rotation movement along their vertical body axis (Fig. 9G-L). In contrast to wild-type larvae, in which tail movements obey a strict rhythm of alternating left-right movements, mutant larvae have a less

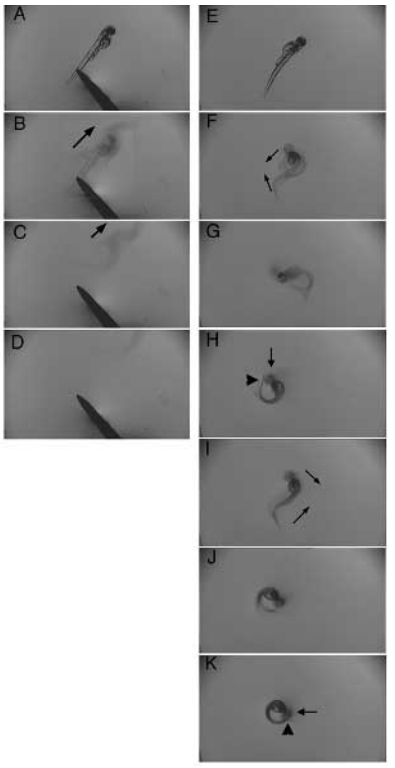

Fig. 10. *tnt* larvae show exaggerated alternating tail movements. Single frames from a video showing a fast response upon tactile stimulus of a wild-type larva at around 60 hours (A-D). An arrow indicates the direction in which the larva is moving. *tnt* mutant larvae show alternating, but hyperactive tail movements (E-K). One such cycle is shown, where the tail touches the head (H) on one side, then the embryo moves its tail to the other side and again touches the head (K). In H and K: head, arrow; tail, arrowhead.

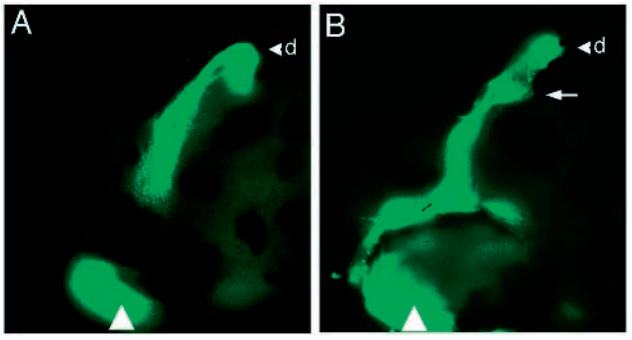

Fig. 11. Retinotectal projection defect in *nev* mutants. Retinotectal projections of dye-labeled nasodorsal axons in wild-type (A) and *nev* (B) larvae at 120 hours. In wild type the retinotectal projections terminate in the posteriodorsal optic tectum (small arrowhead in A), while in *nev* mutants (B) they terminate on both posteriodorsal (small arrowhead) and posterioventral positions (arrow). The large arrowheads in A and B indicate the position of the dye injection. d, dorsal tectum.

precise rhythm. Video-filming of the mutants revealed that in mutant larvae a few cycles of normal left-right tail movements are interrupted by a series of abnormal cycles. During one abnormal cycle several successive tail bends to the same side occur without obvious preference for any side. Some homozygous mutant *spo*^{s239} larvae survive to adulthood with the same swimming defect as observed in larvae.

(C3) 'Crazy fish' mutants

The 'crazy fish' group of mutants is defined by four genes, *techno trousers* (*tnt*), *roller coaster* (*roc*), *wavy* (*way*) and *hertz* (*her*). Mutations in *tnt* and *roc* have very similar phenotypes. Around 48 hours, wild-type embryos are not very active but

react with a fast escape response upon a tactile stimulus (Fig. 10A-D). In contrast, *tnt* mutant embryos hyperreact to tactile stimuli, with a series of exaggerated left-right tail movements (Fig. 10E-K). The number of these consecutive cycles (1 cycle = 1 left-right movement) is increased as well as the amplitude of the tail movements. The amplitude is increased such that the tail tip touches the head of the embryo (Fig. 10H,K); this is not observed in wild-type embryos. At around 72 hours, mutant *tnt* larvae become touch-insensitive and at 96 hours mutant larvae are 10-30% shorter than wild-type larvae and remain touch-insensitive. Mutants start swimming spontaneously for a short time period, however, again with higher amplitude of the tail or with slight contraction along their body axis. At this stage focal lesions in the notochord are visible (data not shown). These notochord lesions, the reduced body size and the inability to react to touch might be consequences of constant and simultaneous muscle tensions in the left and right somitic musculature. Similar behavioral phenotypes have been described in mice and humans and are correlated with the lack of adult glycine receptor function. When we applied the glycine blocking agent strychnine (140 nM) on 72-hour wild-type larvae we observed defects similar to those seen in *tnt* or *roc* mutants: larvae were about 10% shorter, had lesions in the notochord and were touch-insensitive. Thus, similar to the 'accordion' phenotype, the *tnt-roc* phenotype can be mimicked by blocking the inhibitory neurotransmitter glycine. Blocking glycine functions at 28 hours, however, results in an 'accordion' phenotype, while blocking at 72 hours results in a 'spastic'-like phenotype.

The *her* and *way* mutant phenotypes only become apparent around 120 hours. Upon tactile stimuli, mutants swim with reduced frequency of tail movements. During a swimming episode mutant larvae increase the frequency of tail movement and thus swim faster. Often they start to swim in loops and change swimming direction frequently.

(C4) Circling mutants

The circler phenotype is similar to the 'crazy' fish phenotype. 18 mutants have balance-defective swimming behavior around 96 hours. At this stage wild-type larvae are very active, change swimming directions spontaneously and are able to direct their swimming towards targets. Mutant larvae are not very active, and rest on their side or on their back. Upon tactile stimulation the larvae start swimming, but fail to maintain body balance. Some of the mutants follow a corkscrew-like path (*ta229f*, *tc317e*, *tm246a*, *r230b*, *tr279a*, *ty220d*, *tz300a*); others swim in vertical loops. A similar circling behavior is correlated to abnormal ear morphology in *backstroke* (*bks*), *what's up*, *keinstein* and *little ears* mutants (see Table 4; Whitfield et al., 1996). In none of the circling mutants described here is ear morphology noticeably affected, but some of them (*tc256d*, *tc320b*, *th263b*, *tj264a*, *tm246a*, *tr202b*, *ty220d*) do not react to a vibration stimulus applied to the rim of the dish. Muscle mutants without an air-filled swim bladder react in a similar way to wild-type larvae, with a 'fast-start' movement away from the stimulus, indicating that lack of a functional swim bladder alone cannot fully account for this phenotype. Since tactile-vibrational stimuli activate the auditory system, including the Mauthner cell (Eaton and Farley, 1975), and probably tactile as well as lateral line receptors of the larvae, these mutants might have functional defects in these organs.

We noticed that in one mutant (nev^{tr230b}), circling behavior correlates with a defect in retinotectal axon projection (see also Trowe et al., 1996). Therefore we tested 13 of the 22 circling mutants for normal retinotectal projection (Fig. 11A) according to the protocol described by Baier et al. (1996). In one additional circling mutant, nev^{ta229f}, we find that axons emanating from nasodorsal positions terminate on both the posteriodorsal and posterioventral side in the optic tectum (Fig. 11B). This defect is less pronounced in nev^{ta229f} than in nev^{tr230b} mutant larvae (see also Trowe et al., 1996). Ventral axons terminate in the dorsal tectum, as in wild-type larvae. Both mutants fail to complement each other, demonstrating that the behavioral and retinotectal phenotypes are caused by mutations in a single gene, *nevermind* (*nev*).

Table 4. Other mutants affecting embryonic motility

Gene	Abbreviation	Motility defect	Main defect(s)	Reference
spock	*spk*	Circling	Ears	a
backstroke	*bks*	Circling	Otoliths	a
what's up	*wup*	Circling	Otoliths	a
keinstein	*kei*	Circling	Otoliths	a
little ears	*lte*	Circling	Ears	a
u-boot	*ubo*	Reduced motility	Myoseptum	b
sonic you	*syu*	Reduced motility	Myoseptum	b
you-too	*yot*	Reduced motility	Myoseptum	b
chameleon	*con*	Reduced motility	Myoseptum	b,c
white snake	*wis*	Immotile	Brain	d
wirbel	*wir*	Immotile	Body shape	d
boxer	*box*	Reduced motility	Retinotectal projection, fins, jaw	e,f,g
noir	*nir*	Reduced motility	Pigment	h
zwart	*zwa*	Reduced motility	Pigment	h
submarine	*sum*	Balance	Pigment	h
polished	*pol*	Reduced motility	Pigment	h
touch down	*tdo*	Reduced motility	Pigment	h
blanched	*bch*	Reduced motility	Pigment	h
clorix	*clx*	Reduced motility	Pigment, ear	a,j
casanova	*cas*	Reduced motility	Heart	k
still heart	*sth*	Reduced motility	Heart	k, j

References: a, Whitfield et al. (1996); b, van Eeden et al. (1996b); c, Brand et al. (1996b); d, Jiang et al. (1996); e, Trowe et al. (1996); f, van Eeden et al. (1996a); g, Schilling et al. (1996); h, Kelsh et al. (1996); j, Odenthal et al. 1996b; k, Chen et al. (1996).

DISCUSSION

Muscle mutants with defects in trunk muscle development and maintenance

Formation of skeletal muscle involves commitment of mesodermal cells into the myogenic pathway and subsequently differentiation of the skeletal myoblasts into fully differentiated myotubes, resulting in multinucleated muscle fibers. This transition from myoblasts to myotubes is accompanied by functional and morphological changes of these cells. Myotubes lose their mitotic competence, their nuclei fuse and they become more elongated (reviewed by Olson, 1992). The resulting myotubes later become the muscle fibers of the

somitic muscles. The 63 motility mutants with obvious defects in muscle development define four phenotypic groups. The characteristic phenotypes of each group (A1 to A4) become apparent sequentially, suggesting that the various mutations affect consecutive steps of muscle tissue development.

Assignment of mutants into particular phenotypic groups is tentative and may not always include alleles of a given gene into the same group. This is possible for alleles of different strength, which would then not generally be considered for complementation testing. For example, all mutants in group A1 are completely paralyzed, whereas mutants in group A2 still can move. Eight of 24 possible crosses between these two groups complemented. This indicates that assignment of mutants into different phenotypic groups is probably correct, although the matrix is not complete. Further characterization and genetic mapping of the mutants will resolve these uncertainties.

Mutations affecting myoblast differentiation (A1)

By 28 hours, zebrafish somitic trunk muscles are composed of well-formed muscle fibers containing elongated multinucleated myotubes (Fig. 1G). In mutant *slo*, *fro* and *fub* embryos, the expression of the paraxial mesoderm specific gene *sna1* (Hammerschmidt and Nüsslein-Volhard, 1993) and the myoblast specific transcription factor *myoD* (Davis et al., 1987) are indistinguishable from those in wild-type embryos, suggesting that commitment of somitic cells into the myogenic cell lineage is not affected (M. G., F. v. E. and M. H., unpublished data). In all three mutants, muscle fibers are strongly reduced or absent, while *slo* and *fro* muscle nuclei are less elongated and resemble nuclei morphology of younger, less differentiated myoblasts. Therefore, muscle cells of mutant *slo* and *fro* embryos appear arrested in their differentiation program. Muscle development in these two mutants may even be blocked at an earlier stage than in *fub* embryos where the nuclei more closely resemble wild-type muscle fiber nuclei (Fig. 1, compare I and J with H). However, we cannot rule out the possibility that the round shape of *fro* and *slo* muscle nuclei is a secondary result due to the lack of sacromere formation.

In mice, targeted mutations of the *myogenin* gene result in embryos with similar defects in trunk muscle. In embryos mutant for the *myogenin* gene myoblasts are detectable, and some even form myofibers containing myosin and actin; however, the majority of the myoblasts do not differentiate into myotubes (Hasty et al., 1993; Nabeshima et al., 1993). Although we do not know if myoblasts are fused in mutant *slo* and *fro* embryos, their mutant phenotypes suggest a role in myoblast differentiation for the corresponding genes and make them candidates for the zebrafish *myogenin* gene. Alternatively, the *slo* and *fro* gene products might be essential factors required for myoblast fusion or, like the proposed function of the *fub* gene, they might be involved in early stages of myofibril organization.

Mutations affecting muscle fiber development (A2, A3)

Once cells become restricted to the myogenic pathway, they develop further into myotubes. These myotubes express various muscle cell-specific components and eventually form fully differentiated muscle fibers. Mutations in at least eight genes cause a reduction of muscle fibers or less organized muscle fibers. Our initial analysis revealed a general reduction of muscle fibers in *slp* and *dus* mutants and a reduction in sarcomere organization in mutant *buf* embryos. Although analysis of the remaining mutants is not yet complete, these mutants illustrate that reduction of muscle birefringency can reflect specific defects in muscle fiber formation or arrangement.

Mutations affecting muscle tissue maintenance (A4)

Mutations in at least four genes lead to muscle-specific degeneration. The late onset of the mutant phenotype and the tissue specificity may indicate that genes in this phenotypic class function in late differentiation or in maintenance of trunk muscle tissue. There are obvious parallels to human diseases where onset of muscle tissue-specific dystrophy occurs during postnatal stages (Bushby, 1994; Dunne and Epstein, 1991). It would be of interest to analyze the spinal cords of the somite degeneration mutants to see if there are additional defects associated with these mutations or indirect effects due to muscle tissue degeneration.

We have identified a few other muscle mutants with additional phenotypes in other embryonic structures (Table 4, and references therein). For example, in *you-too* (*yot*) mutant embryos, a restricted subpopulation of myotomal cells, the adaxial cells, fails to express *myoD*. In addition *yot* mutants lack a horizontal myoseptum, separating the ventral and the dorsal somites. Muscle fiber formation, however, appears normal in *yot* mutants (van Eeden et al., 1996b).

Mutants with defects in the different patterns of embryonic and early larval locomotion (B1-B6, C1-C4)

A total of 105 motility mutants showed no reduction of muscle striation and thus no obvious defect in muscle development. We expect a substantial portion of these 105 mutants to affect the different neuronal structures mediating and controlling embryonic and early larval locomotion, although some of these mutants might have subtle defects in muscle development. A variety of different behavioral phenotypes were observed among these mutants: touch-insensitive mutants (B5), 'spastic' mutants (*roc* and *tnt* in C3), circling mutants (C4) and motor circuit defective mutants (C1, C2), which together might reflect the diversity of neuronal components and connections required to perform the different patterns of embryonic motility. We tried to correlate these mutant phenotypes with the various processes of locomotion behavior during the different stages of development.

Mutations affecting general motility of the embryo (B1, B2, B4, B6)

The earliest patterns of embryonic locomotion are visible around 22 to 30 hours, when the first alternating tail movements to both sides of the body occur. In contrast to later stages, these activities do not result in net movements of the embryo. Mutations in at least seven genes abolish or strongly reduce this early behavioral pattern. Mutant *sop*, *led*, *nic*, *hat* and *hel* embryos are completely immotile (B1, B2), whereas in 26 other mutants motility is not completely abolished, but is reduced by variable degrees (B4, B6). The primary causes of these behavioral phenotypes might be very diverse, so that

the mutant phenotypes do not provide much information about the function of the mutated genes. Analysis using specific antibodies or labeled mRNA probes will be helpful in characterizing these mutants.

Interestingly, mutations in the two genes *het* and *hal* (B2) also affect heart function. Defects in heart function and embryonic motility are also observed in several other mutants isolated in our screen. Mutant *still heart* (*sth*) and *casanova* (*cas*) embryos also have heart and motility defects (Chen et al., 1996), while *white snake* (*wis*) mutant embryos show additional defects in brain ventricle formation (Jiang et al., 1996).

Mutations affecting touch perception (B5)

From about 22 hours on, zebrafish embryos react to tactile stimuli. In the head region it is probable that a subset of trigeminal ganglion cells and Rohon-Beard cells in the dorsal part of the spinal cord mediate the response to touch (Kimmel et al., 1991; Metcalfe et al., 1990). In *Xenopus* embryos sensory inputs from skin receptors activate a specific class of interneurons, which in turn excite motorneurons (Sillar and Roberts, 1988). We found six mutants in which this touch response pathway might be impaired. In all six cases the mutants can swim, but fail to react to touch. The behavioral phenotype suggests that the sensory part of the pathway, rather than the motor system, is affected in these mutants. Some of the genes we have identified might function in a similar pathway that has been identified in *C. elegans* for touch receptor formation. Mutations in the *mec*hanosensory-defective (*mec*) genes affect development (*mec-3*), structure (*mec-1*, *mec-5*, *mec-7*, *mec-12*) or function (*mec-4*, *mec-10*, *mec-6*) of six of the touch cells used by *C. elegans* to avoid gentle touch (reviewed by Bargmann, 1994; Duggan and Chalfie, 1995).

Mutations affecting motor circuits (C1, C2, C3)

Once sensory input from the skin activates the motor system, the embryos react with alternating tail movements. Alternation of burst activities is achieved by an inhibitory activity located mainly in the spinal cord. Experiments with *Xenopus* embryos (Dale et al., 1986; Roberts et al., 1988; Sillar and Roberts, 1988) and goldfish (Fetcho, 1990; Fetcho and Faber, 1988) have identified a class of glycinergic commissural interneurons, involved in mediating reciprocal inhibition, which prevent simultaneous contraction of the motor systems on both sides of the midline. We have identified 12 genes in the 'accordion', 'crazy fish' and twitch twice phenotypic groups, where the mutant phenotype suggests that they play a role in this motor circuit regulation. Mutations in the seven 'accordion' group genes result in simultaneous contraction of the motor systems on both sides. The phenotype can be mimicked by applying the glycine antagonist strychnine to 28-hour wild-type embryos, suggesting that the 'accordion' phenotype is caused by disruption of a glycine-mediated inhibition mechanism.

In many multicellular animals, reciprocal inhibition is found to coordinate antagonistic motor centers. For example, in *C. elegans*, mutations in five genes result in a 'shrinker' phenotype reminiscent of the 'accordion' phenotype in the zebrafish embryo (McIntire et al., 1993a). One of these genes, *unc-30*, encodes a homeodomain protein and is required for terminal differentiation of a subset of neurons, mediating reciprocal inhibition (Jin et al., 1994; McIntire et al., 1993b). Thus the 'accordion' group mutants are good candidates for controlling neuronal specification of a specific subtype of commissural interneurons that mediate reciprocal inhibition in the zebrafish embryo.

Mutant *tnt* and *roc* embryos also have defects in axial motor circuit regulation, but at a different stage of development. Mutations in the two genes *tnt* and *roc* lead to a spastic behavioral phenotype from 48 hours on, similar to behavioral abnormalities caused by a lack of glycine receptor function observed in other vertebrates. Molecular analysis of the mouse *spastic* and *spasmodic* mutants has shown that they encode the β and α1 subunit of the adult glycine receptor, respectively (Mülhardt et al., 1994; Ryan et al., 1994). 2 weeks after birth mutant mice develop muscle rigidity, tremors and pronounced startle reactions. This behavioral defect also resembles the human dominant neurological syndrome hyperekplexia, which is caused by point mutations in the human GlyR α1 subunit (Shiang et al., 1993). The similarity to the phenotypes in mice and humans might indicate a lack of adult glycine receptor function in mutant *tnt* and *roc* embryos. As with the 'accordion' phenotype, the *tnt* or *roc* phenotypes can be phenocopied by strychnine application, albeit only during later stages of development. This evidence taken together suggests that a lack or reduction of glycine function is also the cause of the zebrafish 'spastic' phenotypes. Thus, glycine might be the common inhibitory neurotransmitter in the neuronal circuits in which the 'accordion' genes and the *tnt* and *roc* genes participate in wild-type embryos. However these circuits function at different stages of development and have distinct phenotypes when blocked, suggesting that they are independent. Correspondingly, 'accordion' group mutants do not show 'spastic' phenotypes and all 'accordion' group mutations tested so far complement the *roc* and the *tnt* mutations. A simple interpretation of these results is that the genes in both phenotypic groups have different functions in controlling axial movements, although they both use glycine as an inhibitory neurotransmitter.

The mutant phenotypes of the three genes *spc*, *spo* and *twi* (C2) also suggest that these genes have a role in a neuronal circuit regulating alternating tail movements. In contrast to the 'accordion' group, mutations in the twitch twice group genes do not affect the early swimming behavior, but do affect rhythmic tail movements during the later free-swimming stages. In mutant larvae tail movements occur without alternating rhythm. The tail of the affected larva bends successively to the same side, suggesting an uncoupling of the alternating rhythm of the left and right side motor systems. In *Xenopus* embryos, experimental separation of the two sides of the nervous system leads to an uncoupling of the rhythmic alteration of the left and right motor systems, but does not affect the rhythmic burst production on either side (Roberts et al., 1981). This has indicated that rhythmic activity is generated independently in the left and right half motor systems and that interneurons crossing the midline connect both motor systems to phase their rhythms (Roberts et al., 1981). These results, combined with our behavioral analysis of the twitch twice phenotype, suggest that the *spc*, *spo* and *twi* genes might control generation or maintenance of rhythmic and alternating activity between antagonistic motor centers on both sides of the midline.

Mutations affecting the balance systems of the embryo (C4)

Locomotion behavior of zebrafish larvae changes dramatically at about 96 hours, when the larvae start swimming freely, and their movements become more like the swimming behavior of adult fish. Due to the air-filled swim bladder, the larva starts moving in three dimensions. Sensory inputs from the vestibular organ and visual information are processed in different parts of the brain to maintain balance. A total of 17 mutants with circling behavior were isolated, suggesting a defect in their balance system. Complementation analysis in this group of mutants was difficult due to the relatively large number of mutants with very similar phenotypes. Only one complementation group, *nev*, was identified, because both alleles showed additional defects of their retinotectal projections (see below). Two of the circling mutants, *tr279a* and *te370e*, are adult-viable. Whereas *tr279a* adults constantly circled around their axis, *te370e* adults behaved as if they were in a weightless environment. As in other vertebrates, the inner ear enables the zebrafish to hear and to maintain balance. Our preliminary analysis, based so far on the morphological appearance of the organ, did not reveal defects in ear development in these circling mutants. We also identified 20 genes with specific defects in ear development, however, and some of the corresponding mutants have similar circling behaviors (Whitfield et al., 1996; Table 4). Molecular probes and dyes labeling specific structures in the ear were used to examine those ear mutants (Whitfield et al., 1996) and should also be helpful for revealing vestibular defects among the circling mutants. In general, the phenotype of the circling mutants is reminiscent of some mice mutants. For example in *kreisler* mice, which have severe motor dysfunction as adults, rhombomere segmentation is affected during embryonic stages (Cordes and Barsh, 1994; Frohman et al., 1993), whereas in *waltzer* mutants formation of the inner ear is affected (Deol, 1956).

Motility mutants as a tool for the study of neuronal development in the zebrafish?

One expectation we had was that a large portion of the 105 motility mutants without visible muscle defects would be caused by defects in the nervous system. Thus we have analyzed some of the mutants using specific antibodies or dye labeling of axonal projections. We found that mutations in the four genes, *unplugged (unp)*, *diwanka (diw)*, *macho (mao)* and *nevermind (nev)*, cause both a behavioral defect and a specific defect in the developing nervous system.

Mutations in the two genes *unp* and *diw* cause abnormal motorneuron development. Whereas in mutant *diw* embryos all the three primary motorneurons are affected, in *unp* only ventral axons fail to elongate normally. In both mutants axonal pathfinding or cell-type specification of motoreurons might be impaired. Recently, a number of transcription factors sharing a common LIM homeobox motif have been implicated in motorneuron specificity (Tsuchida et al., 1994; Lumsden, 1995). Different motorneuron subtypes initially express *Isl-1* and later a combination of LIM domain genes. Combinatorial expression of these genes in motorneurons is detectable before their axonal pathways are established and before their segregation into motor columns is evident. Thus, these genes are also good candidates for specifying motorneuron in zebrafish, and it will be of great interest to characterize the expression of these genes in *unp* and *diw* mutants. Mutant *diw* embryos also show an 'accordion' phenotype, probably caused by a lack of reciprocal inhibition. It is not clear how the motorneuron defect relates to the lack of reciprocal inhibition.

Mutations in the *mao* and *nev* genes cause abnormal retinotectal projections. Whereas in *nev* mutants the dorso-ventral polarity of the termination field in the optic tectum is disturbed, in mutant *mao* larvae the anterior-posterior topography of the field is affected. The behavioral defect in *nev* larvae becomes visible when mutant larvae swim in a corkscrew path. How this behavioral defect is related to the retinotectal defect is not clear, because mutant *don't care* (*don*) larvae swim normally, although they exhibit a similar retinotectal defect (for further discussion, see Trowe et al., 1996). In *mao* larvae we found nasal axons defasciculating prematurely, whereas temporal axons are not affected. The phenotype suggests that *mao* activity is required in the anterior half of the tectum to prevent nasal axons from defasciculating. Alternatively, *mao* activity might be required on nasal axons for guidance towards the posterior part of the tectum.

Although at this point the correlation between the neuronal defects of the mutants and their behavioral phenotypes is not completely understood, our initial characterization shows that analysis of the motility mutants can uncover specific defects in neuronal development. Further characterization and molecular analysis of these mutants, and comparison with existing mice mutants that have impaired motor coordination, should increase our understanding of neuronal development and control of embryonic vertebrate locomotion.

We would like to thank C. Kimmel, J. Eisen and M. Westerfield for providing the *nic* and *fub* mutant strains used for complementation and for the znp1 antibody. In addition we thank Tanja Whitfield and Suresh Jesuthesan for critical reading of the manuscript. This work was supported by a DFG postdoctoral fellowship Gr 1370/1-1 to M.G.

REFERENCES

Ahnn, J. and Fire, A. (1994). A screen for genetic loci required for body-wall muscle development during embryogenesis in *Caenorhabditis elegans*. *Genetics* **137,** 483-98.

Baier, H., Klostermann, S., Trowwe, T., Karlstrom, R. O. Nüsslein-Volhard, C. and Bonhoeffer, F. (1996). Genetic dissection of the retinotectal projection. *Development*, **this issue.**

Bargmann, C. I. (1993). Genetic and cellular analysis of behavior in C. elegans. *Annu. Rev. Neurosci.* **16,** 47-71.

Bargmann, C. I. (1994). Molecular mechanisms of mechanosensation? *Cell* **78,** 729-31.

Bernhardt, R. R., Chitnis, A. B., Lindamer, L. and Kuwada, J. Y. (1990). Identification of spinal neurons in the embryonic and larval zebrafish. *J. Comp. Neurol.* **302,** 603-616.

Brand, M., Heisenberg, C.-P., Jiang, Y.-J., Beuchle, D., Lun, K., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D., Kelsh, R., Mullins, M., Odenthal, J., van Eeden, F. J. M. and Nüsslein-Volhard, C. (1996a). Mutations in zebrafish genes affecting the formation of the boundary between midbrain and hindbrain. *Development* **123**, 179-190.

Brand, M., Heisenberg, C.-P., Warga, R., Pelegri, F., Karlstrom, R. O., Beuchle, D., Picker, A., Jiang, Y.-J., Furutani-Seiki, M., van Eeden, F. J. M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D., Kelsh, R., Mullins, M., Odenthal, J. and Nüsslein-Volhard, C. (1996b). Mutations affecting development of the midline and general body shape during zebrafish embryogenesis. *Development* **123**, 129-142.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77,** 71-94.

Buckingham, M. (1994). Molecular biology of muscle development. *Cell* **78,** 15-21.

Buckingham, M., Houzelstein, D., Lyons, G., Ontell, M., Ott, M. O. and Sassoon, D. (1992). Expression of muscle genes in the mouse embryo. *Symp. Soc. Exp. Biol.* **46,** 203-217.

Bushby, K. M. (1994). The muscular dystrophies. *Baillieres Clin. Neurol.* **3,** 407-430.

Chen, J.-N., Haffter, P., Odenthal, J., Vogelsang, E., Brand, M., van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C. and Nüsslein-Volhard, C. (1996). Mutations affecting the cardiovascular system and other internal organs in zebrafish. *Development* **123**, 293-302.

Chitnis, A., Henrique, D., Lewis, J., Ish-Horowicz, D. and Kintner, C. (1995). Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene *Delta*. *Nature* **375,** 761-766.

Cordes, S. P. and Barsh, G. S. (1994). The mouse segmentation gene *kr* encodes a novel basic domain-leucine zipper transcription factor. *Cell* **79,** 1025-1034.

Culotti, J. G. (1994). Axon guidance mechanisms in *Caenorhabditis elegans*. *Curr. Opin. Genet. Dev.* **4,** 587-595.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I. and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant *reeler*. *Nature* **374,** 719-723.

Dale, N., Ottersen, O. P., Roberts, A. and Storm-Mathisen, J. (1986). Inhibitory neurons of a motor pattern generator in *Xenopus* revealed by antibodies to glycine. *Nature* **324,** 255-257.

Davis, R. L., Weintraub, H. and Lassar, A. B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51,** 987-1000.

Deol, M. S. (1956). The anatomy and development of the mutants *pirouette*, *shaker*-1 and *waltzer* in the mouse. *Proc. R. Soc. London* **B 145,** 206-213.

Dodd, J. and Schuchardt, A. (1995). Axon guidance: a compelling case for repelling growth cones. *Cell* **81,** 471-474.

Duggan, A. and Chalfie, M. (1995). Control of neuronal development in *Caenorhabditis elegans*. *Curr. Opin. Neurobiol.* **5,** 6-9.

Dunne, P. W. and Epstein, H. F. (1991). Molecular biology of human muscle disease. *Biotechnology* **9,** 41-46.

Eaton, R. C. and Farley, R. D. (1975). Mauthner neuron field potential in newly hatched larvae of the zabrafish. *J. Neurophysiol.* **38,** 502-512.

Eisen, J. S., Myers, P. Z. and Westerfield, M. (1986). Pathway selection by growth-cones of identified motoneurons in live zebrafish embryos. *Nature* **320,** 269-271.

Felsenfeld, A. L., Curry, M. and Kimmel, C. B. (1991). The *fub-1* mutation blocks initial myofibril formation in zebrafish muscle pioneer cells. *Dev. Biol.* **148,** 23-30.

Felsenfeld, A. L., Walker, C., Westerfield, M., Kimmel, C. and Streisinger, G. (1990). Mutations affecting skeletal muscle myofibril structure in the zebrafish. *Development* **108,** 443-59.

Fetcho, J. R. (1990). Morphological variability, segmental relationships, and functional role of a class of commisural interneurons in the spinal cord of Goldfish. *J. Comp. Neurol.* **299,** 283-298.

Fetcho, J. R. (1992). The spinal motor system in early vertebrates and some of its evolutionary changes. *Brain, Behavior and Evolution* **40,** 82-97.

Fetcho, J. R. and Faber, D. S. (1988). Identification of motoneurons and interneurons in the spinal network for escaspes initiated by the mauthner cell in goldfish. *J. Neurosci.* **8,** 4192-4213.

Frohman, M. A., Martin, G. R., Cordes, S. P., Halamek, L. P. and Barsh, G. S. (1993). Altered rhombomere-specific gene expression and hyoid bone differentiation in the mouse segmentation mutant, *kreisler* (*kr*). *Development* **117,** 925-936.

Furutani-Seiki, M., Jiang, Y.-J., Brand, M., Heisenberg, C.-P., Houart, C., Beuchle, D., van Eeden, F. J. M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J. and Nüsslein-Volhard, C. (1996). Neural degeneration mutants in the zebrafish, *Danio rerio*. *Development* **123**, 229-239.

Ghetti, B. and Triarhou, L. C. (1992). Degeneration of mesencephalic dopamine neurons in *weaver* mutant mice. *Neurochem. Int.* **20,** 305-307.

Grunwald, D. J., Kimmel, C. B., Westerfield, M., Walker, C. and Streisinger, G. (1988). A neural degeneration mutation that spares primary neurons in the zebrafish. *Dev. Biol.* **126,** 115-128.

Haffter, P., Granato, M., Brand, M., Mullins, M. C., Hammerschmidt, M., Kane, D. A., Odenthal, J., van Eeden, F. J. M., Jiang, Y.-J., Heisenberg, C.-P., Kelsh, R. N., Furutani-Seiki, M., Vogelsang, E., Beuchle, D., Schach, U., Fabian, C. and Nüsslein-Volhard, C. (1996). The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development* **123**, 1-36.

Hammerschmidt, M. and Nüsslein-Volhard, C. (1993). The expression of a zebrafish gene homologous to *Drosophila- snail* suggests a conserved function in invertebrate and vertebrate gastrulation. *Development* **119,** 1107-1118.

Hasty, P., Bradley, A., Morris, J. H., Edmondson, D. G., Venuti, J. M., Olson, E. N. and Klein, W. H. (1993). Muscle deficiency and neonatal death in mice with a targeted mutation in the *myogenin* gene. *Nature* **364,** 501-506.

Hedgecock, W. G. (1992). Guidance of neuroblast migrations and axonal projections in *Caenorhabditis elegans*. *Curr. Opin. Neurobiol.* **2,** 34-41.

Hemmati-Brivanlou, A., Kelly, O. G. and Melton, D. A. (1994). Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell* **77,** 283-295.

Hemmati-Brivanlou, A. and Melton, D. A. (1994). Inhibition of activin receptor signaling promotes neuralization in *Xenopus*. *Cell* **77,** 273-281.

Jiang, Y.-J., Brand, M., Heisenberg, C.-P., Beuchle, D., Furutani-Seiki, M., Kelsh, R. N., Warga, R. M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D. A., Mullins, M. C., Odenthal, J., van Eeden, F. J. M. and Nüsslein-Volhard, C. (1996). Mutations affecting neurogenesis and brain morphology in the zebrafish, *Danio rerio*. *Development* **123**, 205-216.

Jin, Y., Hoskin, R. and Horvitz, R. H. (1994). Control of type-D GABAergic neuron differentiation by *C. elegans* UNC-30 homeodomain protein. *Nature* **372,** 780-783.

Joyner, A. L. and Guillemot, F. (1994). Gene targeting and development of the nervous system. *Curr. Opin. Neurobiol.* **4,** 37-42.

Kelsh, R. N., Brand, M., Jiang, Y.-J., Heisenberg, C.-P., Lin, S., Haffter, P., Odenthal, J., Mullins, M. C., van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Kane, D. A., Warga, R. M., Beuchle, D., Vogelsang, L. and Nüsslein-Volhard, C. (1996). Zebrafish pigmentation mutations and the processes of neural crest development. *Development* **123**, 369-389.

Kennedy, T. E., Serafini, T., de la Torre, J. R. and Tessier-Lavigne, M. (1994). Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* **78,** 425-435.

Kernan, M., Cowan, D. and Zucker, C. (1994). Genetic dissection of mechanosensory transduction: Mechanoreception-defective mutations of *Drosophila*. *Neuron* **12,** 1195-1206.

Kimmel, C. B., Grunwald, D. J., Walker, C., Westerfield, M. and Streisinger, G. (1985). Neuronal degerneration mutations that spare primary neurons in the zebrafish. *Soc. Neurosci. Abstr.* **11,** 647.

Kimmel, C. B., Hatta, K. and Eisen, J. S. (1991). Genetic control of primary neuronal development in zebrafish. *Development* **Supplement 2,** 47-57.

Kuwada, J. (1995). Development of the zebrafish nervous system: genetic analysis and manipulation. *Curr. Opin. Neurobiol.* **5,** 50-54.

Lalonde, R. and Thifault, S. (1994). Absence of an association between motor coordination and spatial orientation in *lurcher* mutant mice. *Behav. Genet.* **24,** 497-501.

Lumsden, A. (1995). A LIM code for motor neurons. *Curr. Biol.* **5,** 491-495.

Marx, J. (1995). Helping neurons to find their way. *Science* **268,** 971-973.

McIntire, S. L., Jorgensen, E. and Horvitz, R. H. (1993a). Genes required for GABA function in *Caenorhabditis elegans*. *Nature* **364,** 334-337.

McIntire, S. L., Jorgensen, E., Kaplan, J. and Horvitz, R. H. (1993b). The GABAergic nervous system of *Caenorhabditis elegans*. *Nature* **364,** 337-341.

Messersmith, E. K., Leonardo, E. D., Shatz, C. J., Tessier-Lavigne, M., Goodman, C. S. and Kolodkin, A. L. (1995). Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord. *Neuron* **14,** 949-959.

Metcalfe, W. K., Myers, P. Z., Trevarrow, B., Bass, M. B. and Kimmel, C. B. (1990). Primary neurons that express the L2/HNK-1 carbohydrate during early development in the zebrafish. *Development* **110,** 491-504.

Mülhardt, C., Fischer, M., Gass, P., Simon-Chazottes, D., Guénet, J.-L., Kuhse, J., Betz, H. and Becker, C.-M. (1994). The spastic mouse: aberrant splicing of glycine receptor β subunit mRNA caused by intronic insertion of L1 element. *Neuron* **13,** 1003-1015.

Mullins, M. C., Hammerschmidt, M., Haffter, P. and Nüsslein-Volhard, C. (1994). Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Curr. Biol.* **4,** 189-202.

Nabeshima, Y., Hanaoka, K., Hayasaka, M., Esumi, E., Li, S., Nonaka, I. and Nabeshima, Y. (1993). *Myogenin* gene disruption results in perinatal lethality because of severe muscle defect. *Nature* **364,** 532-535.

Norman, D. J., Feng, L., Cheng, S. S., Gubbay, J., Chan, E. and Heintz, N. (1995). The *lurcher* gene induces apoptotic death in cerebellar Purkinje cells. *Development* **121,** 1183-1193.

Odenthal, J., Rossnagel, K., Haffter, P., Kelsh, R. N., Vogelsang, E., Brand, M., van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Mullins, M. C. and Nüsslein-Volhard, C. (1996). Mutations affecting xanthophore pigmentation in the zebrafish, *Danio rerio*. *Development* **123**, 391-398.

Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H. and Mikoshiba, K. (1995). The *reeler* gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14,** 899-912.

Olson, E. N. (1992). Interplay between proliferation and differentiation within the myogenic lineage. *Dev. Biol.* **154,** 261-272.

Olson, E. N. and Klein, W. H. (1994). bHLH factors in muscle development: dead lines and commitments, what to leave in and what to leave out. *Genes Dev.* **8,** 1-8.

Ontell, M., Ontell, M. P., Sopper, M. M., Mallonga, R., Lyons, G. and Buckingham, M. (1993a). Contractile protein gene expression in primary myotubes of embryonic mouse hindlimb muscles. *Development* **117,** 1435-1444.

Ontell, M. P., Sopper, M. M., Lyons, G., Buckingham, M. and Ontell, M. (1993b). Modulation of contractile protein gene expression in fetal murine crural muscles: emergence of muscle diversity. *Dev. Dyn.* **198,** 203-213.

Papan, C. and Campos-Ortega, J. A. (1994). On the formation of the neural keel and neural tube in the zebrafish *Danio* (*Brachydanio*) *Rerio*. *Roux's Arch. Dev. Biol.* **203,** 178-186.

Roberts, A. (1989). The neurons that control axial movements in a frog embryo. *Amer. Zool.* **29,** 53-63.

Roberts, A., Dale, N., Ottersen, O. P. and Storm-Mathisen, J. (1988). Development and characterization of commisural interneurons in the spinal cord of *Xenopus laevis* embryos revealed by antibodies to glycine. *Development* **103,** 447-461.

Roberts, A., Kahn, J. A., Soffe, S. R. and Clarke, J. D. W. (1981). Neural Control of swimming in a vertebrate. *Science* **213,** 1032-1034.

Rudnicki, M. A. and Jaenish, R. (1995). The *myoD* familiy of transcription factors and skeletal myogenesis. *BioEssays* **17,** 203-209.

Ryan, S. G., Buckwalter, M. S., Lynch, J. W., Handford, C. A., Segura, L., Shiang, R., Wasmuth, J. J., Camper, S. A., Schofield, P. and O'Connell, P. (1994). A missense mutation in the gene encoding the alpha 1 subunit of the inhibitory glycine receptor in the *spasmodic* mouse. *Nature Genet.* **7,** 131-135.

Salzberg, A., D'Evelyn, D., Schulze, K. L., Lee, J.-K., Strumpf, D., Tsai, L. and Bellen, H. (1994). Mutations affecting the pattern of the PNS in *Drosophila* reveal novel aspects of neuronal development. *Neuron* **13,** 269-287.

Schilling, T. F., Piotrowski, T., Grandel, H., Brand, M., Heisenberg, C.-P., Jiang, Y.-J., Beuchle, D., Hammerschmidt, M., Kane, D. A., Mullins, M. C., van Eeden, F. J. M., Kelsh, R. N., Furutani-Seiki, M., Granato, M., Haffter, P., Odenthal, J., Warga, R. M., Trowe, T. and Nüsslein-Volhard, C. (1996). Jaw and branchial arch mutants in zebrafish I: branchial arches. *Development* **123**, 329-344.

Serafini, T., Kennedy, T. E., Galko, M. J., Mirzayan, C., Jessell, T. M. and Tessier-Lavigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans UNC-6*. *Cell* **78,** 409-424.

Shiang, R., Ryan, S. G., Zhu, Y. Z., Hahn, A. F., O'Connell, P. and Wasmuth, J. J. (1993). Mutations in the alpha 1 subunit of the inhibitory glycine receptor cause the dominant neurologic disorder, hyperekplexia. *Nature Genet.* **5,** 351-358.

Sillar, K. and Roberts, A. (1988). A neuronal mechanism for sensory gating during locomotion in a vertebrate. *Science* **331,** 262-265.

Soffe, S. R. (1987). Ionic and pharmacological properties of reciprocal inhibition in *Xenopus* embryos. *J. Physiol.* **382,** 436-473.

Tessier-Lavigne, M. (1994). Axon guidance by diffusible repellants and attractants. *Curr. Opin. Genet. Dev.* **4,** 596-601.

Thomas, J. H. (1994). The mind of a worm. *Scienec* **264,** 1698-1699.

Trevarrow, B., Marks, D. L. and Kimmel, C. B. (1990). Organization of hindbrain segments in the zebrafish embryo. *Neuron* **4,** 669-679.

Trowe, T., Klostermann, S., Baier, H., Granato, M., Crawford, A. D., Grunewald, B., Hoffmann, H., Karlstrom, R. O., Meyer, S. U., Müller, B., Richter, S., Nüsslein-Volhard, C. and Bonhoeffer, F. (1996). Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, *Danio rerio*. *Development* **123**, 439-450.

Tsuchida, T., Ensini, M., Morton, S. B., Baldassare, M., Edlund, T., Jessell, T. M. and Pfaff, S. L. (1994). Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* **79,** 957-970.

van Eeden, F. J. M., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J., Warga, R. M. and Nüsslein-Volhard, C. (1996a). Genetic analysis of fin formation in the zebrafish, *Danio rerio*. *Development* **123**, 255-262.

van Eeden, F. J. M., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J., Warga, R. M., Allende, M. L., Weinberg, E. S. and Nüsslein-Volhard, C. (1996b). Mutations affecting somite formation and patterning in the zebrafish, *Danio rerio*. *Development* **123**, 153-164.

van Mier, P. (1988). Reticulospinal neurons, locomotor control and the development of tailswimming in *Xenopus*. *Acta Biologica Hungaria* **39,** 161-177.

van Mier, P., Armstrong, J., Roberts, A. (1989). Development of early swimming in *Xenopus laevis* embryos: Myotomal musculature, its innervation and activation. *Neuroscience* **32,** 113-126.

Van Raamsdonk, W., Pool, C. W. and Kronnie, G. T. (1978). Differentiation of the muscle fiber types in the teleost *Brachydanio rerio*. *Anat. Embryol.* **153,** 137-155.

Van Raamsdonk, W., Van der Stelt, A., Diegenbach, P. C., Van der Berg, W., DeBruyn, H., Van Dijk, J. and Mitzen, P. (1974). Differentiation of the musculature of the teleost *Brachydanio rerio*. I. Myotome shape and movements of the embryo. *Z. anat. Entwickl. Gesch.* **145,** 321-342.

Van Raamsdonk, W., Van Veer, L., Veeken, K., Heyting, C. and Pool, C. W. (1982). Differentiation fo muscle fiber types in the teleost, *Brachydanio rerio*, the zebrafish. *Anat. Embryol.* **164,** 51-62.

Van Vector, D., Sink, H., Fambrough, D., Tsoo, R. and Goodman, C. (1993). Genes that control neuromuscular specificity in *Drosophila*. *Cell* **73,** 1137-1153.

Waterman, R. E. (1969). Development of the lateral musculature in the teleost, *Brachydanio rerio*: A fine structural study. *Am. J. Anat.* **125,** 457-493.

Weintraub, H. (1993). The *MyoD* family and myogenesis: redundancy, networks, and thresholds. *Cell* **75,** 1241-1244.

Westerfield, M., Liu, D. W., Kimmel, C. B. and Walker, C. (1990). Pathfinding and synapse formation in a zebrafish mutant lacking functional acetylcholine receptors. *Neuron* **4,** 867-874.

Whitfield, T. T., Granato, M., van Eeden, F. J. M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J. and Nüsslein-Volhard, C. (1996). Mutations affecting development of the zebrafish inner ear and lateral line. *Development* **123**, 241-254.

Williams, B. D. and Waterston, R. H. (1994). Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. *J. Cell Biol.* **124,** 475-490.

(Accepted 14 February 1996)