"All the Zebrafish News That's Fit to Print"

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CARDIOVASCULAR DEVELOPMENT IN THE ZEBRAFISH: I. MYOCARDIAL FATE MAP AND HEART TUBE FORMATION

By D.Y.R. Stainier, R.K. Lee, and M.C. Fishman, Cardiovascular Research Center, Massachusetts General Hospital, 149 13th St., Charlestown, MA 02129, USA and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

We have examined the origin of cardiac progenitors in the zebrafish embryo by injection of single blastomeres with a lineage tracer dye, and examined the formation of the zebrafish heart tube by serial sectioning of immunostained embryos. At the 512-cell stage (early blastula), most cardiac progenitors lie in a marginal zone that extends from 90 longitude (midway between the future dorsal and ventral axis) through 180 longitude (the future ventral axis) to 270 longitude. By focusing on myocardial progenitors located at 90 (and 270) longitude, we found that a single cell injected in the early blastula can contribute progeny to both the atrium and ventricle. A cell injected in the midblastula contributes progeny to either the atrium or ventricle, but not both. This analysis suggests that, at least for these myocardial progenitors, the atrial and ventricular lineages separate in the midblastula.

Precardiac cells involute early during gastrulation and turn towards the animal pole with other early involuting cells. These cardiogenic cells reach the embryonic axis around

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THE VENTRAL AND POSTERIOR EXPRESSION OF THE ZEBRAFISH HOMEOBOX GENE *EVE1* IS PERTURBED IN DORSALIZED AND MUTANT EMBRYOS

By J.-S. Joly[1], C. Joly[2], S. Schulte-Merker[3], H. Boulekbache[2], and H. Condamine[1; 1] Unité de Génétique des Mammifères, Institut Pasteur, 25 rue du Docteur Roux, F-75724 Paris Cedex 15, FRANCE; [2] Laboratoire de Biologie du Développement, Université Paris 7, 7 quai St-Bernard, F-75251 Paris Cedex 05, FRANCE; [3] Laboratory of Developmental Biology, NIMR, The Ridgeway, Mill Hill, London NW7 1AA, UNITED KINGDOM.

We have identified and characterized zebrafish *eve1*, a novel member of the *Drosophila even-skipped* (*eve*) gene family. *eve1* RNAs are expressed initially in late blastulae with a peak during the gastrula stage, at which time expression is confined to ventral and lateral cells of the marginal zone of the zebrafish embryo. Later, *eve1* transcripts are located in the most posterior part of the extending tail tip. We show that LiCl, known to dorsalize *Xenopus* embryos, has the same effect in zebrafish, resulting in embryos with exaggerated dorsoanterior structures. In LiCl-treated embryos, *eve1* transcripts are completely absent. *eve1* is therefore a marker of ventral and posterior mesoderm.

In the light of its ventroposterior expression domain, the localization of *eve1* transcripts was analyzed in *spadetail* (*spt*) and *no tail* (*ntl*), two mutants with abnormal caudal development. In spt^{b140} homozygous mutants, there is an accumulation of cells in the tail region, resulting from inadequate migratory behavior of trunk somite cells. These cells, in their abnormal environment, express *eve1*, emphasizing the correlation between ventro-posterior position and *eve1* expression. In homozygous mutant embryos for the gene *ntl* (the homologue of mouse Brachyury, originally called Zf-T), posterior structures are missing (M.E. Halpern and C.B. Kimmel, personal communication). While mutant and wildtype embryos do not differ in their *eve1* transcript distribution during gastrulation, *eve1* expression is absent in the tail bud of mutant embryos during early somitogenesis, indicating a requirement for *ntl* in the maintenance of *eve1* expression.

Our findings suggest that *eve1* expression is correlated with a ventral and posterior cell fate, and provide first insights into its regulation.

MARK THESE DATES ON YOUR CALENDAR!

By J.S. Eisen*, D. Grunwald, and W. Driever, *Institute of Neuroscience, University of Oregon, Eugene, OR 97403 USA

April 27-May 1, 1994 will be the first community-wide zebrafish meeting at Cold Spring Harbor Laboratory. The organizers want to thank everyone who responded so promptly to our queries about the meeting.

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GENTLE FIXATION BY FREEZE SUBSTITUTION GIVES EXCELLENT HISTOLOGICAL RESULTS WITH ZEBRAFISH EMBRYOS

By K. Griffin, Developmental Biology Research Centre, Randall Institute, King's College, 26-29 Drury Lane, London WC2B 5RL

Freeze substitution is a previously described technique for the gentle fixation and dehydration of tissue. I originally employed it solely because it was reputed to result in minimal loss of antigenicity and had been used by other groups with similar research interests (Shiurba *et al*, 1991; Michael *et al*, 1984). However, I wish to draw it to the attention of other zebrafish workers because it gives such excellent histological results with zebrafish embryos which are far superior to other methods, such as paraformaldehyde fixation for example (fig 1).

The main features of the technique are as follows. By freezing the tissue at a temperature well below the eutectic point (-85°C; the minimum temperature at which ice crystallization occurs), rapid and uniform ice formation occurs and tissue damage due to crystal formation is eliminated, so long as the tissue sample is small and the cryogen volume is large. The cryogen recommended here, isopentane, has high specific heat and thermal conductivity, so that heat from the sample is conducted away fast and does not adversely affect the temperature of the cryogen. After freezing, the tissue is transferred to methanol kept cold on dry ice. The methanol slowly dissolves (substitutes) the ice from the tissue, but has very little fixative activity because fixation is indirectly proportional to the ambient temperature.

Freeze substitution is, therefore, a convenient method for generating high quality histological material without the use of cross-linking fixatives and with only minimal denaturation. Since it is compatible with the use of wax sectioning, it is also more convenient than cryofixation. It might also be the method of choice for the dehydration and embedding of previously fixed embryos, e.g. sectioning of whole-mounts or prior to sectioning for *in situ* hybridization or immunostaining.

Method

1. Place 100 ml of isopentane in a small Duran bottle into an ice box or wide-mouthed thermos filled with dry ice. When the temperature is equilibrated (1-2 hours), pour ~200 mls of liquid nitrogen directly over the bottle. It is best to monitor the temperature of the isopentane with a thermo-couple, but a rule of thumb is to wait 10-15 min after pouring the nitrogen before freezing the tissue. It

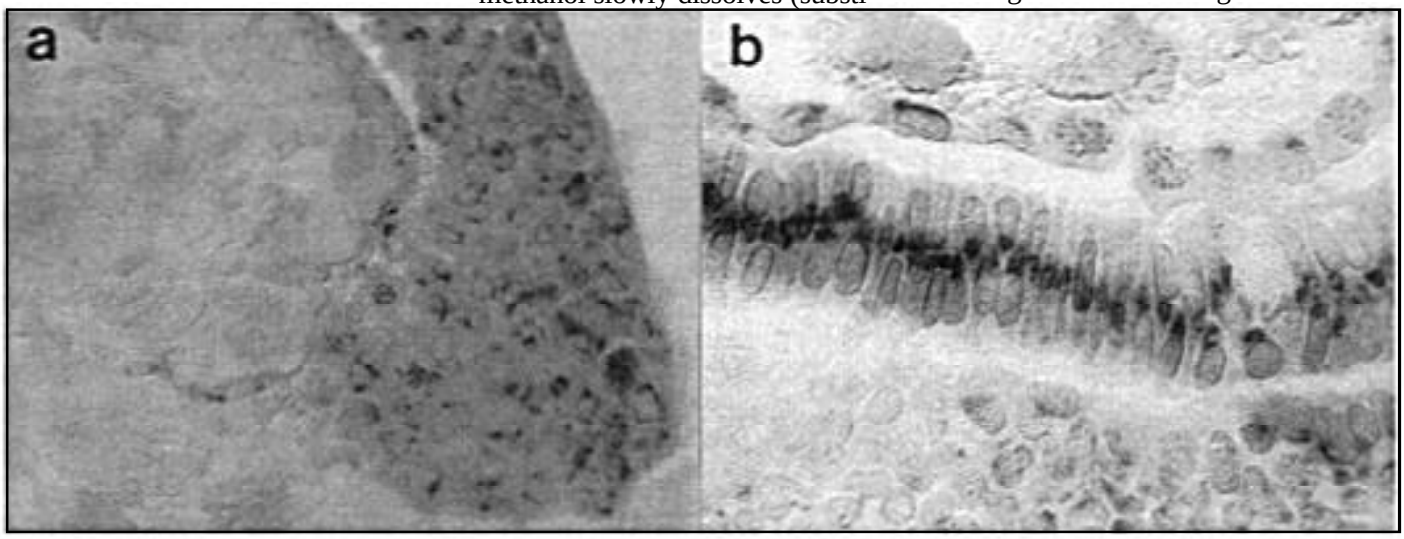

Figure 1. **(a)** Sagittal section of a zebrafish embryo at 50% epiboly immunostained with an antibody to bFGF. The future dorsal side is shown at the onset of involution (animal pole is uppermost). The EVL, deep cells and the YSL are all easily distinguished. The yolk platelets appear brownish and refractile after equilibration in glycerol and under Nomarski optics. The numerous brown intracellular spots are HRP staining products. **(b)** Longitudinal section of a ten somite embryo immunostained using the same antibody. The notochord is well displayed in this section, showing a heavy central area of staining in what appears to be the Golgi apparatus. The yolk cell and YSL can be seen immediately underneath, and the neural tube above.

is a good idea to swirl the isopentane periodically to equalize the temperature. In a separate ice bucket, place as many 5-10 ml labeled vials of methanol as required on dry ice. (Screw-top scintillation vials are ideal; flip-top lids are very difficult to remove when cold.)

2. Pipette embryos (in their chorions) on to one end of a 5x1 cm piece of nylon gauze (or other suitable material). Dab off excess water through the underside of the gauze; the embryos will adhere quite strongly to the gauze through surface tension. Embryos can also be dechorionated and embedded in agarose (e.g. 0.8% low-melting point agarose for support; excess agarose should be trimmed with a scalpel prior to freezing.

3. Hold the gauze by the other end with forceps and plunge the embryos into the center of the isopentane for 1 min.

4. Transfer the embryos (now securely frozen onto the gauze) to the methanol. Store at -70˚C (at least), preferably colder, for 5 days or longer*****. I have not tried shorter times.

5. When required, slowly warm the tissue by transferring it to -20˚C (freezer) for 2-3 hrs, the fridge for 2-3 hrs, and to room temperature for 2-3 hrs.

6. The embryos are ready for embedding. I routinely use polyester wax (BDH) because this is molten at 37˚C and is gentler on the tissue. It is also soluble in methanol or ethanol, avoiding the need for additional organic reagents. This wax can conveniently be cut in a cool room (<20˚C).

The one drawback of this method seems to be the short shelf life of the tissue. I have stored tissue in the fridge for 2 months and found a considerable increase in non-specific nuclear staining (looks similar to toluidine blue counterstaining) and a decrease in signal. However, the results are sufficiently good to offset this minor complaint.

***** At -70˚C ice crystals will be able to form, but this does not seem to be a problem here, perhaps because zebrafish embryos dehydrate sufficiently before crystals form. However, should material of higher quality be required, e.g. for EM work, then it is recommended to use 15% propylene glycol as a cryoprotectant during the freezing, and to freeze substitute in ethanol at -100˚C throughout. This is a slightly more complicated procedure but does not require expensive equipment; the temperature is attained using a slurry of dry ice and nitrogen kept in a thermos in the freezer with periodic monitoring and addition of more dry ice and nitrogen as required. Consult Campbell *et al* (1991) for a description of the technique.

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AUTONOMOUS EXPRESSION OF THE *NIC1* ACETYLCHOLINE RECEPTOR MUTATION IN ZEBRAFISH MUSCLE CELLS

By D.S. Sepich, R.K. Ho, and M. Westerfield, Institute of Neuroscience, University of Oregon, Eugene, OR 97403

The *nic1*^{b107} (*nic1*) mutation blocks expression of both functional and clustered acetylcholine receptors (AChRs) in zebrafish muscle. Normally, signaling between motoneurons and muscles regulates AChR clustering. To learn if signaling is affected and to identify the primary cellular target of the *nic1* mutation, we made mosaic embryos by transplanting motoneurons and muscle precursors from wild-type to mutant embryos. Genotypically mutant muscles cells fail to cluster AChRs even when contacted by wild-type motoneurons, whereas genotypically mutant motoneurons induce AChR clustering on wild-type muscle cells. Moreover, mutant muscle cells fail to cluster AChRs under culture conditions that induce AChR clustering on wild-type cells. We conclude that the *nic1* mutation acts autonomously in muscle cells rather than by affecting signaling between motoneurons and muscle. The wild-type *nic1* gene is necessary in muscle for expression and clustering of AChRs.

Cardiovascular... *cont'd from Page 1*

the 8-somite stage, and there they coalesce to form a pair of myocardial tubular primordia on either side of the midline. By the 21-somite stage, the tropomyosin-immunoreactive myocardial tubes have moved closer to each other, and a distinct group of cells the endocardial progenitor cells, sits medially between them. The myocardial tubes then fuse to enclose the endocardial cells and form the definitive heart tube. By 22 hours postfertilization (26-somite stage), the heart tube is clearly beating. The regionalization of cardiac myosin heavy chain expression distinguishes the cardiac chambers at this stage, although they are not morphologically delineated until 36 hours.

This work shows that cardiogenic regions can be identified in the early blastula, and that chamber restriction seems to arise in the midblastula. Additionally, it provides the basis for embryological perturbation at the single cell level, as well as for the genetic analysis of heart tube formation in the zebrafish.

A SIMPLIFIED RIBONUCLEASE PROTECTION ASSAY FOR EMBRYOS

By G.M. Kelly and R.T. Moon, Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195

We use a ribonuclease protection assay based on the methods of Thompson and Gillespie (*Anal. Biochem.* **163**:281-291, 1987) and Haines and Gillespie (*Biotechniques* **12**:736-740, 1992) to characterize the expression of various transcripts during zebrafish embryogenesis. This rapid technique, which eliminates the need to isolate purified RNA, is extremely sensitive such that an overnight exposure is sufficient to detect a *wnt1* signal from ten 12-hr embryos (Kelly and Moon, in preparation). In addition, with the generation of a standard curve, this assay can also be used to determine the abundance of a specific transcript.

1. Collect embryos at particular developmental stages in 1.5 ml microcentrifuge tubes, and remove the medium. Add 45 l of lysis buffer (4 M guanidine thiocyanate, 25 mM sodium citrate and 0.5% sarcosyl) for every ten embryos.

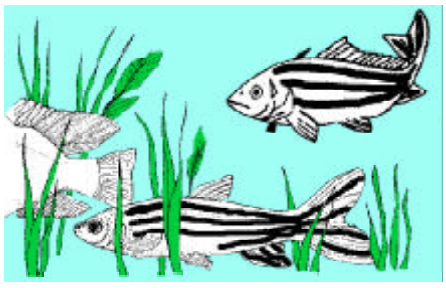
2. Draw embryos and lysis buffer through a 22 gauge needle into a syringe, expel and repeat three times, then vortex and store at -20˚C.

3. Centrifuge the embryonic lysates for 1 min to pellet the broken chorions. For hybridization, transfer 45 l of each sample to tubes containing 1x10^5 cpm of ^{32}P-UTP-labeled antisense probe diluted in 5 l of lysis buffer. Varying the amount of labeled probe may be necessary to optimize signal to background. We prefer the Maxiscript kit available from Ambion (Austin, TX) for making the probes.

4. As a positive control and to generate a standard curve, hybridize 45 l of lysis buffer containing either 1.0, 0.1, 0.01, or 0.001 ng of synthetic sense strand RNA with the radioactive antisense probe. Similarly, hybridize the antisense probe with 45 l of lysis buffer containing 10 l of *torula* tRNA to ensure that the RNAse will digest any unprotected single-stranded RNA molecules.

5. After an overnight hybridization at 55˚C, mix the sample with 500 l of RNAse cocktail (20 l RNAse A, Sigma R-5503, previously boiled for 5 min, aliquoted and stored at -20˚C plus 500 U of RNAse T1, Sigma R-1003, in 10 mM Tris-HCl, pH 7.5, 300 mM NaCl and 5 mM EDTA), and incubate at 37˚C for 1 hr. Varying the hybridization temperature may have an effect of the sensitivity of the assay, we begin at 55˚C and then, if necessary, alter the temperature in 5˚C intervals.

6. To isolate nucleic acids after hybridization, digest the lysate with 10 l of 20% SDS and 5 l of 20 mg/ml proteinase K, for 45 min at 37˚C. Add Isopropanol (500 l) and 3 ng of *torula* RNA to each sample before centrifuging. Air dry the pellets, resuspend in 10 l of loading buffer (88% formamide, 10 mM EDTA, 1 mg/ml each of xylene, cyanol, and bromophenol blue), heat at 75˚C for 4 min, then place on ice before loading onto a 5% polyacrylamide/urea/taurine gel (details in the Sequenase kit, US Biochemical). Electrophorese samples next to 2000 cpm of undigested probe and DNA sequencing reactions to provide size markers, and process the gel for standard autoradiography.



GENOMIC LIBRARY

By Scott E. Stachel, Department of Molecular and Cell Biology, Division of Biochemistry, and Molecular Biology, 401 Barker Hall, University of California at Berkeley, Berkeley, CA 94720.

I produced a zebrafish genomic library last fall, and now feel that this reagent has been tested enough so that it can be distributed to other laboratories. The specifics of the library follow:

The library was made from genomic DNA obtained from 72 h zebrafish embryos (post-fertilization). The embryos were from commercially-obtained Twild-typeU fish (Ekkwill Tropical Fish Breeders, Gibsonton, FL). The DNA was partially digested with Sau3A to an average size of 20 kb, the first two nucleotides of the Sau3A ends were filled-in, and the DNA was cloned into Xho-1 digested and partially filled-in Lambda Fix II (Stratagene). The ligation was packaged with Gigapack II XL Packaging Extract (Stratagene), which preferentially size selects for 47-51 kb recombinants (18-22 kb inserts). A primary library of 1.8 x 10^6 clones was amplified in SRB/P2 to a titre of 4 x 10^9 pfu/ml. As of March 1993 the titre had dropped to 2 x 10^9 pfu/ml.

The supplied phage stock should be amplified in XL1-Blue/MRA or SRB (Stratagene), or an equivalent strain. Upon arrival, 70ml of DMSO should be added per ml of stock, and the library subsequently stored at -70 C and the titre checked.

I have found that the amplified library contains all genes for which I have screened, including three separate retinoic acid receptor genes, goosecoid, noggin, dor3, otx, zhox21, and alpha-tropomyosin. Inserts have been in the 20 kb size range.

TIDBITS (NIBBLES) ON RAISING ZEBRAFISH BABIES

By K.C. Cheng, E. Kauffman, and E. Gestl, Department of Pathology, Penn State, M.S. Hershey Medical Center, Hershey, PA 17033

Use of fine nylon mesh to remove undesirable protozoa from zebrafish embryo cultures.

We have found that paramecia fed to zebrafish babies are, on occasion, accompanied by an overgrowth of small ciliated protozoa (5-50 microns) that are inevitably present in paramecium cultures. This overgrowth can produce a rapid loss of zebrafish. We have recently saved several cultures by pouring the entire culture with the zebrafish larvae through 5 micron nylon netting (we used Nitex brand, available through Argent Laboratories, 1-800-426-6258); this allows most of the smaller protozoa to squeeze through. Filtration is slow enough to keep the fish in water at all times. Most of the volume of the tank can be decanted to minimize the time required to wash the zebrafish culture, and the collected fish can then be rinsed with an excess of clear water. We began with three cultures, each of about 25 nine-day old leopard zebrafish in 24 x 14 x 13 cm mouse cages half-filled with water in which small, ciliated protozoan overgrowth was present. Twelve embryos were lost in an hour. These cultures were washed, replaced in their original tanks after washing with dechlorinated tap water (during filtration), filled half full with fresh prewarmed dechlorinated water, and fed with powdered food. No embryos were lost over the next 24 hours.

Small brine shrimp for feeding baby zebrafish.

After feeding of young (4-12 day old) embryos with paramecia or powdered food, brine shrimp feeding is used to accelerate growth. During the early days of feeding (after about day 9), we have noted that the brine shrimp are often too large for many of the zebrafish to eat. There are two solutions to this problem. First, Grade 0 Platinum label Argentemia from Argent Chemical Laboratories yields smaller brine shrimp than other sources. This is a relatively expensive solution. An alternative is to take advantage of the large variation in size of brine shrimp (from 250-700 micron length) by differential filtration. To separate the smaller from larger brine shrimp for those first feedings, we use 220 micron nylon netting. The brine shrimp that pass through the 220 micron mesh are collected using either 100 or 120 micron mesh and fed to the babies. The larger ones are fed to the larger babies. For the first brine shrimp feedings, we simultaneously continue feeding Microfeast L-10 and spirulina powder until all the fish are eating brine shrimp.

NUCLEIC ACID EXTRACTION PROCEDURE FOR ZEBRAFISH EMBRYOS

By Debbie Ellies, Loeb Institute for Medical Research, Ottawa Civic Hospital, 725 Parkdale Avenue, Ottawa, Ontario, CANADA K1Y 4E9

This rapid, simple procedure yields DNA and RNA which can both be seen on a normal agarose gel. We remove the unwanted nucleic acid with the appropriate nuclease.

Buffer: 100mM Tris-HCl (pH 8.0)
100 mM EDTA
250 mM NaCl
1% SDS
use RNase free reagents!

You do not need to remove embryos from their chorions.

For a single embryo:

- Rinse embryo in sterile water.
- Homogenize in 10µl of buffer by hand with a sterile pipette tip.
- Add 90µl of buffer and homogenize again.
- Extract with 50µl of phenol:chloroform:isoamyl-alcohol (50:48:2). Mix gently by inversion, centrifuge and collect top aqueous phase.
- Extract aqueous phase as above but with 50µl of chloroform:isoamyl-alcohol (24:1).
- Add 25µl of NaAcetate 3M pH 7 and precipitate with 200µl of ethanol.
- Resuspend pellet in 20µl of TE.

For 5 or more embryos:

- Rinse embryos in sterile water.
- Homogenize in 100µl of buffer by hand with a sterile plastic pestle
- Add 300µl of buffer and homogenize again.
- Extract with 200µl of phenol:chloroform:isoamyl-alcohol (50:48:2). Mix gently by inversion, centrifuge, and collect top aqueous phase.
- Extract aqueous phase as above but with 200µl of chloroform:isoamyl-alcohol (24:1).
- Add 100µl of NaAcetate 3M pH 7 and precipitate with 800µl of ethanol. *Note: with 5 or more embryos, the nucleic acid precipitate is immediately visible and can be centrifuged right away.*
- Centrifuge and resuspend pellet in 20µl of TE.

MUTANT STRAINS AT THE UNIVERSITY OF OREGON

By the University of Oregon zebrafish group

Following is a reasonably complete list of (recovered) mutations now at the Oregon facility. All of the mutants were isolated at Oregon, in the Streisinger, Kimmel, and Weston labs, except for *lof* and *leo*, which were obtained from the Nüsslein-Volhard lab at Tübingen, and *flh*, obtained from the Jowett lab at Newcastle. Unless otherwise noted, and except for the *reg* mutants, phenotype descriptions refer to embryos. Allelic mutations (as determined by failure of complementation) have the same names. Complementation analysis has not yet been carried out for the *reg* mutants. Names of unpublished mutations should be regarded as tentative. Some mutations have not yet been named.

Abbreviations D: dominant, e: enu, g: gamma, rl: recessive lethal (usually by 7 days), s: spontaneous, ts: temperature-sensitive, v: viable

Locus	Name	Allele	Origin	Description
albino	*alb*	*b4*	s	Unpigmented melanocytes at 48 h, adult has pink eye, v
beaky	*bky*	*b188*	g	Protuberance at nose, gray yolk, rl
blackhead	*blh*	*b191*	s	Tiny head & eyes, dark dorsal pigment, no lateral line neuromasts, no jaw, rl
brass	*brs*	*b2*	s	Lightly pigmented melanocytes at 48 h, adult has ruby (dark) eye. v
chinless	*chw*	*b146*	g	Jaw defect, small eyes, rl
coral	*crl*	*b257*	e	Lacks most adult iridophores, irregular stripes, v
cyclops	*cyc*	*b16*	g	Lacks floor plate, fused eyes, ventral curving tail, rl
cyclops	*cyc*	*b213*	g	Phenotype like *b16*, probably a translocation, rl
cyclops	*cyc*	*b229*	g	Phenotype like *b16*, rl
feeble	*feb*	*b130*	s	Feeble movements, lacks pigment, neural degeneration, rl
floating head	*flh*	*nl*	s	No notochord, partial floor plate, fused somites, rl
fused eye	*fsy*	*b173*	g	Fused eye, lacks floor plate, probably a translocation, rl
fibers unbundled	*fub*	*b45*	g?	Skeletal muscle Myofibrils not organized into striations, partly paralyzed, rl
fibers unbundled	*fub*	*b126*	g	Phenotype like *b45*, rl
fibers unbundled	*fub*	*b153*	g	Phenotype like *b45*, rl
golden	*gol*	*b1*	s	Lightly pigmented, brown melanocytes at day 2, v
golden	*gol*	*b13*	g	Pigmentation like *b1*, degeneration in late embryo, rl
golden	*gol*	*b204*	g	Pigmentation like *b1*, degeneration in late embryo, rl
ghost	*gst*	*b259*	e	Condensed melanin granules, v
jaguar	*jag*	*Db230*	s	Homozygote has few stripes, heterozygote has splotchy stripes with missing pigment in the center of tail, v
jawless	*jwl*	*b163*	g	Jaw defect, lightly pigmented melanocytes, rl
leopard	*leo*	*t1*	s	Late appearing spotted pigment pattern, v
lively dead	*lid*	*b222*	g	Neural degeneration, hyperactive, rl
long fin	*lof*	*Dt2*	s	Late appearing defect in regulation of fin growth, v
mother of pearl	*mop*	*b258*	e	Variegated dorsal-ventral transformation, extra dorsal stripes & dorsal fin
	max	*b214*	g	Haploid has missing or very tiny Mauthner axon
neural degeneration	*ned*	*b39*	g	Neural degeneration at 30 h, primary neurons survive, rl
nicotinic receptor1	*nic1*	*b107*	g	Paralyzed, no functional nicotinic acetylcholine receptors, rl
no tail	*ntl*	*b160*	g	No notochord or tail. Somites not chevron shaped, rl
no tail	*ntl*	*b195*	s	Phenotype like *b160*, rl

poof	*pof*	*b201*	g	Disintegrates during somitogenesis, rl
poor pec fins	*ppf*	*b217*	g	Pectoral fins small and poorly formed, rl(?)
	reg1	*b260*	e	Fin regeneration defect, ts v
	reg3	*b261*	e	Early arrest of fin regeneration, ts v
	reg4	*b262*	e	Fin regeneration defect, ts v
	reg5	*b263*	e	Early arrest of fin regeneration, ts v
	reg6	*b264*	e	Fin regeneration defect (late regenerate tumors), ts v
	reg8	*b265*	e	Fin regeneration defect (fragile stump), ts v
	reg9	*b266*	e	Slow fin regeneration, ts v
	reg10	*b267*	e	Fin regeneration defect, ts v
	reg11	*b268*	e	Fin regeneration defect, (V-shaped regenerate), ts v
	reg12	*b269*	e	Fin regeneration defect (cleft defect), ts v
	reg13	*b270*	e	Early arrest in fin regeneration, ts v
	reg16	*b273*	e	Fin regeneration defect (cleft defect), ts v
	reg17	*b274*	e	Late defect in fin regeneration, ts v
	reg18	*b275*	e	Late defect in fin regeneration, ts v
rose	*rse*	*b140*	e	Late lack of some irodophores and melanocytes, v
silent heart	*sih*	*b109*	g	Lacks a beating heart, rl
sparse	*spa*	*b5*	s	Embryo lacks normal number of melanocytes especially in head, adult has mottled stripes and few dorsal melanocytes, v
sparse	*spa*	*b134*	g?	Phenotype like *b5*, v
spiral tail	*spi*	*b193*	g	Dorsally spiraling tail, neural degeneration, reduced yolk tube, rl
short fin	*shf*	*b123*	s	All fins are short in adult, v
spadetail	*spt*	*b104*	?	Trunk muscle precursors migrate to tail, which forms a terminal mass of cells, rl
short tail	*stl*	*b180*	g	Short, pyramidal tail, small eyes, missing jaw structures, reduced pigment, rl
throbless	*tbl*	*b212*	g	No heart beat, touch insensitive, rl
transparent	*tra*	*b18*	s	Lacks iridophores at 72 h, adult has huge black eyes and almost transparent body
		b136	g	Haploid has bulb tail, neural degeneration, rl
		b147	g	24 h embryo is necrotic, hyperactive, long kinked tail, rl
		b152	g	No jaw or pectoral fins, small head, dorsally curving axis, reduced pigment, thin yolk tube, rl
		b158	g	Lightly pigmented, grayish cast, little movement, retarded? rl
		b174	g	Haploid has spinal bifida, diploid necrotic at 72 h, rl
		b177	g	Melanocytes lightly pigmented, kink at end of very thin tail, retarded? rl
		b181	g	Dark dorsal pigment, jaw defect, rl
		b186	g	Gastrulation defects producing severe necrosis, possible translocation, rl
		b187	g	Midbrain and hindbrain degeneration, thin yolk tube, rl
		b197	g	Visceral arches degenerate, rl
		b202	g	Neural degeneration, rl
		b205	g	Jaw defects, midbrain degeneration, rl
		b215	g	Ventral tail defect, partially v
		b218	g	Lacking jaw structures, small poorly formed pectoral fins, rl?
		b225	g	Haploid has bulb tail, diploid has spiral tail and neural degeneration, rl
		b226	g	Early necrotic axis, rl
		b227	g	Necrotic, has planar tail, possible involved in translocation with *b226*

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