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Zebra Fish, the Reliable Vertebrate Model Organism-A Review.

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Abstract:

The mouse has long been favored as a model organism for the study of vertebrate development, cancer biology as well as toxicology research, but in 1980's Streisinger introduced zebra fish as a model organism for developmental biology and genetics. The zebra fish has advantage over mouse with its high fecundity, small size, rapid regeneration time and transparency at an early embryogenesis which favored the study of developmental biology. This technique was further developed to study comparative zebra fish biology for modeling human disease through several transgenic models. In present review, overview for current state of knowledge and resources for zebra fish study.

Keyword: - Zebra fish, disease modeling, resources, technique.

Introduction:

Zebra fish (*Danio rerio*.) is a tropical freshwater teleost belonging to Cyprinidae family representing only four species indigenous to South Asia such as *Danio dangila, Danio jaintianensis, Danio meghalayensis* and *Danio rerio,* broadly distributed across India, Bangladesh, Nepal, Myanmar, and Pakistan (Rahman, 1989; Barman, 1991; Talwar and Jhingran, 1991; Menon, 1999; Bhat, 2003). In the late 80's and 90's zebra fish received widespread attention in study of vertebrate developmental biology (Anderson and Ingham, 2003 and Mayden *et al*., 2007) and well suited to both developmental and genetic analysis (Streisinger *et al*., 1981, Fishman, 2001, Grunwald and Eisen, 2002). Later on Streisinger *et al*. (1981) comparatively studied all tropical species and selected finalized Zebrafish *Danio rerio* as a model organism for the study of vertebrate developmental pattern, which was a breakthrough in zebra fish research era. There are various factors for employing Zebra fish as a model organism, as it is small (2-3 cm long), translucent, high fecundity, rapid regeneration time and transparency at an early embryogenesis which is useful for study of different developmental stages (Driever *et al*., 1994). Several investigations in this field are

going considering zebrafish as a model organism in human welfare. The morphological differences between male and female are very clear. The males are translucent with black stripe, slender and torpedo shaped, followed by black longitudinal stripes and usually a gold colouration on the belly and fins. Females are fat when laden with eggs and have little, if any, gold on their undersides. Eggs are fertilized and embryos develop (in 2-4 days). Embryos are clear and easily observed under the microscope.

Development of the zebra fish embryo (Kimmel *et al*., 1995) has been studied in detail, from pre-gastrula to gastrula stages (Driever, 1995; Kuwada, 1995; Solnica-Krezel *et al*., 1995; Woo *et al*., 1995) through organogenesis (Schmitt and Dowling, 1994; Streisinger and Fishman, 1994) and gives solid information for the detection and interpretation of mutant phenotypes. Further studies on Zebrafish by various researcher revealed the development of modern genetic techniques, such as 'cloning'(Streisinger *et al.,* 1981), mutagenesis (Chakrabarti 1983, Walker *et al.,* 1983, Grunwald *et al.,* 1992 and Solnica-Krezel *et al.,* 1994), transgenesis (Stuart *et al.,* 1988) and mapping approaches, underpinned the use of zebra fish to apply invertebrate-style forward genetics to questions of vertebrate development.

Zebra fish Strain

Table 1. Zebra fish Strains

AB (AB)	HK/AB (HK/AB)	SJA (SJA)
AB/C32 (AB/C32)	HK/Sing (HK/SING)	SJD (SJD)
AB/TL (AB/TL)	Hong Kong (HK)	SJD/C32 (SJD/C32)
AB/Tübingen (AB/TU)	India (IND)	Tübingen (TU)
C32 (C32)	Indonesia (INDO)	Tupfel long fin (TL)
Cologne (KOLN)	Nadia (NA)	Tupfel long fin nacre (TLN)
Darjeeling (DAR)	RIKEN WT (RW)	WIK (WIK)
Ekkwill (EKW)	Singapore (SING)	WIK/AB (WIK/AB)

Table 2: Historical journey of Zebrafish through scientific world

Years	Events
1960	Streisinger obtains zebra fish from commercial suppliers
1970	Gynogenetic technique developed for mapping and gene linkage studies. Haploid embryos from eggs activated with ultraviolet irradiated.
1980	Cell-lineage studies in the early embryo, visualization of neurite outgrowth in a living zebrafish embryo, and mutagenesis and mapping reported.
	The method for producing clonal lines of homozygous zebrafish is published. Gynogenetic procedures and their potential applications are described.
	The first description of an induced embryonic-lethal mutation in the zebrafish is published
1990	The fate map of the zebrafish gastrula, Cell transplantation to generate genetically mosaic embryos is used to test autonomy of gene function.
1993	Systematic large-scale screens for embryonic lethal mutations begin in Tübingen, Germany, and Boston, USA
1994	First Cold Spring Harbor Conference on Zebrafish Genetic sand Development is held. No tail is the first mutation to be identified molecularly, using a candidate-gene approach.

1996	The results of the 'Big Screen' are published in a single issue of Development, volume 123. Development of linkage map and genomic resources. Insertional mutagenesis is established and large-scale screens for insertional mutants begin. The Trans-NIH Zebra fish Initiative is launched. Establishment of a centralized, web-based database, ZFIN, and a stock centre, ZIRC.
1997	Eyed pinhead is the first mutation to be positionally cloned.
2000	The beginning of whole genome sequencing.
2001	Sanger Centre initiates Zebrafish Genome Sequencing
	Efficient germline transgenesis using transposons
2002	1st zebrafish TILLING report published
	1st Zebrafish Genome Assembly released
2003	European 6th Framework funds zebrafish models.
	1st zebrafish transgenic model of a human cancer
2004	1st chemical genetic screen published.
2005	1st Strategic Conference of Zebrafish Investigators
2006	6th version of the Zebrafish Genome Assembly released.
	7th International Zebrafish Meeting, 1000 participants

(Reference –Nature review genetics Jonathan et.al. 2002 and Graham J. Lieschke and Peter D. Currie 2007)

Table 3: Comparative Zebrafish biology for modeling human disease

Characteristics	Key similarities to humans	Key differences and unknowns
Genome structure	Diploid; essentially contains the full vertebrate repertoire of genes	Gene duplication resulting from ancestral whole-genome, duplication, resulting in subfunctionalization and neofunctionalization
Anatomy	Vertebrate body plan	Aquatic adaptations include streamlined body plan and different locomotor strategies
Diet and metabolism	Omnivorous	Poikilothermic, grows optimally at 28.5°C
Growth	Growth is determinate exhibits a ☐alutatory growth pattern	regeneration capacity of many tissues and organs, for example, heart, fin, retina
Lifespan	Juvenile and adult phases of growth around the point of reproductive maturity;	Lifespan of 3–5 years; generation time of 3 months
Embryology	Stages and processes of cleavage, early patterning, gastrulation, somitogenesis, organogenesis	Very rapid; non-placental, occurs ex vivo; influence of maternal transcripts; involves hatching.
Skeletal system	Complex ossified skeleton comprising cartilage and bone	Lack long bone, cancellous bone, and bone marrow; joints are not weight-bearing
Muscle	Axial and appendicular muscle groups; skeletal, cardiac and smooth muscle cell types; fast and slow skeletal muscle fibres	Fast- and slow-twitch muscle are topographically separate; tail-driven locomotion depends on alternating contractionof myotomal muscle; appendicular muscle bulk is proportionately small
Nervous system and behaviour	Representative anatomy: fore-, mid- and hind-brain, including diencephalon, telencephalon and cerebellum; peripheral nervous system with motor and sensory components; enteric and autonomic nervous systems; specialized sensory organs: eye,	Telencephalon has only a rudimentary cortex; fish-specific sensory organs, such as the lateral line; fish behaviours and cognitive function are abstracted or simplified compared with human behaviour

	olfactory system and ear; exhibit 'higher' behaviours and integrated neural function: memory, conditioned responses and social behaviours	
Haematopoietic and lymphoid/ immune systems	Multiple haematopoietic cell types,T- and B-lymphocytes; coagulation cascade for haemostasis; innate and adaptive humoral and cellular immunity	Erythrocytes are nucleated; possess thrombocytes rather than platelets; kidney interstitium is the haematopoietic site; could have evolved fish-specific immune system components
Cardiovascular system	Multi-chamber heart with an atrium and ventricle; circulation within arteries and veins; separate lymphatic circulation	Has left–right distinctions in cardiac anatomy, but does not have separate left–right circulations, that is, the heart has only two chambers; so far no evidence for secondary heart field derivatives; lymph nodes have not been described
Reproductive system	Molecular and embryological biology of germ-cell development; cellular anatomy of germ-cell organs, the testis and ovary	No sex chromosomes; mechanism of sex determination is uncertain; fertilization is ex vivo; non-lactating; no breast equivalent
Endocrine system	Most endocrine systems represented, for example, hypothalamic/hypophyseal axis (glucocorticoids, growth hormone, thyroid hormone, prolactin), parathyroid hormone, insulin and rennin	Differences in anatomical distribution of glands, prolactin has a primary role in osmoregulation
Skin and appendages	Ectodermal derivative; pigmentation pattern is due to melanocytes	Have structures unique to fish that are specialized for the aquatic environment (for example, elasmoid scales, mucous cells); possess two additional pigment cell types: xanthophores and iridophores

Table 4 Zebra fish genome map from Zebra Fish Information Network (ZFIN)

Organism	BioProject	Assembly	Status	Chrs	Organelles	Size (Mb)	GC %	Gene	Protein
Danio rerio	PRJNA1392, PRJNA11776	Zv9	Complete	25	1	1,412.47	36.7	28,770	27404

Resource, Nature review genetics, Lieschke and Currie 2007 (Ref: ZFIN)

Scientific Application:

Recently Zebrafish has been accepted as a model that is widely used to investigate a wide variety of diseases (Rubinstein, 2003). Research with *D. rerio* has yielded advances in the fields of developmental biology, oncology (Berghmans *et al.,* 2005), toxicology (Xiang 2009, Hill *et al.,* 2005), reproductive studies (Hill, A. J. 2005), teratology, genetics, neurobiology, environmental sciences, stem cell and regenerative medicine (Major 2007), Drug discovery (Zon and Peterson, 2005) and evolutionary theory (Parichy, 2006).

Acetylcholine is a neurotransmeter required for central nervous system functioning. It play critical role in memory function and its defect cause Alzheimer´s disease (AD). The brain organization between human and zebrafish is quite similar (Wullimann and Reichert, 1996). Furthermore zebrafish is used to solve the problem in Alzheimer´s disease (Santana *et al.,* 2012), mammalian osteogenesis and tissue

mineralization (Spoorendonk *et al.,* 2010). Several scientists isolated and sequenced the genes for AD disease from zebrafish which showed similarity with human genome for example, psen1 (Liemer *et al.,* 1999),Aph1(Francis *et al.,* 2002) , Pen2 (Groth *et al.,* 2002), APOE (Babin. 1997), Nicastrin (Sanger *et al.,* 2010), etc. Not only disease treatment, it is randomly used as an alternative model for mice and rat in brain organization and behavior studies. Use of rodent model in neuroscience is much more successfully, but it is expensive due to cost. That's why researchers are now interested in zebrafish as a complimentary model to detect the particular region of brain which control the hungriness, water trust, learning etc. (Guo, 2004). Few researchers reported the presence of different types of receptor in zebrafish such as GABA receptor (Kim *et al*., 2004), NMDA receptor (Nam *et al.,* 2004). Like acetylcholine, glutamate is the another important neurotransmitter that has been already characterized in zebrafish by many scientists (Portavella 2002; Cox 2005) and that would be used in future neurological disease treatment.

In 21st century new technique developed for reverse genetics to reduce gene expression or modify splicing using Morpholino oligonucleotide (MO) antisense technology (Nasevicius and Ekker, 2000). In recent year Lamason (2005) identified a gene using morpholino knockdown technique from golden strain (SLC24A5) which is responsible for unusual pigmentation that is required for melanin biosynthesis. Transgenesis is a popular approach to study the function of genes in Zebrafish. Zebrafish as a human genetic model is reliable due to similar genome construction and similar homology (Postlethwait 2000). Sprague (2008) reported that Zebrafish showed a 50%-80% homology with the available human genome. Construction of transgenic Zebrafish is rather easy by a method using the Tol2 transposon system (kawakami, 2004). The *Tc1*/mariner family transposable element sleeping beauty (SB) for transgenesis and long-term expression studies in the Zebrafish (Davidson, 2003). Researchers at Boston Children's Hospital developed a new strain of Zebrafish (Casper) with unique phenotypic character i.e. adult bodies with transparent skin (White, 2008).

Now a days, Zebrafish have been widely used to make several transgenic models for biomedical studies like cancer, including melanoma (Ceol Craig, 2011), leukemia (Langenau 2005, Langenau 2003, Langenau, 2005), pancreatic cancer and hepatocellular carcinoma.

Table 5: The Zebrafish tools and technique

Technology	Description	References
Forward genetics		
Chemical mutagenesis	High mutation rates, large-scale screens	Solnica-Krezel et al 1994
Insertional mutagenesis	Efficient cloning of mutations	Amsterdam 2003
Reverse genetics		
Morpholinos	Rapid, inexpensive gene knock-downs	Nasevicius, A. & Ekker 2002
TILLING	Directed identification of permanent mutations	Wienholds et al 2002
Expression profiling		
Gene chip	Zebrafish Affymetrix chip	Affymetrix GeneChip Array
Spotted microarrays	cDNA and oligonucleotide microarrays	Stickney,et al. 2002,Ton,2002
Other tools		
Transgenesis	Rapid production of stable transgenic lines	Meng, et al 1999 Davidson,et al(2003) Kurita,et al (2004).

cDNA collections	Full-length cDNA collections	www.ncbi.nlm.nih.gov/genome
Mutant collections	Thousands of catalogued mutant lines Hundreds of lines available through public stock centres	www.zfin.org/zirc www.eb.tuebingen.mpg.de
Physical and genetic maps	Radiation hybrid and microsatellite genetic linkage maps	Kwok,et al.(1998). Hukriede, N. A. et al. (1999), Geisler, R. et al. (1999)
Genomic sequence	5.7-fold coverage of the zebrafish genome Substantial genome annotation	www.sanger.ac.uk, www.nih.gov

(Reference, Reviewed by Lieschke and Currie, 2007 nature review Genetics)

Database resources

Database	Online resources
NCBI	http://www.ncbi.nlm.nih.gov/genome/danio rerio
Zebra Gene collection	zgc, http://zgc.nci.nih.gov
Zebra fish model organism Database, ZFIN	http://zfin.org
Zebra fish Resources,Well come Trust Sanger institute	http://www.sanger.ac.uk/resources/zebrafish
Zebra fish Zv9	http://asia.ensembl.org/Danio rerio
This site allows browsing of zebrafish BAC and PAC clones that were sequenced, analysed and manually annotated within the Sanger zebrafish genome project.	(Vega) Zebrafish Genome Annotation Browser
This site allows browsing of the latest zebrafish whole genome assembly Zv9	Zebrafish Genome Browser at Ensembl
A joint collaboration between UCSC and the Zebrafish Genome Initiative at Children's Hospital Boston	Zebrafish Genome Browser at UCSC
A community resource for Zebrafish Genomics	FishMap Zebrafish Genomics Knowledgebase
Tübingen Map of the Zebrafish Genome	
Stock Centers	a) Zebrafish International Resource Center (ZIRC) b) Tübingen Zebrafish stock center
Morpholinos	a) Morpholino Database b) Quality control testing of Gene Tools morpholinos
Mutagenesis Projects	a) Targeted mutations using zinc-finger nucleases (ZFNs) b) Zebrafish Mutation Resource (Sanger Institute)
FishMap is a unified and centralized resource for storage, retrieval, and display of genomic information of zebrafish.	FishMap (IGIB,INDIA)

India scenario of Zebrafish research

Zebra fish is native to Indian subcontinent which is fresh water dweller. First time CSIR-IGIB laboratories initiate Zebrafish research and announced first whole genome sequencing in 2009 (Sivasubbu 2010). IGIB Zebra fish research group member and their respective research area viz. Sridhar Sivasubbu (Zebrafish Functional Genomics), Chetana Sachidanandan (Zebrafish Chemical Genetics)

and Vinod scaria (Genome informatics). FishMap Database developed by IGIB group which is a unified and centralized resource for storage, retrieval, and display of genomic information of Zebrafish. Another organization which is actively engaged with Zebra fish research, TATA institute of fundamental research (TIFR), Mumbai, Research team lead by Dr. Mahendra sonawane, Zebrafish Epidermis Research Laboratory, mainly focus on Epidermis development using zebra fish as a model organism. Another CSIR-CCMB Center for cellular and Molecular Biology Hyderabad started of Zebra fish facility (2012). Their thrust research area mainly Zebrafish regeneration, Dr. Mohammed M Idris and colleagues actively engaged with Zebra fish fin regeneration research (Mohammed et al 2008).

Future Perspective: -

Zebra fish (*Danio rerio*) is widely used for fundamental research such as pattern formation, developmental biology and biomedical research purpose for generating human diseases model. Biomedical research depends on the use of animal models to understand the pathogenesis of human disease at a cellular and molecular level and to provide systems for developing and testing new therapies (Lieschke and Currie, 2007). In many Human disease, zebra fish based modeling for human Duchenne muscular dystrophy (Bassett, 2003), Photosensitive erythrocytes and altered porphyrin profile models of hepatoerythropoietic porphyria and erythropoietic protoporphyria (Wang 1998, Childs 2000), human DiGeorge syndrome, (Piotrowski 2003). Biomedical disease modeling is active research area, which explore mechanism of various genetic disorders as well as unanswered question of health.

Certain lower vertebrates such as urodele amphibians and teleost fish have a greater capacity for regeneration than mammals. However, Zebra fish also having regeneration capacity in some organs such as fin, skin and heart. Little is known about molecular mechanisms of regeneration, and cellular mechanisms are incompletely defined (Poss and Nechiporuk, 2003).

A number of non-mammalian vertebrate species are able to regenerate their hearts including the Zebrafish which can fully regenerate its heart following amputation of up to 20% of the ventricle (Raya et al.2003, Poss et al 2002). Now a day's many researchers vigorously unravel mysteries of regeneration mechanism by exploiting proteomics and genomics.

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