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Transposable elements: Strategies and mechanism of transposition in *Danio rerio*, a genetic model

DHARMENDRA KUMAR MEENA[1], B.K. BEHERA[1], PRONOB DAS[1], A.K. PRUSTY[2], SATENDRA KUMAR[3], M. SEKAR[3] AND KANTI MEENA[4]

[1]Central Inland Fisheries Research Institute (CIFRI), Barrackpore, KOLKATA (W.B.) INDIA
[2]Project Directorate of Farming System Research, MEERUT (U.P.) INDIA
[3]Central Institute of Fisheries Education, MUMBAI (M.S.) INDIA
[4]Central Research Institute for Jute and Allied Fibres, Barrackpore, KOLKATA (W.B.) INDIA
Email: dkmeenafnb@gmail.com

Zebra fish (*Danio rerio*) is an excellent model animal to study vertebrate development by various genetic approaches. External development and transparent nature of its embryo makes it feasible to visualize and manipulate a cell in the living organism. Genetic engineering is well suitable in this organism due to the availability and easy collection of large number of embryos from the adult fish. Several approaches have been implemented to manipulation the zebra fish at DNA, RNA and protein level by employing different technologies (EMU and trapping based mutagenesis, TILLING, site specific recombination, transgenics and so on). Identification of several transposon in this model organism has opened the doors for genetic manipulation with a new approach which can be effectively used in other animals. Taking these into consideration it can be used in transgenic development which would increase sustainability by manifolds in term of production and vigour traits production. This study highlight the recent developments in the field of insertional mutagenesis in zebra fish, the mechanisms and concepts of new transposon-based mutagenesis approaches under development which may be used as reference approach for adopting in commercially important fish or gene silencing for disease diagnosis in higher vertebrates . This review is an eye opening signal for researcher which would ignite deep interest to carry forward these modern tools and techniques to address the short falls of genetic improvement strategies indispensable for sustainable aquaculture.

Key words : Zebra fish, (*Danio rerio*), Transposones, Mutagenesis, Genetic model, Gene trapping

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INTRODUCTION

Transposons or transposable elements (TEs) are sequences of DNA that can move around to different positions within the genome of a single cell; the process of their movement is called transposition. The event of transposition can cause mutations and change in the size of genome. TEs make up a large fraction of genome, which is evident through the C-values of eukaryotic species and these elements are part of sheer volume of seemingly useless material termed as "Junk DNA". Barbara Mc Clintock discovered TEs, early in her career for which she was awarded a Nobel Prize in 1983. TEs are present both in prokaryote and eukaryotes. The mobile genetic elements can be grouped based on their mechanism of transposition into Class I and Class II TEs. Class I mobile genetic elements, or retrotransposons, transpose themselves by being first transcribed into RNA, then require enzyme reverse transcriptase to transcribed back into DNA, travel to another position at the accepter site, and integrate into the genome. However, class II mobile genetic elements move directly from one position to another using an enzyme transposase to "cut and paste" themselves at donor and acceptor site, respectively within the genome. Similarly, based on their self sufficiency to transpose, TEs can also be categorized into autonomous TEs and have sequence which encodes for transposase and are self sufficient to transpose and integrate into the genome whereas non autonomous TEs require the transposase from other source for their transposition, non autonomous transposons.

The recent introduction of several transposable elements in *Danio rerio* (zebra fish) opens new frontiers for genetic manipulation in this important vertebrate model. This article discusses TEs as mutagenesis tools for fish

functional genomics, briefing various mutagenesis strategies that were previously applied in other model organisms, such as *Drosophila melanogaster* (drosophila), *Arabidopsis thaliana* (arabidopsis) and *Mus musculus* (mouse) and similarly may be beneficial if applied in fish genetic research.

Zebra fish - An ideal genetic model for research:

Zebra fish exhibits many qualities that are essential in a good genetic model organism such as large, robust and transparent embryo (Dahm 2006), high fecundity, reasonably short generation time, relatively small size, low maintenance costs, amenability to large-scale saturating mutagenesis screening, availability of mutants, genetic maps, diurnal sleep cycle similar to mammals (Jones 2007), easy drug administration, external fertilization and fully sequenced genome. The lack of some classical tools in genome manipulation has been a serious drawback.

However, in recent years, several transposable elements have been demonstrated to be capable of transposing, among them many are from heterologous hosts (*Tc3*, *mariner*, *Tol2*, *Sleeping Beauty*, *Frog Prince*, and *Ac/Ds*) and have been successfully applied in zebra fish (Fadool *et al.* 1998; Raz *et al.,* 1998; Davidson *et al.* 2003; Emelyanov *et al.,* 2006). In contrast, so far only three of them *viz., SB* (Davidson *et al.,* 2003, Balciunas *et al.* 2004), Tol2 (Kawakami *et al.* 2004; Parinov *et al.,* 2004) and Ac/Ds (Emelyanov *et al.,* 2006; Parinov and Emelyanov, 2007) have been utilized for mass production of transgenic fish and further efforts are required to scale up their numbers. Research with zebra fish has allowed advances in the fields of developmental biology, oncology (Xiang, 2009), toxicology (Hill *et al.,* 2005) reproductive studies, teratology, genetics, neurobiology, environmental sciences, stem cell, regenerative medicine (Major and Poss, 2007) and evolutionary theory (Parichy *et al.,* 2006).

Mutagenicity of transposon insertions:

A promising modular mutagenesis approach has been recently developed in zebra fish (Sivasubbu *et al.,* 2006). This strategy combines the mutagenic capacity of 5' gene trap with the high trapping capacity of polyA trap vectors. The 'gene-breaking' transposon vector developed (Sivasubbu *et al.,* 2006) harbours a 5' mutagenicity cassette and a 3' polyA trap cassette in the same transposon vector. The 5' mutagenicity cassette produces strong termination of transcription by combining an effective splice acceptor sequence with a polyA signal of ocean pout antifreeze protein.

To date, only a few examples of successful gene inactivation using gene trap vectors have been reported in zebra fish (Kawakami *et al.,* 2004; Kotani *et al.,* 2006). Unbiased systematic studies using a statistically significant number of tagging events are required to estimate the actual mutagenic ability of the various gene trap designs and to compare it with the mutagenicity of transposon insertions carrying simple reporters or enhancer traps.

Transposon vectors can also be designed to screen for gain-of-function mutations by random activation and/or over-expression of genes (Rorth *et al.,* 1998; Weigel *et al.,* 2000). In these screens, usually referred to as 'activation tagging' or 'modular mis-expression', transposons are equipped with enhancer or promoter sequences that can induce transcription of the endogenous gene adjacent to the insert. Several laboratories have begun testing these tools in zebra fish, but there are no published reports yet.

Mutagenesis of closely linked genes by re-mobilization of genomic transposon insertions:

A classic gene trap vector (5' gene trap) harbours a splice acceptor site upstream of a promoter less reporter (Stanford *et al.,* 2001). When inserted into an intron of a transcribed gene it can cause abnormal fusion transcripts and expression of the reporter gene under regulation of the promoter of the gene into which it is inserted. Because gene trap vectors target introns, they are effective in species with large introns such as vertebrates. However, the rate of alternative splicing through the splice acceptor sequence of the gene trap cassette can be insufficient to prevent fully the synthesis of the original transcript, resulting in generation of hypomorphic alleles (McClive *et al.,* 1998) or no detectable effect on gene function. Multiple splice acceptor sequences and multiple transcription terminator sequences (Vallier *et al.,* 2001) can be introduced

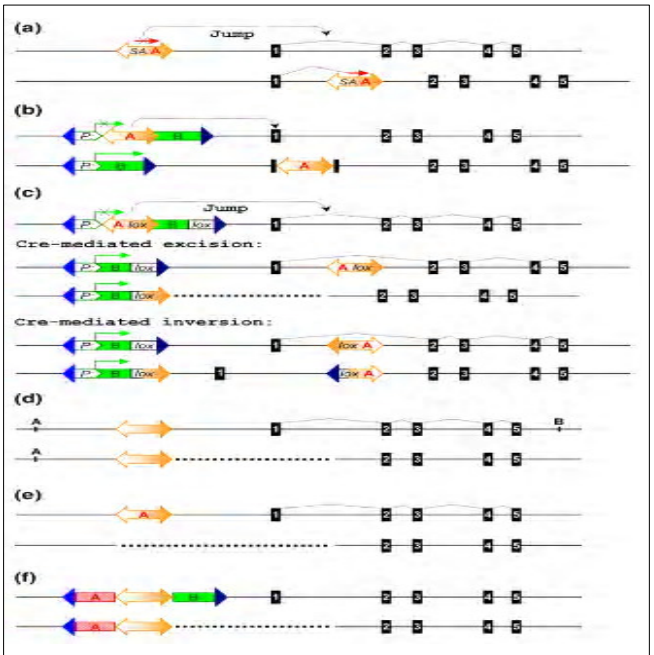

Fig. 1: Strategies for mutagenesis of nearby genes by re-mobilization of genomic transposon insert

into gene trap vectors to improve their mutagenicity and tagging efficiency. The selection scheme had shown (Fig. 1) which can only detect transposition events that cause detectable reporter gene expression.

A more effective strategy for selecting re-transposition events uses a combination of two selection markers: one to detect the excision events and another to retain the re-transposed element (Fig. 1). This double selection scheme utilizes two types of transposable elements. A 'carrier' element harbours a 'jumper' element and an excision marker. The 'carrier' is used to generate insertions in the genome that are subsequently used as the donor sites for transposition of the 'jumper' element. In plant genetics, such donor sites, usually called 'launch pads', have successfully been used to saturate specific regions of the genome with insertions (Smith *et al.,* 1996; Ito *et al.* 1999; Panjabi *et al.,* 2006). Because zebra fish exons are small, the mutagenesis efficiency of this approach is expected to be low. Arming the jumper element with an additional 'mutagenicity cassette' (Sivasubbu *et al.,* 2006) should be beneficial.

– Regional mutagenesis using gene trap elements- Here, the reporter gene at the donor site is silent. Transpositions can be detected when reporter gene expression is activated.
– Regional mutagenesis from a 'launch pad'- A jumper element carrying a selection marker 'A' (for example, GFP under regulation of a constitutive promoter) is inserted between an open reading frame of a marker gene 'B' (for example, red fluorescent protein) and a suitable promoter. When the jumper element is excised, the expression of the 'B' is switched on. Animals carrying an empty donor site and retaining the jumper element can be analyzed by polymerase chain reaction.
– Regional mutagenesis from a launch pad combined with site-specific recombination system (Cre/*lox*)- The system is a modification of the method shown in part b but the carrier and jumper elements both carry *loxP* sites. After local re-transposition of the jumper element the region between the *loxP* sites is deleted or inverted according to the orientation of *loxP* sites using Cre recombinase.
– Selecting flanking deletions using flanking marker recombination (from the method of P induced male recombination in *Drosophila*) - This approach requires two closely linked markers (A and B) around the donor site. The TEs is usually retained at one side of the deletion.
– Generating deletions by selecting for 'imprecise excision' events.
– A compound element optimized for screening of flanking deletions- This approach can detect the same events as the methods shown in parts c and d but no additional markers are required. The animals that harbour deletion events can be identified by loss of one flanking marker, whereas retention of the other marker shows presence of the donor site.

Large-scale reverse genetics mutagenesis in fish :

In the post-genome era, the most common problem is gaining insight into the function of genes that have been identified by sequencing but for which mutant information is lacking (reverse genetics). Therefore, reverse genetics approaches to mutant screening must operate with a complete collection of random mutations representing all genes, regardless of the phenotypic manifestation. Until recently, only one reverse genetic approach has been implemented in zebra fish to obtain mutations in the gene of interest. This approach utilizes TILLING (targeting induced local lesions in genomes) and re-sequencing of ENU-mutagenized fish for target-selected screening of point mutations in the gene of interest (Wienholds *et al.,* 2003). Although this approach is very powerful, considerable screening efforts are required each time to obtain mutations in every new gene. Furthermore, this method of mutagenesis generates predominantly missense mutations, which may or may not result in phenotypes.

An alternative approach to reverse genetic mutagenesis screening was recently pioneered by Znomics, Inc. (Portland OR USA), where a large-scale library of retroviral insertions in zebrafish was produced (*http://www.znomics.com*). The company has been identifying the locations of retroviral insertions from cryopreserved sperm samples containing, on average, 25 insertions per sample by sequencing the flanking DNA adjacent to the insertions. With 100,000 insertions already identified, Znomics hopes to map a total of half a million insertions. Thus, their bank of retroviral insertions promises to be a valuable resource for the zebra fish research community. However, the actual efficiency of the Znomics screen and the utility of the strains remain to be determined. Despite the recent vector improvements, the frequency of transposon insertions still remains lower than that of retroviral insertions. It is therefore important to determine whether transposon vectors have the potential to complement the retroviral projects in the field of large-scale insertional mutagenesis. One way to increase the number of transposon insertions per germline is to use the donor lines carrying multiple inserts for re-transposition (Speulman *et al.,* 1999).

Mechanisms of transposition :

There are two mechanisms by which transposons move.

Replicative transposition :

The majority of eukaryotic transposable elements are more complex transposons called retrotransposons. The host

cell recognizes the retrotransposon as a normal DNA sequence within its genome, and synthesizes an RNA copy of it. However, the retrotransposon codes for an enzyme called reverse transcriptase which is able to convert the RNA copy of the retrotransposon into an exact DNA duplicate of the original. Transposase (which has been coded for by the retrotransposon) then finds a suitable location to insert the new retrotransposon into the genome. This process is called replicative transposition. If a retrotransposon is left functional, replicative transposition will produce multiple copies of the retrotransposon within the genome (Fig. 2).

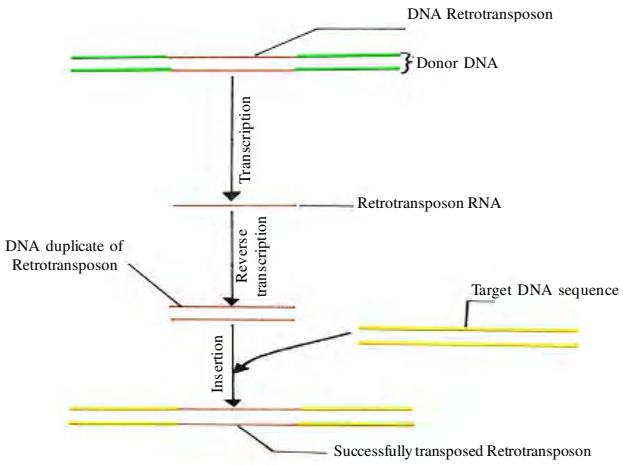

Fig. 2: Replicative transposition

It plays important role in evolution due to its ability to produce rearrangement in the genome and have attracted attention as vector for mutagenesis (Bourchis and Bestor, 2004; Bestor, 2005). But some of the major drawbacks which limit the use of retrotransposons as vectors are A) Rapid increase in its copy number which may hamper the maintenance of stable transgenics. B) Retrotransposons as a vector carry smaller size of gene than reporter gene of fluorescent protein C) These are unable to remobilize and D) certain risks are involved while handling. These factors make it inadequate to be a preferred vector or ETs.

Simple transposition (Non-replicative transposition) :

Transposition of some transposable elements occurs by a non-replicative, cut-and-paste» mechanism. This is also known as direct or conservative transposition. First, the transposase makes a double-stranded cut with the help of a transposon encoded enzyme, transposase in the donor DNA at the ends of the transposon and makes a staggered cut in the recipient DNA. Each end of the donor DNA is then joined

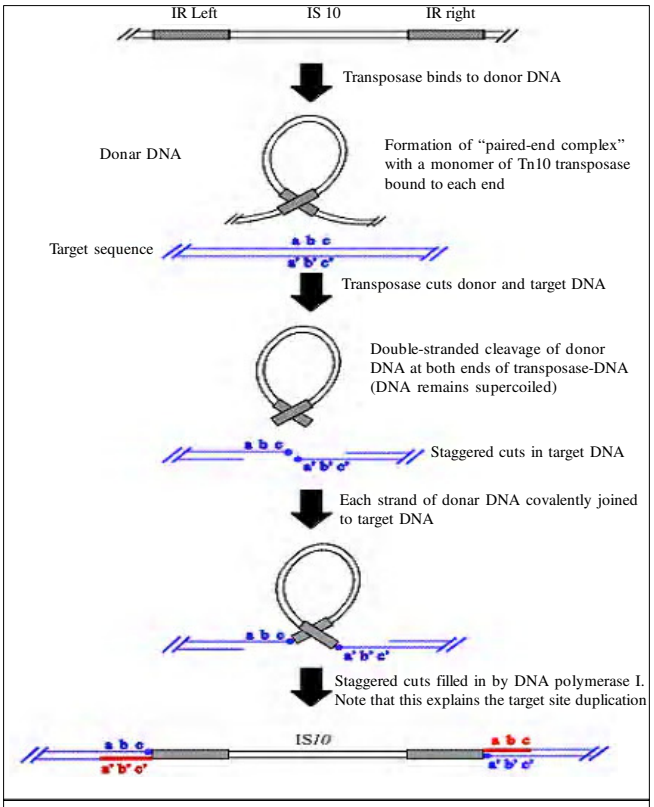

Fig. 3: Simple transposition (Non-replicative transposition)

to an overhanging end of the recipient DNA. DNA polymerase fills in the short, overhanging sequences, resulting in a short, direct repeat on each side of the transposon insertion in the recipient DNA (Fig. 3). However, the gap created at the donor position can be filled in either by element replacement with transponson or without it.

All of these 'cut-and-paste' transposon vectors are designed to operate as two component systems: a non-autonomous element that inserts into the genome of the host and a transposase from other sources (as it transcribes faulty polypeptide) catalyzes transposition of the non-autonomous element). A non-autonomous element can carry a cargo inserted between the two end sequences that are required in *cis* for transposition. The TEs is usually supplied in the form of mRNA by microinjection into fertilized eggs to induce transposition of the non-autonomous element. The ease of microinjection in fish is advantageous; the injected transposase mRNA is degraded after some hours of injection, and new insertions of the non-autonomous element in the genome remain immobilized. Whereas another system work on transposase from self or transposase gene encoded in DNA code for its own transposase.

Selection of TEs to be used would depend on whether after insertion TEs should remain at the insertion site or transpose to different location in the genome. Non

autonomous elements integrate and remain at the same position generation after generation. The system is very useful in development of stable transgenic (Korzh, 2007) and developmental study of embryo in fishes. Whereas in autonomous system we take advantage of their mobility and are useful tool for gene mining.

Prospects of transposons :

- Vector for diverse species, single TEs vector can be used in heterologous hosts for transgenesis and mutagenesis. These vectors are also useful in development of stable transgenics.
- Advantage of using TEs insertions in transgenesis are for easy identification and isolation at large number of sites. In principle, it is possible to find TEs insertions in any nonessential gene in the genome.
- A TEs insertion in a gene usually causes complete loss of function, with a few rare exceptions, transposon insertion mutations are null alleles.
- The phenotype of the insertion mutation is completely linked to antibiotic resistance in genetic crosses. Therefore, it is possible to transfer the mutation into a new strain by selecting for antibiotic resistance.
- Site directed mutagenesis can be performed using TEs at conserved terminal base.
- Insertion mutants can be recovered at high frequency after low-level mutagenesis. Thus, the resulting mutant phenotype is unlikely to be due to multiple transposon insertion mutations. It is possible show that the mutant phenotype is due to the transposon insertion by a genetic backcross.
- Insertions in operons are usually strongly polar. Thus, TEs insertions can be used to determine whether genes are in an operon.
- Insertions can be obtained which are near but not within a gene of interest. (Such insertions are useful for constructing deletions, and for genetic mapping.)
- TEs can provide a portable region of homology. Thus, recombination between TEs can be used to construct deletions or duplications with defined endpoints, or to form co-integrates between different genetic elements.
- Special TEs can be used to construct operon or gene fusions to reporter genes.
- These TEs vector could be useful in gene therapy or replacement of affected gene (s).
- TEs act as an enhancer trap or gene trap when transposon vector carry reporter gene, promoter or splice site in it. This tool can analyze expression pattern of reporter gene or marker gene in different organ or cells. However, examination of expression patterns is comparatively easier and effective in transparent embryo, such as fish embryo.
- Identification, development and analysis of organs which is yet to be described, could be done using ET lines (ex.: corpuscles of stannius (CT) in many fishes). These ET lines can be an excellent tool to study detail anatomy of fishes in vivo.
- Marker assisted screening can be performed using TEs, where the host will be inserted with a marker gene like green fluorescent protein (GFP) and later the same animal could be selected easily.

Disadvantage :

Disadvantage of 5' gene trap is that only insertions in transcriptionally active genes are selected. Genes that are expressed at low levels or are not expressed during the stages observed in the screen remain undetected even if a tagged gene is successfully inactivated by the gene trap. To circumvent this, polyA trap vectors have been designed to tag genes regardless of their transcription status (Zambrowicz *et al.,* 1998). A basic poly A trap vector (sometimes termed 3' gene trap) generally harbors a promoter driving the transcription of a reporter gene, which has no polyA signal but a downstream 3' splice donor sequence. Such reporters normally produce unstable transcripts, which after successful splicing are polyadenylated using polyA signals of the tagged gene.

Conclusion :

At the present time, there appears to be some variability from laboratory to laboratory in cataloging transposon lines. Because various TEs screens are designed for different purposes (mutagenesis, enhancer and gene trapping, driver lines, and so on), it is important to develop a set of standard requirements for the lines that are deposited at the stock center. These requirements should define the alternative descriptions to be provided with each line that would allow a searchable database to be established. At the same time, these requirements should not deter the community from depositing their collections into the stock center. Arguably the simplest way to characterize an insertion line is by sequencing DNA flanking the insertions. This information can be used to map the insertions along the identified genome sequence (Znomics model). Alternatively, flanking sequences can be used to build-up the BLAST dataset, which would allow checking for the availability of insertions inside any DNA fragment using the BLAST search programme. It is much more difficult to produce comprehensive expression pattern information, and to systematize and organize this information into a searchable database. High-throughput reverse genetics screening using transposable elements can generate a huge resource for zebra fish research, but the actual benefit of large-scale projects will depend on the subsequent utilization of this resource. This

challenge will require coordinated efforts from the whole community.

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