

## Transposons – the useful genetic tools

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Mobile DNA elements, originally discovered in maize more than fifty years ago, have become the indispensable tools for bacterial genetics: so many different types of specialised transposon derivatives were constructed so far. The main aim of this article is to summarise the major types of transposon constructs and their application in molecular genetic techniques. Classical *in vivo* transposition applications include insertional mutagenesis, gene fusion and mapping techniques as well as DNA sequencing strategies. Recent applications have extended transposition-based techniques to the analysis of genomes, proteins, protein-DNA complexes and proteomes.

Key words: transposon, transposition, *in vivo* cloning, functional genomics.

Abbreviations: ATC, altered target specificity; DR, direct repeat; IR, inverted repeat; IS, insertion sequence; oriT, origin of transfer; Tn, transposon; Tnp, transposase protein; Ap<sup>r</sup>, Km<sup>r</sup>, Tmp<sup>r</sup>, Str<sup>r</sup>, Cm<sup>r</sup>, Tc<sup>r</sup>, Er<sup>r</sup>, resistance to following antibiotics: Ampicillin, Kanamycin, Trimetoprim, Streptomycin, Chloramphenicol, Tetracycline, Erythromycin.

### General features of transposons

Transposable elements are discrete DNA segments that can be repeatedly inserted into several sites in genome. This process is independent of previously recognised mechanisms for the integration of DNA molecules and occurs without need of DNA sequence homology. The extensive studies – identification and characterisation of mobile elements in bacteria started in the late 1960s and early 1970s. Transposons revealed as the elements diverse in size, structure, specificity of insertion, mechanism of transposition, and regulation of movement and might possess several phyloge-

netic origins (SHAPIRO et al., 1977). The simple insertions, in contrast, are characterised by specific structural features: each insertion contains exactly the same set of non permuted transposition sequences; they are accompanied by duplication of a short target DNA sequence; the ends of transposons are terminated by short inverted repeats (CALOS & MILLER, 1980).

KLECKNER (1981) has divided the transposable elements into three distinct classes, based on the structural properties, mechanism of transposition and DNA sequence homology:

*Class I* – the insertion sequence (IS) modules and composite elements formed from them.

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IS modules are short elements, less than 2 kb in size, encoding only determinants relevant to their own transposition (IS1–IS5, IS102, ISR1). Two copies of certain ISs flanking a DNA segment were termed the composite transposons.

*Class II* – the transposon (Tn) family, sized more than 5 kb, containing 38–40 bp inverted repeats at their ends, which generate 5 bp repeats of target DNA during insertion. These usually encode, in addition to transposition functions, the accessory determinants, such as antibiotic and heavy metal resistance.

*Class III* presents the transposing bacteriophages, such as Mu or its derivatives. These possess the genes and sites for transposition as well as the genes for DNA replication, phage development and cell lysis.

The first mobile elements described were the simple insertion sequences (IS). General features of insertion sequences are that they encode no function except of those genes involved in their own mobility (CAMPBELL et al., 1979). These include the factors required in *cis* recombination, in particular the recombinationally active DNA sequences that define the ends of the element accompanied with enzyme – transposase, which recognises and processes these ends. The majority of ISs exhibit the short terminal inverted repeats (IRs) sized between 10 and 40 bp. The sequence, encoding the transposase gene is often located partially within these IRs accompanied by the upstream promoter with the conventional IR sequences. This arrangement provides the binding mechanism for autoregulation of synthesis. The sequence-specific DNA binding activities of transposase are generally located in the N-terminal region, while the catalytic domain is often localized towards the C-terminal region. An additional characteristic of many transposases is the capacity to generate multimeric forms, essential for their activity. Another general feature of IS elements is the generation of short directly repeated sequences (DRs) of the target DNA flanking the IS during the insertion. The length of DRs, usually between 2 and 14 bp, is characteristic for each element (MAHILON & CHANDLER, 1998).

Early studies have shown two ways of intermolecular insertion mechanisms – the conservative and replicative transposition. The conservative (nonreplicative) transposition known in Tn10 and Tn5 (originated from lambda phage) was described as a transposition of element, separated from vector DNA, by a double-strand break at each end. This insertion into the target DNA sequence is realized without prior replication, and

the linearised vector-donor is destroyed (BERG et al., 1984). The replicative transposition, known for example in Tn1000 (from F factor), is realized by co-integrates that consist of vector and target DNAs (WEINERT et al., 1984). The co-integrates can be resolved in the second recombination step. While the intermolecular transposition yields in the simple insertion and co-integrates, the intramolecular transposition usually result in deletions and/or inversions.

Many of the acquired antibiotic resistance genes, found in enterobacteria and pseudomonades, are the part of small mobile elements known as gene cassettes, but similarly other virulence related genes are likely to be found in these cassettes. Together they form integrons, the mobile elements often responsible for the lateral gene transfer. The origin of these genes is not known, but recent analyses of available data suggested that the gene cassettes might be the ancient structures (RECCHIA & HALL, 1997). Several transposable elements, carrying antibiotic resistance genes, e.g. Tn1 [Ap<sup>r</sup>] (HEDGES & JACOBS, 1974); Tn3 [Ap<sup>r</sup>] (KOPECKO & COHEN, 1975); Tn5 [Km<sup>r</sup>] (BERG et al., 1975); Tn7 [Tnp<sup>r</sup>, Str<sup>r</sup>] (AMYES & SMITH, 1978); Tn9 [Cm<sup>r</sup>] (GOTTESMAN & ROSNER, 1975); Tn10 [Tc<sup>r</sup>] (FOSTER et al., 1975) were recognized almost simultaneously in the mid 1970s as the natural components of R-factor plasmids executing their ability of transposition. The recombinant DNA methods became widely available shortly after the discovery of resistance transposons and the resistance genes were frequently incorporated into the plasmid cloning vectors, currently common in use.

Conjugative transposons are the important determinants of antibiotic resistance, mainly in Gram-positive bacteria. They are remarkably promiscuous providing the conjugation between bacteria, especially those of different species and genera. Transposon-promoted conjugation reminds the F plasmid one, thus only the single strand of the transposon DNA is transferred from donor to recipient. The mechanism of recombination during the conjugative transposition differs from that of other transposons, as it was shown for example by the absence of duplications of the target sequence upon the integration (BRINGELL et al., 1992). The site-specific recombinases, encoded by the conjugative transposons, belong to the integrase family. Alike the phage lambda integrase, the integrase of Tn916 has two DNA-binding domains that recognize different sequences, one within the ends of the element and one that includes target DNA-not specific sequences, but apparently con-

sist of bent DNA. The similarity between the conjugative transposons and phage lambda is striking and suggests that both use the same mechanism of recombination, with the exception of the recombining sites that are nearly always different in the conjugative transposition (SCOTT & CHURCHWARD, 1995). The first step in the conjugative transposition of Tn916 is the excision from donor DNA molecule, followed by the circularisation of transposon and its transfer to a new host. The studies have demonstrated that in Gram-positive hosts, both the Xis protein and the site-specific recombinase Int are required for the excision of Tn916 (RUDY et al., 1997). Neither protein alone is the rate limiting for excision, but overexpression of Int and Xis together resulted in the increased excision. After excision, the frequency of Tn916 circle formation was found to be the same as the frequency of repair of donor DNA molecule. This suggested the close similarity of a single reaction in both molecules (MARRA & SCOTT, 1999).

Lots of transposons are the normal constituents of the most bacterial genomes and of many extrachromosomal plasmids and bacteriophages. The worldwide research indicates that these DNA insertion elements play a special evolutionary function (CAMPBELL, 1981; KIDWELL & LISCH, 2000).

### Transposons as the molecular genetic tools

Recently, there took place the explosive development of *in vitro* DNA technology. Joining of DNA segments *in vivo*, however, has been possible for a number of years. Transposons have been evolved as the natural tools for genetic engineering (BUKHARI, 1977). The most widely used constructs were derived from the insertion sequences, IS containing composite elements transposons, or from bacteriophages. The classical applications of transposable elements in bacterial genetics can be distinguished, based on the type of insertion. In general, the transposition from one DNA molecule to another is usually utilized for the stable maintenance of genetic marker present on the transposon as the source of selectable marker; on the other hand the transposons are often used as the insertion mutagens generating deletions and inversions during their transposition.

Additional application of transposons is based on their ability of the portable reporter genes expression control, the transcriptional-translational fusions as well as for the transpositional fusion. Transposable elements were acquitted as the useful tools for molecular genetics also for

the gene and large-scale genome mapping, for the *in vivo* cloning and the cloned DNA sequencing (BERG & BERG, 1996).

DNA transposition studies up to date have involved strictly *in vivo* approaches, in which the transposon of choice and the gene encoding transposase, the enzyme responsible for transposition, had to be introduced into the cell together. However, the *in vivo* systems have many technical limitations. Therefore, a large number of *in vitro* transposition systems for Tn5, Tn7, Mu, Himar1 and Ty1, which bypass many limitations of *in vivo* systems, have been constructed. For this purpose also, a technique for transposition that involves the formation *in vitro* of release Tn5 transposition complexes, followed by the introduction of complexes into the target cell of choice by electroporation (GORYSIN et al., 2000) was developed.

The strategy useful for transposon delivery depends on the target strain, on whether the target is the bacterial chromosome, a phage, or plasmid. (BERG & BERG, 1996). For chromosomal targets, transposon is usually delivered on phage, suicide vector or F factor. Usually, the suicide vectors are used, when a variety of suicide strategies based on mutant plasmids are unable to replicate under the special condition, for example – high temperature.

Delivery vehicle determines the choice of target molecule. Bacteriophages are the most convenient type of delivery vehicles for insertion into the bacterial chromosome. The phage carrying a transposon can be introduced into the host cell under the conditions, disabling the phage genome replication, cell lysis, or even its stable integration into the host cell. Lambda vehicles are used for the isolation of Tn10, Tn5 and Mu insertions. Mu is also used directly.

Nonconjugative multicopy plasmids and bacteriophages are also the delivery vehicles of choice for the isolation of insertions. The specific plasmid vehicles have also been constructed for this purpose.

In the case the target molecule is phage or conjugative plasmid, the transposon delivery vehicle can be utilized, any type of molecule or replicon other than the target itself. General features of transposon derivatives are important if the primary goal is the isolation of stable insertion into a target gene or region of interest.

For stability it is always preferable to use a mini-transposon construct whenever other considerations permit. Mini-transposons are referred as transposons that do not contain a transposase gene within their boundaries and are generally

smaller than a corresponding wild-type transposon.

### Transposon Tn10 and its derivatives

Tn10, the composite bacterial transposon comprising of two IS10 elements (R and L) plus internal sequences including tetracycline resistance, can move into and out of chromosomes or plasmids in a non-replicative fashion (HANIFORD & CHACONAS, 1992; KLECKNER et al., 1996). Recently, the complete nucleotide sequence of Tn10 has been determined (CHALMERS et al., 2000). Using Tn10 for generation of mutations by transposon insertion can be a powerful analytical technique. Historically derivatives of bacterial transposon Tn10 were described that were useful for defining the functional limits and regulatory sites of bacterial genes (WAY et al., 1984).

Wild-type Tn10 preferentially inserts into the so-called hotspots. Potential sites of Tn10 insertion cannot be predicted, but generally four from six base pairs in the consensus sequence are GC pairs. Mutant IS10 transposase strains with altered target specificity (ATS) exhibit significantly lower degree of insertion specificity than a wild type (HALLING & KLECKNER, 1982).

A lot of Tn10 derivatives, useful for various types of mutagenesis carried on plasmid and/or phage vehicles, have been constructed by KLECKNER et al. (1991). These constructs named NK can be divided into several types (Fig. 1): (i) those carrying the wild-type Tn10 (101), bearing an IS10 Left and Right, the internal sequence and tetracycline resistance genes; (ii) derivative 102, which contains the *ats* mutation and transposase gene fused to the strong, IPTG inducible Ptac promoter useful tool for complementation in *trans* mini-Tn10; (iii) derivatives 103–108 mini-Tn10 with ATS are small (400–3000 bp) and can be used for stable insertions, because they do not carry a transposase gene (these are available with a variety of selectable markers); and (iv) finally the mini-Tn10 construct, which generates the translational fusion of *lacZ* to target gene and the translation fusion to *kan* gene.

Later the plasmid-based vehicles were described, which can be used for delivery of IS10-derived transposons into Gram-negative and also capable to replicate in Gram-positive bacteria (MAHILLON & KLECKNER, 1992). All the derivatives described above use the standard delivery vehicles based on bacteriophage lambda or plasmid pBR322. The transposons carried on plasmid can be introduced by electroporation or transforma-

tion and selected with the appropriate antibiotic. All these systems possess a number of useful features: the variety of antibiotic markers (Er<sup>r</sup>, Cm<sup>r</sup>, Km<sup>r</sup> or Tc<sup>r</sup>); the polylinker containing the restriction sites for rare-cutting endonucleases to facilitate the physical mapping of chromosomal insertions; the mutant transposase that confers relaxation in insertion specificity and positioning of the transposase-encoded gene outside of the transposing segment to ensure the stability of insertions once isolated.

The new derivative, based on a mini-transposon Tn10 named NKBOR, has been constructed (ROSSIGNOL et al., 2001). This mini-transposon contains a conditional R6K-suicide vector that permits the random insertions into the chromosome of Gram-negative bacteria and the subsequent rapid cloning of sequences flanking the insertion site in *E. coli*.

HARE et al. (2001) have developed the *in vivo* genetic foot-printing system based on Tn10 transposon for *E. coli*, a model bacterium for rapid discovering the genes that affect the cell fitness under the variety of growth conditions. This system enables the high frequency of randomly distributed transposon insertions, utilizing the conditionally regulated Tn10 transposase, with relaxed sequence specificity and the conditionally regulated replicon for the vector, containing the transposase and mini-Tn10 transposon with an outwardly oriented promoter using the bacteriophage lambda delivery system.

### Transposon Tn5 and its derivatives

Wild type of Tn5 is a 5700 base pair composite element, in which a pair of simpler mobile elements, e.g. 1534 bp insertion sequences IS50L and IS50R, are present in an inverted orientation with a bracket of central region that contains genes encoding resistance to kanamycin (*kan*) and/or streptomycin. Tn5 transposes with high frequency and inserts into many sites, including a small number of hotspots. Alike several other bacterial elements, Tn5 generates a direct 9-bp duplication of the target sequence at its site of insertion. One of the two IS elements in Tn5, IS50R encodes a *cis*-acting protein, transposase, that is necessary for IS50 and Tn5 movement. Transposase is thought to act directly by binding to distinctive 19-bp nucleotide sequences near the ends of its recognized elements (JOHNSON & REZNIKOFF, 1983). IS50L differs from IS50R in that it contains the promoter used for expression of *kan* gene in Tn5's central region and also it possess an ochre allele of the trans-

## Tn10 derivatives

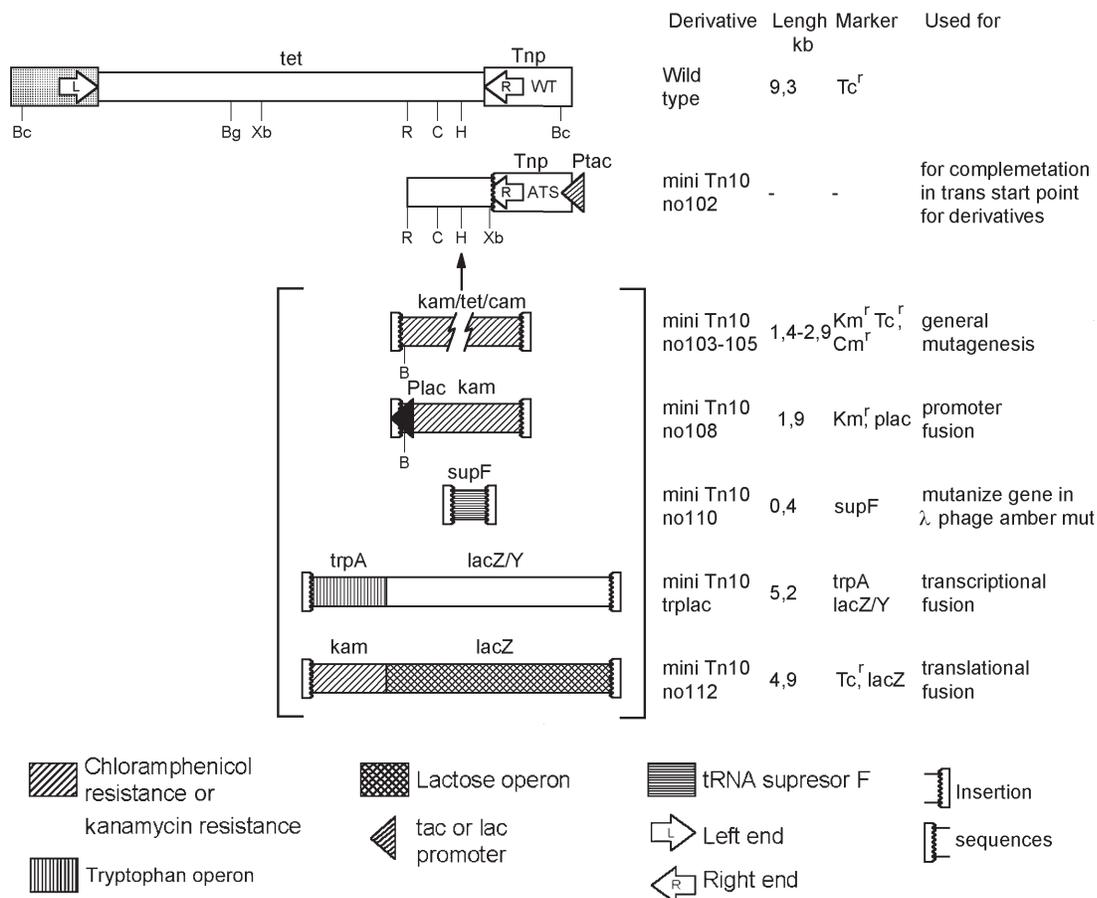


Fig. 1. Genetic and physical maps of the different mini-Tn10 derivatives and their using. Cleavage recognition sites for restriction enzymes are: Bc, BclI; Bg, BglII; C, ClaI; H, HindIII; R, EcoRI; Xb, XbaI. Structure of each transposon is drawn to scale.

posase (*tnp*) gene. Both the *kan* promoter and the mutant *tnp* allele arose from a substitution of single nucleotide pair 112 bp from IS50's inside end (BERG & BERG, 1983; BERG et al., 1984).

Tn5 has become the model for development of an efficient *in vitro* transposition system. The key component of such system was the use of hyperactive mutant transposase. The inactivation of the wild type transposase is likely to be related to the low frequency of *in vivo* transposition.

The *in vitro* experiments have demonstrated the following: the only required macromolecule for the most of steps in Tn5 transposition is transposase; the specific 19-bp Tn5 end sequences, and the target DNA; transposase may not be

able to dissociate itself from a DNA product; Tn5 transposes by a conservative "cut and paste" mechanism; Tn5 release from the donor backbone involves the precise cleavage of both 3' and 5' strands at the ends of the specific end sequences (ZHOU & REZNIKOFF, 1997; GORYSHIN & REZNIKOFF, 1998).

The Tn5 transposition process involves the following steps: (i) binding of transposase monomers to the 19 bp end sequences; (ii) oligomerization of the end-bound transposase monomers, forming a transposition synaptic complex; (iii) blunt end cleavage of the transposition synaptic complex from adjoined DNA, resulting in formation of the released transposition complex or trans-

poson; (iv) binding to target DNA; and (v) strand transfer of transposon 3'-ends into a staggered 9 bp target sequences (ZHOU & REZNIKOFF, 1997; GORYSHIN et al., 2000, REZNIKOFF, 2003).

The Tn5 *in vitro* transposition system provides a highly efficient one step reaction, containing two macromolecular components: a hyperactive form of Tn5 transposase (Tnp) and DNA containing two inverted 19 bp Tnp recognition sites. Synthetic transposons have to contain an *ori*, an antibiotic resistance gene marker, a multicloning site and two hyperactive end sequences. This transposon-plasmid, containing no target sequences should be incubated in the presence of purified transposase protein and transformed into desired target strain to undergo transposition (YORK et al., 1998). Construction of such mini-Tn5 vector – pUT was developed by LORENZO et al. (1998). Tn5 is a composite transposon; its mobility is determined by two insertion sequences (IS50L and IS50R) flanking the DNA region encoding the Km<sup>r</sup> genes. Interestingly, as in Tn10, the transposase determined by IS50R (*tnp* gene) is still functional, even if the gene is artificially placed in *cis* to the cognate terminal sequences. Mini-Tn5 possesses all elements essential for transposition (IS terminal sequences and *tnp* gene) and delivering system into the target strain, based on plasmid, e.g. R6K, which requires a specific replication protein PI, maintained only in host strains. Some mini transposon vectors have the origin of replication as well as the origin of transfer (*oriT*) from F factor (or pRK2 in pUT plasmid). The *tnp* gene is lost shortly after insertion and mini-transposons are anchored preventing the DNA rearrangements or any form of genetic instability.

### **Tn1721 family transposons and their derivatives**

Tn1721 transposon as well as Tn501 and Tn21 belong to a subgroup of Tn3 family, although their sequence homology is relatively low. Transposition of Tn3-like elements is characteristic for involving the formation of co-integrates, catalysed by transposase and co-integrates resolution catalysis by resolvase. The second step is strictly site-specific and requires two directly oriented copies of the resolution sequence in the co-integrate (SCHMITT et al., 1981; ROGOWSKY & SCHMITT, 1985).

Originally isolated Tn1721 transposon contains tetracycline resistance; many of new derivatives carrying resistances to chloramphenicol, tetracycline, kanamycin and streptomycin were described. These elements are provided on various

plasmid vehicles and as chromosomal insertions to extend the range of targets for Tn mutagenesis. Single *EcoRI* sites at the ends of these transposons have been proved as the most useful for physical mapping, for the generation of new *EcoRI* sites in cloning experiments, for end-labelling and for sequencing of DNA adjacent to the insertion (UBBEN & SCHMITT, 1986).

Derivatives of Tn1721 are useful because of their properties, i.e. random insertion and generation of transcriptional fusions at the site of insertion: transposable promoters (Tn1735) carry a strong, inducible Ptac promoter that turns on adjacent (cryptic) genes; and transposable promoter probes (Tn1736, Tn1737) carrying promoterless genes encoding chloramphenicol acetyl transferase or  $\beta$ -galactosidase frequently used for accurate determination of external promoters. These elements are available with four different selectable resistance markers and on conjugative, temperature-sensitive and multicopy plasmid vehicles. Experiments were described that demonstrate the advantage of random insertions for various genes expression and for the gene regulation studies (UBBEN & SCHMITT, 1987).

### **Mu and mini-Mu derivatives use for genetic analysis**

Bacteriophage Mu has been described as a temperate phage, which upon lysogenization generated mutation in host with the highest transposition frequency and the most random insertion specificity of any known transposons. A striking difference between Mu and other temperate phages is that the Mu genome integrates in the host chromosome whether it enters the lytic cycle or the lysogenic state. Its transposition requires the presence of *cis*- and *trans*-acting Mu DNA sequence, especially the left end of Mu phage. Mu was the first transposable element with the evidence of replicative transposition mechanisms. Products of three genes, e.g. *c*, *ner* and *MuA*, which encodes transposase, control transposition. Product of *MuB* gene is required for transposition in high level. Packing proceeds from the *pac* sites – the left end towards the right end, by the head full system up to 35–58 kb (KAHMANN & KAMP, 1979).

Bacteriophage Mu based vectors, used for genetic engineering purpose, harbour a *cis* action terminal DNA sequence, necessary for transposition, the *pac* site and a selectable marker. Most of derivatives contain a thermo-sensitive (ts) allele of repressor (FAELEN & TOUSSAINT, 1976). Mu derivatives, which can still grow as phage,

are designated by the letter “p”, indicating that they can form plaques (e.g., MupAp1); the letter “d” indicates that they are defective and unable to grow as phages, but they can cause the rearrangements, typical for these transposable elements (e.g., MudII). Mu derivatives that can form transcriptional or transcriptional-translational fusions are usually designated MudI and MudII, respectively (GROISMAN, 1991). Mu elements can be introduced into bacteria by transduction, conjugation, or transformation. Transduction has been widely used because Mu has a very broad host range and because all derivatives possess the *pac* sites. Mu lysates are often prepared from double lysogens, harbouring both the defective Mu derivatives to be selected for and a helper Mu phage that will complement the Mu derivatives for the morphogenic functions. Transformation has been usually used to introduce mini-Mu plasmids (GROISMAN et al., 1984).

Transposon mutagenesis has become the procedure of choice to isolate mutants – inactivation of gene, mutagenesis of open reading frames, transcriptional and translational fusions (*in vivo* mutagenesis) or *in vivo* cloning and mapping DNA. For chromosome as a target it usually can be interesting to obtain the stable insertion by performing the mutagenesis with a derivative that is defective for transposition but that can be complemented in *trans*. This can be accomplished by using phage that only transpose in suppressor backgrounds, by using the phage that is *MuA*<sup>+</sup> and co-infecting with a phage that carries the *MuB*<sup>+</sup> gene in *trans* or by using the *MuA*<sup>-</sup>*B*<sup>-</sup> derivatives. Mu phage is especially suitable for the *in vivo* cloning, because it transposes hundreds of times as it replicates when derepressed for its lytic functions (BREMER et al., 1984). Many mini-Mu derivatives with *lac* fusion elements were prepared for gene structure and expression studies (CASTILHO et al., 1984). These can be useful for the *in vivo* cloning so far, because they possess plasmid high-copy (or low-copy), broad-host-range replicons, different resistance markers, some of them also an *oriT* (GROISMAN & CASADABAN, 1986; STUHLÍK et al., 1993b; OSUSKÝ et al., 1994). Low-copy mini-Mu vectors were used successfully for cloning of toxic genes (LACZAOVÁ et al., 1995; GRONES et al., 1996; BURIAN et al., 1998; TU et al., 2001). See also Fig. 2 for more details.

The derivatives of Mu bacteriophage thus became useful molecular biology tools both for the *in vivo* and *in vitro* techniques. Mini-Mu phage cloning methods have many advantages, besides

economical (skipping DNA isolation, restriction and ligation steps), the main advantage being the ability to clone relatively large regions of a chromosome and those bearing toxic or harmful genes that are difficult to clone using the *in vitro* system (STUHLÍK et al., 1993a; VIZVÁRYOVÁ et al., 2002).

### Transposons used in post genomic era

In this post genomic era, complete DNA sequences of many microbial genomes are available, but nearly half of the putative genes lack identifiable functions. Identification of genes with their function is necessary to convert the sequence data into meaningful biological information.

Transposon mutagenesis revealed a powerful technique for the minimal *Mycoplasma genitalium* genome estimation (HUTCHINSON et al., 1999) as well as for the identification of bacterial virulence factors (AKERLEY et al., 1998; GUO et al., 2001; GAO et al., 2003).

A new family of transposons with a broad host range and short recognized sequence has been developed, based on the eukaryotic transposons, providing the identification of essential genes by *in vitro* transposition – marine mutagenesis.

So-called *mariners* are the widespread animal transposons, present in many different insect species. *Mos1* (from fruit fly *Drosophila melanogaster*, ROBERTSON & LAMPE, 1995) and *Himar1* (from horn fly *Haematobia irritans*, LAMPE et al., 1996) are among the best-studied *mariner* transposons targeting the AT dinucleotide recognition site that are able to insert into diverse genomes, including both eukaryotes and prokaryotes (FADOOL et al., 1998; GUEIROS-FILHO & BEVERLEY, 1997).

*MaT*, another clade of *mariner* transposons, originated from housefly *Musca domestica*, is flanking the gene involved in organophosphate insecticide resistance, but highly similar homologue was found also in silkworm moth *Bombyx mori*, and even 16 copies of *maT* sequence were found in the genome of *Caenorhabditis elegans* (CLAUDIANOS et al., 2002). Since the identical *mariners* were isolated and sequenced in several different species, they are supposed to take part in primary selective sequence evolution spread by the horizontal gene transfer (LAMPE et al., 2003).

Despite the fact that the palette of *in vitro* techniques has been growing rapidly together with the genomics generated data, the use of *in vivo* techniques, including transposons will still remain the powerful tool of functional genomics studies.

## Mini-Mu derivatives

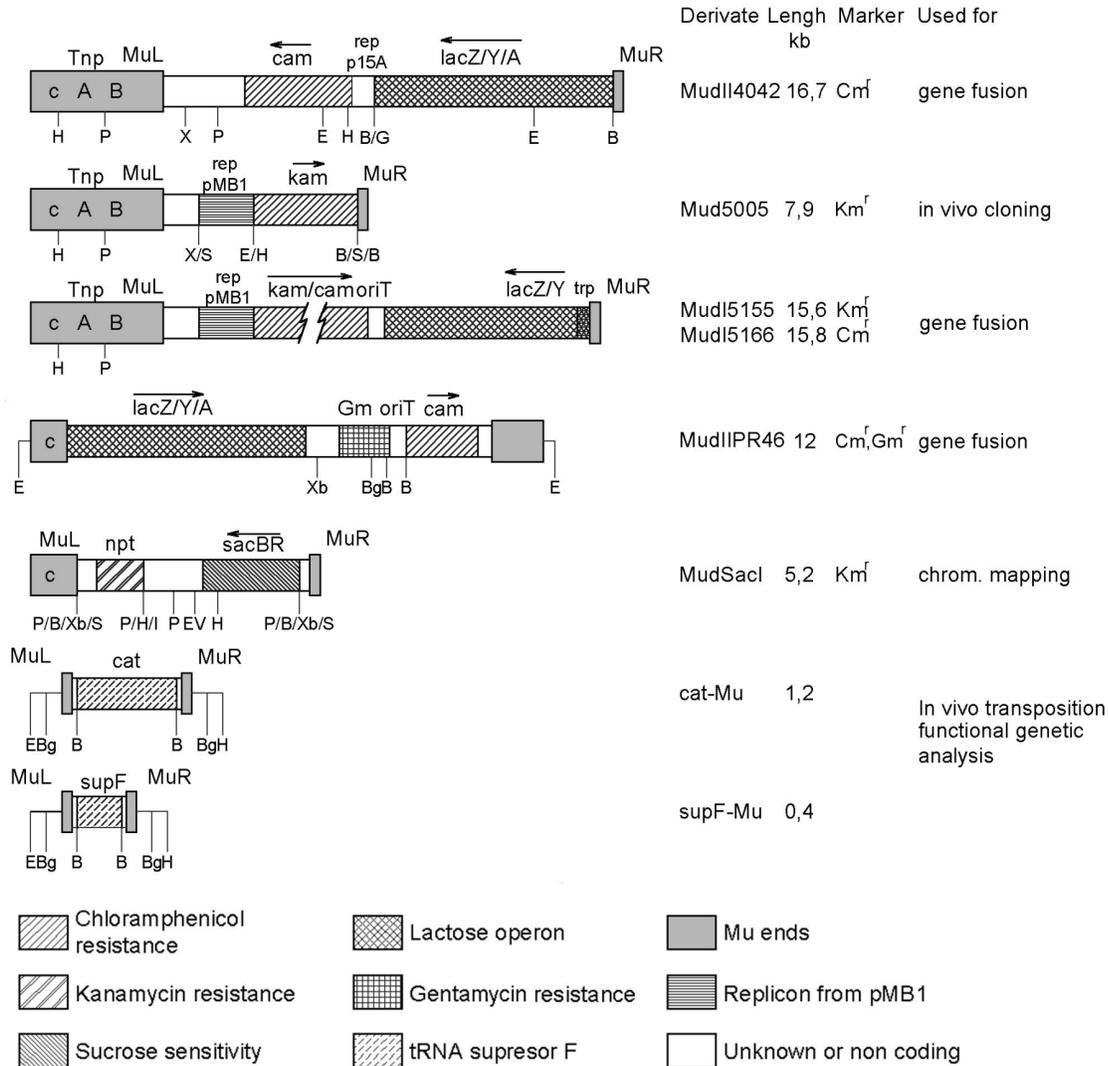


Fig. 2. Genetic and physical maps of the different mini-Mu derivatives and their using. Cleavage recognition sites for restriction enzymes are: B, BamHI; E, EcoRI; P, PstI; S, Sall; V, PvuII, X, XhoI. The other sites are as in Fig. 1. Structure of each mini-Mu is drawn to scale.

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