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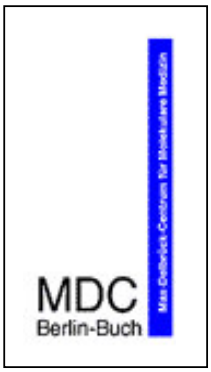

Improvement of the *Sleeping Beauty* Transposon System

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Dedication

To the soul of my Parents

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List of Abbreviations

Abbreviation	Full expression
°C	degree(s) Celsius
A	adenine
aa	amino acid
bp	base pair
BSA	bovine serum albumen
cDNA	complementary deoxyribonucleic acid
CpG	cytosine and guanine dinucleotide
dNTP	deoxynucleoside triphosphate
dsb	double stranded break
DTT	dithiothreitol
EDTA	ethylenediaminetetra acetic acid
IHF	integration host factor
Fig	Figure
Kb	kilobase
kV	kilovolts
l	liter
LB medium	luria Bertani medium
LTR	long terminal repeat
M	molar
mer	oligomer
mF	milli Farad
µg	microgram
mg	milligram
$MgCl_2$	magnesium chloride
min	minute(s)
µl	microliter
ml	milliliter
µm	micrometer
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
ng	nanogram
nmol	nanomole
OD	optical density
PBS	phosphate buffered saline
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulphate poly-acrylamid gel electrophoresis
sec	second(s)
TE	Tris EDTA
TEMED	N,N,N',N'tetramethylethylenediamine
tRNA	transfer RNA
U	unit
UTR	untranslated region
UV	ultraviolet
V	volt

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Abstract

Sleeping Beauty (*SB*) is the most active Tc1/*mariner*-type transposon in vertebrates. It is a synthetic transposable element that has been reconstructed from defective copies of an ancestral Tc1-like element in fish (Ivics at al., 1997). It is a 1.6-kb element that is flanked by ~230-bp terminal inverted repeats (IRs), and encodes a single protein, the transposase, that catalyzes transposition of the element from one genomic locus to another. SB transposes by a cut-and-paste mechanism that requires binding of the transposase to its binding sites within the IRs. Each IR contains two transposase-binding sites (DRs), a feature termed the IR/DR structure.

SB shows high transpositional activity in a number of vertebrate cell lines *in vitro* (Izsvak et al., 2000), and in both somatic and germline tissues of the mouse *in vivo* (Yant *et al*., 2000; Dupuy et al., 2002). Thus, *SB* is being developed as a gene vector for transgenesis and insertional mutagenesis in vertebrate model systems as well as for human gene therapy. However, biological evidences indicate that the maximal activity of the *SB* transposon system has not yet been reached. To improve the transpositional activity of the *SB* transposable element, I followed three experimental approaches: 1) find the optimum conditions under which *SB* can transpose, by investigating the role of host factors which may directly or indirectly be involved in *SB* transposition; 2) increase the recombinational activity of the SB transposase; 3) modify the structure of the SB transposon DNA.

Most transposons do not function (well) without accessory (host) factors (Sherrat, 1995). The involvement of cellular proteins in the regulation of *SB* transposition was investigated in this thesis. I show that the DNA-bending high-mobility group protein, HMGB1, is a host-encoded cofactor of SB transposition. Transposition was severely reduced in mouse cells deficient in HMGB1. This effect was rescued by transient over-expression of HMGB1, and was partially complemented by the closely related HMGB2, but not with the unrelated HMGA1 protein. Over-expression of HMGB1 in wild-type mouse cells enhanced transposition, indicating that HMGB1 can be a limiting factor of transposition. SB transposase was found to interact with HMGB1 *in vivo*, suggesting that the transposase may recruit HMGB1 to transposon DNA. HMGB1 stimulated preferential binding of the transposase to the DR further from the cleavage site, and promoted bending of DNA fragments containing the transposon IR. The role of HMGB1 is proposed to ensure that transposase-transposon

complexes are first formed at the internal DRs, and to subsequently promote juxtaposition of functional sites in transposon DNA, thereby assisting the formation of synaptic complexes.

Transposases are not selected for maximal activity in nature, because high transpositional activity may be detrimental to the host. Indeed, replacements of some of the acidic (negatively charged) amino acids to basic (positively charged) amino acids in both the bacterial transposase Tn5 (Zhou and Reznikoff, 1997) and the *mariner* element Himar1 transposase (Lampe et al., 1999) were found to elevate the recombinational activities of the transposases. Similar, we hypothesized that the intrinsic activity of the SB transposase can be increased by amino acid substitutions. Following the lessons of Tn5 and Himar1 mutagenesis, I systematically replaced all aspartic acid (D) and glutamic acid (E) residues (that are not conserved within the Tc1 family) of the SB transposase with lysine (K) or arginine (R) residues. One such mutant, D260K, consistently increased the jumping efficiency of SB with about 30%. D260K works synergistically with other hyperactive mutations to elevate the overall transposition efficiency to about 370% over the wild-type SB transposase. The success of this limited range of site-directed mutagenesis indicates that large-scale, random mutagenesis of the SB transposase will likely yield hyperactive versions with as high as possibly a 100-fold increase in activity.

The other component of the transposon system where modifications might improve activity is the transposon DNA. Indeed, a combination of four mutations in the IRs was shown to increase the activity of the *SB* transposon by about 4-fold (Cui et al., 2002). The efficiency of *SB* transposition decreases with increasing the transposon size (Izsvak et al., 2000). We reasoned that changing the structure of the transposon could increase its ability to mobilize longer DNA fragments. For example, a composite transposon consisting of two identical copies of itself flanking a nonrepetitive sequence (longer than 10kb) in an inverted orientation has been seen to be mobilized in the fly species *Drosophila virilis* (Petrov et al., 1995). This transposon is called the *Paris* element (Petrov et al., 1995). TA target site dinucleotide duplications flanking the particular composite *Paris* transposon (Petrov et al., 1995) indicate that the insertion was generated by transposition. A construct mimicking the structure of the composite *Paris* element was made from two identical copies of the *SB* transposon flanking relatively large pieces of DNA in an inverted orientation. The inner binding sites of the transposase were mutated to ensure that the individual *SB* units cannot transpose. These mutations were proven to only interfere with the transposition capacity but not with the

binding capability of the transposase. This construct is called the sandwich vector (SA). SA was able to jump 3 times more efficiently than similar size marker genes.