Project: Determining the Genes Underlying the Williams-Beuren Syndrome by Generating Allelic Series of Mutations Using Novel Transposon Approaches

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Introduction

Williams-Beuren syndrome (WBS) is a contiguous gene syndrome that usually involves mental retardation, but also an increase of particular capacities, especially musical skills, auditory memory, social use of language and engaging personality. On the other hand, learning of these patients is significantly retarded, and cognitive ability is usually underdeveloped. In addition, heart and kidney phenotypes have been described. The overall life expectance of WBS patients is normal.

In Germany, there is an estimated number of 1500 patients, and with 80 children born per year, WBS has an incidence of 1:8.000 to 1:10.000. In 95% of the cases, patients exhibit a microdeletion in the *Elastin* gene, however, there are about 30 possible modifier genes on chromosome 7q11.23 in a region of 500 kb around the *Elastin* gene. The synthenic region in the mouse is on chromosome 5G. Deletion mutants have been generated for *Elastin (1).* However, none of the observed phenotypes completely recapitulates the human disease phenotype. The genetic mechanism of WBS remains unclear. Microdeletions are obviously frequent, but duplications of critical regions have also been described. Therefore, we propose to study the WBS syndrome in the mouse by generating an allelic series of mutants by transposon mutagenesis.

Transposons are mobile segments of DNA that can move from one locus to another within genomes. These elements move via a "cut-and-paste" mechanism: the transposase catalyzes the excision of the transposon from its original location and promotes its re-integration elsewhere in the genome (Fig. 1). Transposase-deficient elements can be mobilized if the transposase is provided *in trans* by another transposase gene. Transposable elements are very efficient in integrating into DNA, and large mutagenesis screens using transposon vectors have been successfully applied to model organisms such as *Drosophila* and *C. elegans*. However, until recently, transposable elements have not been used for the investigation of vertebrate genomes for two reasons. first, there have not been any well-defined, DNA-based mobile elements in these species. Second, a major obstacle to the transfer of an active transposon system from one species to another has been that of species-specificity of transposition due to the requirement for factors produced by the natural host. To remedy such problem, an active transposon has been reconstructed from inactive elements found in fish genomes using a comparative phylogenetic approach, and was named *Sleeping Beauty* (SB) *(2).* SB jumps efficiently in cells of diverse vertebrate species in culture, as well as in somatic and germ line tissues of the mouse *in vivo*. SB has been successfully used for forward genetic approaches in the mouse. Double transgenic mouse lines were generated bearing chromosomally present transposons and an either ubiquitously expressed or male germ line-specific transposase gene. Segregating the transposition events by mating the founder males to wild-type females revealed that up to 90% of the progeny can carry transposon insertions, and that a single sperm of a founder can contain, on average, two insertion events *(3).* Additionally, a recent paper elegantly showed that the germ line of such a founder can harbor approximately 10,000 different mutations *(4).* Transposition of gene trap transposons identified mouse genes with ubiquitous and tissue-specific expression patterns, and mutant/lethal phenotypes were easily obtained by generating homozygous animals. Thus, SB transposition can be a powerful strategy for insertional mutagenesis in mammals.

Project Status

Hyperactive transposases

Transposases have not been selected for the highest possible activity in nature. This is because transposition can potentially endanger the survival of the host organism by insertionally inactivating essential genes. The *Sleeping Beauty* transposon shows efficient transposition in cells of a wide range of vertebrates, including mice. Nevertheless, one of the key parameters concerning the utility of SB is the overall activity. We are in the process of deriving hyperactive transposase mutants by *in vitro* evolution. Hyperactive mutants likely contain one or a limited number of amino acid replacements. We follow two major strategies for mutagenesis of the transposase gene: 1) site-directed mutagenesis of selected amino acids; 2) random mutagenesis. The rationale behind the first approach is that sequence variants of transposase genes that are isolated from nature likely represent functional sequences, but with potentially different activities. For example, zebrafish- and salmonid-type (SB) transposases are clearly distinct subfamilies that diverged in different species, and the two proteins are about 90% identical in amino acid sequence.

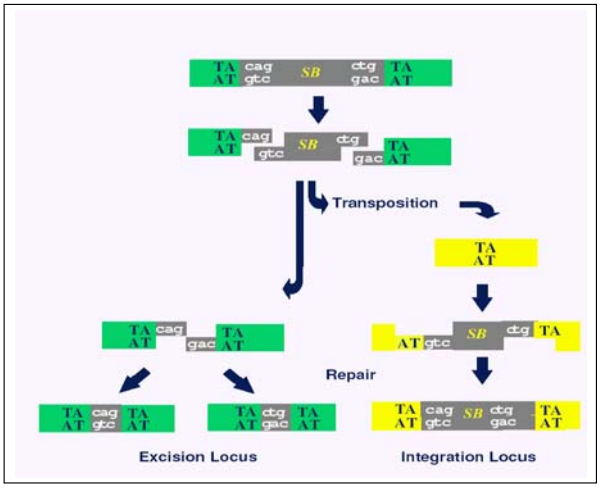

Fig 1: Model for the mechanism of jumping of Tc1/mariner transposons. *The element is excised from the donor locus by staggered cuts and introduced into a new TA target site. The TA will be duplicated and flank the inserted element after repair of the single-stranded gaps.*

The remaining 10% thus likely represent functional sequence variants. We have “transplanted” 32 amino acids, one-by-one, from the zebrafish-type transposase into SB, and have identified three hyperactive mutations resulting in 2-5-fold increase in transpositional activity. The rationale behind the second approach is to screen large numbers of sequence variants that have been generated by random, error-prone PCR mutagenesis. The advantage of random mutagenesis is that it generates a large pool of sequence variants; the disadvantage is that these variants have not been preselected for activity. We will generate about 10,000 mutants, and use a genetic transposition assay to screen for hyperactives. We will combine the hyperactive sequences that we identify by the two approaches using DNA shuffling, thereby generating combinations of mutations that result in

additive or synergistic hyperactive phenotypes. Such hyperactive transposases will expand the utility of DNA transposition in vertebrate genetics, and will be a valuable asset for this project.

Vector design and construction

Our gene trap vector (Fig. 2) will contain a lacZ marker gene fused to a splice acceptor site, followed by a GFP reporter gene driven by a mouse opsin promoter, which drives the expression of GFP in the retina of mutant animals only if transposition into a gene has occurred. Since GFP does not contain a poly-A signal sequence but a splicing donor site instead, expression of GFP will depend on capturing an endogenous poly-A site. We will clone the gene trap cassette into an SB vector that has two left inverted repeats, which ensures high efficiency of transposition.

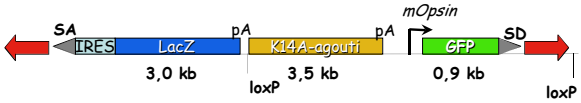

Fig 2: The transposon construct. *The transposon contains a polyA-trap cassette consisting of the mouse opsin promoter-GFP-splice donor (SD) to tag new mutant mouse lines by retinal GFP expression. The GFP does not contain a polyA site, therefore it needs to insert into a gene to produce a stable transcript. The mutagenic part of the vector is a splice acceptor (SA)-IRES-lacZ cassette, which will both disrupt the trapped transcript and monitor the expression of the trapped gene. The K14-agouti cassette will enable to distinguish between heterozygote and homozygote animals by coat colour. Both promoter-containing cassettes may be removed by breeding to Cre deleter mice, leaving only a single loxP site in the locus, which may later serve to induce larger chromosomal rearrangements (deletions and inversions) by crossing two independent mouse lines.*

The most active SB transposase version, showing about 10-fold increase in transpositional activity compared to the first-generation vector, has been cloned into a CAGGS enhancer/promoter construct for transposase expression in the mouse germ line. The expression cassette is being incorporated into a gene targeting vector suitable to insert our construct into the Rosa26 locus by homologous recombination. The gene trap (mutagenic) part of our transposon vectors is ready; we will still have to put together the polyA trap cassette. Once the transposon vector is ready to go, we will introduce it into the WBS critical region using knock-in technology.

Outlook

Two transgenic mouse lines will be generated; a “jumpstarter” stock expressing the SB transposase, and a “mutator” stock containing the a gene trap transposon vector. In the mutator stock, the GFP gene trap must be in an inactive state. These two stocks will be crossed to bring the two components of the transposon system together, and transposition of the gene trap transposons is expected to occur in the sperm cells of F1 double-transgenic males (referred to as “seed” mice). Such males will be bred to wild type mouse to segregate the different insertion events in their sperm cells in separate F2 animals (Fig. 3). To find out whether the construct has landed in a gene, we will screen newborn pups for GFP expression with a UV lamp shortly after birth. Positive pups will be further screened by 3’ RACE PCR in order to determine the exact site of integration.
Based on previous observations, about half of the newly transposed insertions should occur locally, including the WBS target region. Mouse lines with integrations in the WBS critical region will be bred to homozygosity (Fig. 3) and phenotypical alterations, including pathology and behaviour, especially hippocampus-dependent tasks like object recognition memory, social memory, social discrimination and learning, will be studied in more detail. Appropriate tests for a mouse model of WBS are yet to be developed, and will include the 8-arm labyrinth (working memory test), and the Morris-Water-Maze test (spatial navigation). Due to the K14-agouti cassette within the transposon, the agouti gene will be expressed in the fur of all mutant mouse lines. Depending on whether mutant animals carry one or two copies of the transposon, we expect to be able to distinguish between heterozygotes and homozygotes by their coat colour. Therefore, we should be able to detect embryonic lethal phenotypes by simply looking at the offspring without prior investing in molecular genotyping strategies. In case the K-14 agouti system will work only in heterozygotes, we will genotype the offspring by multiplex PCR strategies.

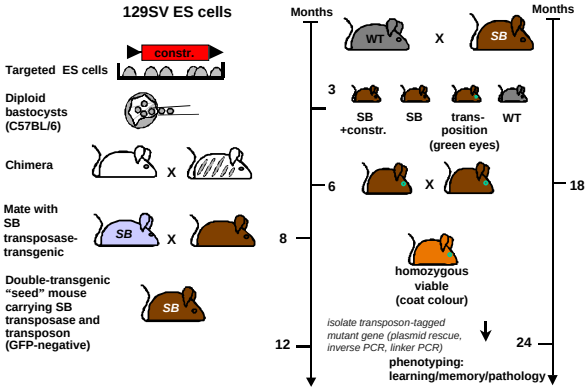

Fig 3: *Breeding scheme for the induction of transposition events in the mouse germline and screening strategy.*

In order to later be able to introduce larger chromosomal deletions, we will introduce loxP sites in the transposon (Fig. 2). The K14-agouti and the opsin-GFP cassettes will be flanked by loxP sites, so that we will be able to remove both using Cre recombinase. This will leave only one loxP site in the respective locus. This will enable us to crossbreed different lines and thereby delete (or invert) the intervening DNA by utilizing a Nestin-Cre recombinase mouse line. Thus, we will be able to generate serial deletions of the region, and experimentally define the critical loci responsible for the WBS phenotype.

*Lit.: **1**. Durkin ME et al. Integration of a c-myc transgene results in disruption of the mouse Gtf2ird1 gene, the homologue of the human GTF2IRD1 gene hemizygously deleted in Williams-Beuren syndrome. Genomics. 2001 73(1):20-7. **2**. Ivics Z et al. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. Cell. 1997 91:501-510. **3**. Dupuy AJ et al. Transposition and gene disruption in the male germline of the mouse. Genesis. 2001 30:82-88. **4.** Horie K et al. Characterization of Sleeping Beauty transposition and its application to genetic screening in mice. Mol. Cell. Biol. 2003 23: 9189-207.*