Functional Significance of WSTF hemizygosity in Williams Syndrome

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Abstract

Williams-Beuren syndrome (WBS) is a devastating genetic disorder characterized by numerous developmental defects, mental retardation and behavioral abnormalities. WBS affects one in every 20,000 live births and is caused by a deletion of approximately 1.5 Mb from one copy of human chromosome 7q11.23 5. Consequently, WBS patients are hemizygous for at least 28 genes, of which some or all contribute to the manifestation of the disease.

Herein lies the challenge presented by all contiguous deletion syndromes: which genes are responsible for the various phenotypes? and therefore those upon which to focus potential therapies and treatments. One such gene, the WBS candidate region-9 (*WBSCR9), encodes a 170kDa bromodomain* containing protein termed the Williams syndrome transcription factor (WSTF). WSTF is a key component of two chromatin remodeling complexes, WINAC and WICH, that are involved in regulating transcription, DNA replication and DNA repair.

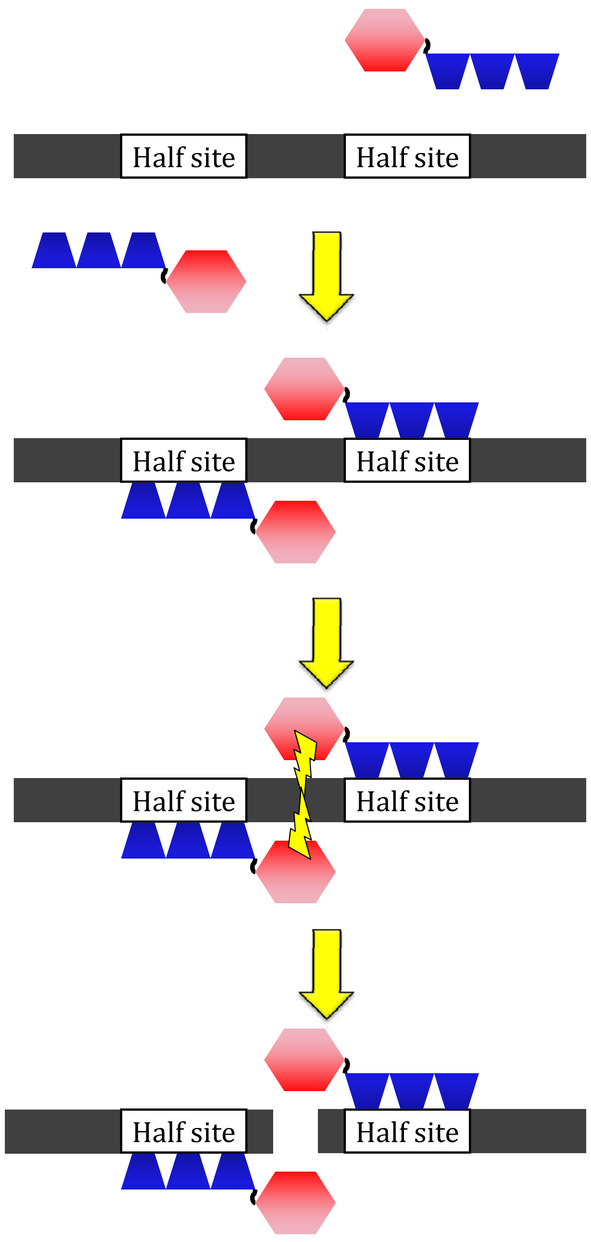
Strategy

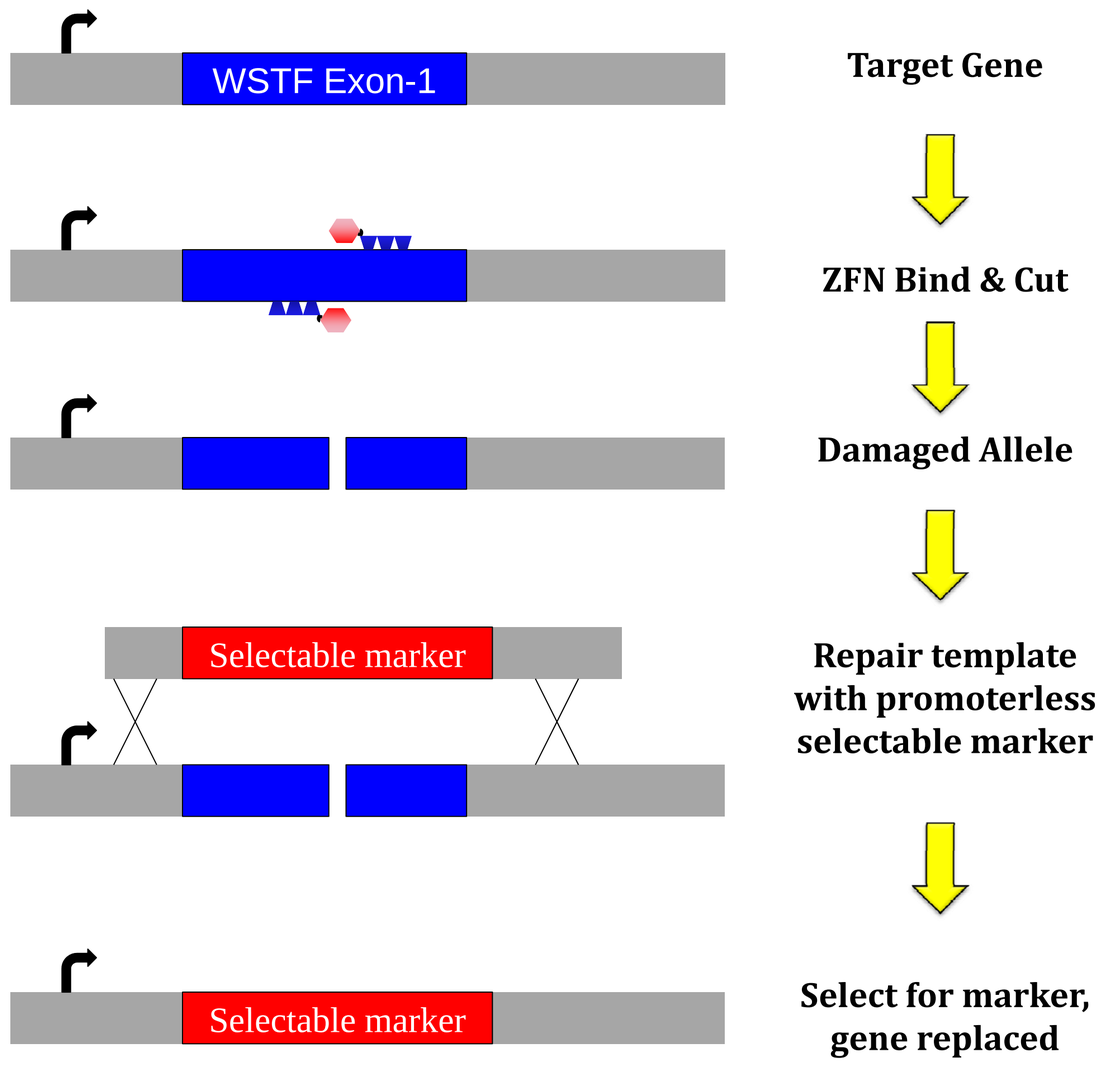
In order to investigate the contribution of WSTF hemizygosity in WBS and identify WSTF regulated genes, we sought to disrupt one allele of WSTF and monitor global changes in gene expression. Such an approach will isolate the impact of WTSF hemizygosity on gene expression away from that of the other 27 genes in the 1.5 Mb hemizygous deletion. Furthermore, the expression pattern of targeted cells can be directly compared to that of the parental cell line as a controlled background.

Gene targeting by homologous recombination in human cells is extremely inefficient, with successful targeting occurring in fewer than in a 10^6 cells. As such, there are few reports of genetic manipulation in human cultured cells. However, several recent advances have reignited interest in human gene targeting. Chief among them is the process of Zinc finger nuclease (ZFN) assisted homologous recombination.

A Zinc Finger is a common DNA binding motif consisting of a zinc-bound protein structure that recognizes specifically 3 contiguous bases in a DNA molecule. It is possible to change specific amino acid residues in this structure in order to alter the specificity of the DNA sequence that the finger will recognize. Almost all zinc finger proteins consist of several adjacent fingers. Therefore, we can engineer a multi-zinc finger protein that will recognize a very specific DNA sequence. Zinc finger nucleases take advantage of this by fusing a nuclease domain to the zinc fingers.

The image opposite shows a schematic of ZFN technology. Essentially two 3-finger zinc finger nuclease proteins are generated that recognize two 9bp Half site sequences separated by a spacer sequence of defined length. When the ZFN recognize this target and bind, they bring the fused nuclease domain (red hexagon) into close proximity and the enzymes proceed to cause a double strand break in the DNA. The cell is forced to repair the damaged DNA and often does this using the other copy of the chromosome in the cell. However, if an artificial template is provided, it is possible to repair the DNA using this sequence instead.





The above model shows a schematic representation of the targeting strategy for the WSTF gene. Essentially, a pair of ZFNs designed to a region in the WSTF gene cause a double strand break. We provide a promoterless selectable marker flanked by regions of DNA with homology to the WSTF gene. Alignment of the homologous regions ensures recombination, repairing the damaged region and sealing the break. The selectable marker uses the WSTF promoter to induce its expression greatly increasing the chance that clones obtained are genuine WSTF targeted cells.

Progress

Design and generation of ZFNs that possess *in vivo* DNA binding activity is complex and involves numerous advanced molecular biology techniques over a 6-12 month period. However, we have successfully obtained active ZFN constructs and have managed to target several human genes using this strategy. Presently we have two sets of targeting constructs for human WSTF and have generated promising ZFN to both that will assist in ZFN mediate homologous recombination.

Future Direction

In the coming months we will begin to use the ZFNs we have generated along with our targeting constructs to disrupt one allele of WSTF in normal cells. This will be the first example of modeling a contiguous gene deletion syndrome in man and identify the target genes impacted by WSTF hemizygosity. Such a model cell line would provide an invaluable manipulable system in which to identify factors that can correct or alleviate phenotypes associated with this gene defect. Furthermore, such an approach would demonstrate the feasibility of targeting each gene in turn to decipher their role and possible therapeutic approaches.

We believe that this powerful experimental approach could be applied to other contiguous gene deletion disorders and provide a human system that would be more relevant than standard mouse models of human disease.

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