**ABSTRACT**

Both the mammalian immune system’s complex defenses and a bacteriophage’s targeting mechanism depend on protein diversification. These models have inspired innovations ranging from targeted drug delivery to protein display. Using the major tropism determinant (Mtd) protein expressed on the Bordetella bacteriophage BPP-1, we aim to develop an in vitro system for generating antibody-like proteins that bind specific targets. The Mtd protein expressed at the phage’s tail fiber is naturally modified at its variable region to produce nearly $10^{12}$ possible binding variants while preserving its structure. Mutating the Mtd’s variable region by PCR can match the massive diversity expressed at the phage’s tail fiber is naturally modified at its variable region to produce nearly $10^{12}$ possible binding variants while preserving its structure. Mutating the Mtd’s variable region by PCR can match the massive diversity expressed at the phage’s tail fiber is naturally modified at its variable region to produce nearly $10^{12}$ possible binding variants while preserving its structure.

**BACKGROUND**

BORDETELLA BRONCHISEPTICA

Bordetella bronchiseptica is the natural host of the BPP-1 bacteriophage, and is responsible for causing bronchitis. BPP-1 binds to the bacteria’s surface protein pertactin to facilitate infection. However, pertactin is not always expressed by the bacteria, which can cycle between the Bvg- and Bvg+ phases – the latter phase inhibiting pertactin expression. Despite the lack of surface pertactin in the Bvg+ phase, BPP-1 is still able to infect the bacteria, albeit at a lower frequency. This suggests that BPP-1 contains a mechanism for changing host specificity, and can change the binding properties of the tail fiber proteins which normally interact with pertactin.

**BORDETELLA PHAGE BPP-1**

BPP-1 belongs to the Podoviridae family, and has a short, non-contractile tail with six “spikes” attached to tail fibers. Each tail fiber contains the major tropism determinant (Mtd) protein at its end, which normally binds to the pertactin protein expressed on Bordetella’s surface. Individual Mtd proteins do not have a high affinity for pertactin, but the flexibility and number of tail fibers on each phage confer an overall high avidity for its host. As the virus is lytic, BPP-1 destroys the bacterial host and releases more copies of itself into the surrounding. When Bordetella switches into the Bvg+ phase and inhibits pertactin expression, BPP-1 relies on a diversity generating retroelement (DGR) system to retain the chance of binding the bacteria.

**DIVERSITY GENERATING RETROELEMENT**

Various species of phage contain a DGR for diversifying tail-fiber proteins. The BPP-1 mtd gene contains a variable repeat (VR) of 134 bases with variations possible at 23 distinct adenine sites. Downstream the VR is the template repeat (TR) region, which does not change. During phage assembly, the TR is transcribed, then reverse transcribed by a reverse transcriptase coded for by the brt gene. The reverse transcriptase introduces site mutations at the 23 nucleotides. A transposition event then integrates the mutated sequence into the VR. This mutagenic shuffling event results in $9.22 \times 10^{12}$ possible Mtd variants, one or many of which can bind onto another surface protein on the Bvg+ Bordetella.

**MAJOR TROPISM DETERMINANT (Mtd)**

The mtd gene is 1146 bases long, and contains two mutagenic islands in the variable repeat region near the gene’s 3’ end. There are 23 specific adenine bases that are modifiable by the native DGR, which alter 12 amino acids on the protein. This variable repeat region is a part of the C-lectin hypervariable region of the C-terminus. The C-lectin fold is found in different phages, acting as a scaffold to provide the protein rigidity and the ability to resist mutations without undergoing structural changes. In vivo, three Mtd proteins form into a globular trimERIC structure, and the variable repeat of each monomer forms a surface on one face of the complex.

**TARGET SCREENING**

We plan on screening our mRNA library against E. coli bacteria. We will suspend E. coli cells in a liquid medium, then add our library of Mtd variants. After incubation, we discard the supernatant, leaving cells and Mtd protein that effectively bound the cells. We then wash several times to remove any remaining unbound Mtd. To amplify bound Mtd variants, we will resuspend the cells in liquid medium to use as template for PCR. The amplicons will be cloned into plasmids and sequenced.

**LIBRARY GENERATION**

Instead of using the DGR’s reverse transcriptase to create variants, we used PCR with a library of synthesized oligonucleotides. We utilized two oligos with random nucleotides at the specific mutagenic sites and a third oligo with the necessary glycine-serine bridge as primers. The oligonucleotides with variations would anneal normally to the mtd template, and the resulting PCR products would be extremely varied.

**PROJECT DESIGN**

Schematic of library generation. Random nucleotides are inserted at the ‘N’ positions marked in yellow. These sites mimic the adenines normally mutated by the DGR after generating the DNA library, we transcribe the mtd-in vitro. Each RNA template is then ligated to a puromycin linker using a single oligo. The mRNA library is then translated in vitro. During translation, the puromycin molecule, which is structurally similar to tRNA but lacks a hydrolysable amide bond, gets incorporated into the polypeptide chain, thereby linking the Mtd variant and its template RNA. This RNA is then reverse transcribed in order to confer more stability to the molecule.

**FUTURE DIRECTIONS**

Diversity is essential for the success of this project. By increasing the number of sites we mutate within the variable region of the mtd, we could achieve much higher levels of diversity, creating much more opportunity for the selection of high affinity binding to our target.

One practical application of our project is to develop it as an antibody analog for protein detection. Obtaining new antibodies that are specific for a new target is time consuming and very expensive. DiversiPhage could be used to generate Mtd proteins with strong affinity for any desired target. These Mtd proteins could then be used for detection as an alternative to antibodies.

Another direction in which we could take this project is to re introduce the mtd gene back into bacteriophage. We would engineer the Mtd in vitro to bind a target and then reinsert the sequence for this variant back into the phage. This would give us a way to program the phage against any desired target. This could be very useful in applications such as phage therapy.

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