Development of TAL Nucleases for Genome Modification in Chlamydomonas
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Abstract
Engineered nucleases have been used as a means of genomic engineering for many years; however, the time consuming construction, and validation process along with poor efficiency and high failure rates make them difficult to work with. Recently, the transcription activator-like effector (TAL) class of DNA binding proteins have been shown to be truly modular in their native design and to possess a simple nucleotide recognition code. Additionally, they are easily adapted as engineered nucleases or TALENs. TALENs have been found to work effectively on a variety of organisms. We intend to use TALENs to modify the genome of Chlamydomonas, which is of interest due to its status as a model organism and its biofuel production potential. This research project was designed to test the construction accuracy and efficiency as well as the functionality of a new TAL library designed to quickly and easily assemble engineered TALENs for use in Chlamydomonas.

Brief History of Nucleases
Transgenic organisms play key roles in agriculture and experimental biology. Transgenic organisms were traditionally created by forcing a random DNA insertion into the genome and screening hundreds and thousands of events to find an organism with an intact gene that is properly located for consistent expression. This process was very time consuming, expensive and had relatively low efficiency.

It has been known for some time that double strand breaks (DSB) in the genome direct both non-homologous end-joining and homologous recombination at that site much like a text editor allows words on a page to be changed at will. The problem was the inability to generate a site specific DSB. A potential solution was to express nucleases that encode long recognition sites so that the nuclease is specific to a single location in the genome. The nuclease then acts like a postal carrier searching for a specific address among billions of potential addresses and delivering a DSB that could be used to precisely modify a genome.

Biologists have since been working to design such a system to more precisely and conveniently create transgenic organisms. Early attempts to use nucleases as an alternative began with the meganucleases. Meganucleases are naturally occurring nucleases that recognize 12-40 base pairs. Meganucleases make a double-stranded base pair cut in cultured cells. Long recognition sequences give high precision when inserting a gene, but only if the organism has the specific DNA sequence recognized by the meganuclease. Attempts to engineer meganucleases has proven difficult, however, there have been successes.

In attempts to broaden the use of site specific nucleases, the zinc finger DNA binding potential was adapted for use as a nuclease by fusing a zinc finger to the nuclease portion of FokI restriction endonuclease. Zinc fingers are engineered to bind DNA at a specific site in the genome. A finished zinc finger contains three to six zinc finger domains, each recognizing a consecutive base pairs of a target, as well as the FokI nuclease, which is activated upon dimerization2. This system works but like engineered meganucleases, zinc finger nucleases are unreliable, inefficient, and difficult to work with.

A simpler nuclease, known as the transcription activator-like effector nuclease (TALEN), was eventually created. This nuclease is truly modular in its design—one repeat variable diresidue (RVD) unit per nucleosome and it uses a very simple DNA recognition code (Fig. 1). The modular nature of the TALEN and elementary recognition code simplifies construction. Like the zinc finger nucleases, TALENs use the nuclease portion of FokI. TALENs can be engineered to specifically recognize targets of 6 to 24 bases within an organism’s genome. TALENs have been found to aid in genome modification in a variety of organisms such as human cells, zebra fish, yeast, C. elegans, rats, and rice.

Figure 1. TALEN Structure

Figure 2. Yeast Recombination Assay

Figure 3. Yeast Assay Results

Figure 4. Expected Results for TALENs in Chlamydomonas

Conclusions
• The library construction method proved to be fast and accurate, which will greatly increase the speed at which TALENs can be generated.
• The right phytoene synthase TALEN is fully functional.
• The left phytoene synthase TALEN is slightly functional, but its decreased functionality poses a problem for work in Chlamydomonas.
• An alternative left TALEN will have to be constructed.
• As demonstrated by the weak left TALEN, the yeast assay remains an important step in TALEN validation.

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References