# A Newly Developed Enzyme May Improve Targeted Genome Modification

January 21, 2014

ND Weber

Genome modification can be used in applications involving either gene disruption or gene correction. The desired outcomes of targeted DNA cleavage depend on the objectives of different genome modification strategies, but most of them are initiated with a DNA double strand break at a specific site. Targeted genome editing is a rapidly emerging field based on the use of rare-cleaving endonucleases to specifically target and cleave unique DNA sequences. Researchers in Seattle, including Dr. Barry Stoddard of the Basic Sciences Division, have developed a new rare-cleaving endonuclease that holds immense promise in addressing some of the main hurdles facing genome modification applications.

The quest to specifically recognize and cleave a DNA sequence safely for therapeutic applications requires addressing three main issues confronting this technology. First, enzymes must be designed to specifically target the desired DNA sequence with a high efficiency. Second, enzyme specificity must be sufficient so that off-target cleavage at similar but non-desired DNA sites, is minimized, as off-target activity can lead to cytotoxicity or worse outcomes. Third, current endonuclease platforms are either too large, require the delivery of multiple peptide chains, or possess other characteristics that result in problems in delivery when using the most common gene therapy delivery vectors. A class of new enzymes reported in a recent paper published in*Nucleic Acids Research*, designated megaTALs, was designed to improve the technology in all three of these areas.

MegaTALs are derived from the combination of two distinct classes of DNA targeting enzymes. Meganucleases (also referred to as homing endonucleases) are single peptide chains that have the advantage of both DNA recognition and nuclease functions in the same domain. However, meganuclease target recognition is difficult to modify, and they often have reduced specificity and lower on-target cleavage efficiency than other genome targeting endonucleases. Transcription activator-like (TAL) effectors are DNA recognizing proteins that have been linked to separate DNA endonuclease domains in order to achieve a targeted DNA double strand break. In contrast to meganucleases, TALs are easily engineered to target specific DNA sequences. Current platforms rely on a pair of TAL effectors, each coupled to a non-specific DNA cleavage domain, in which DNA

cleavage only occurs when both TAL effectors bind their respective sequences and the two endonuclease domains dimerize in order to cleave the DNA. However, TAL effector nucleases can cause off-target activity, are much larger than meganucleases, and require the delivery of two separate proteins. A megaTAL is the unification of a TAL effector with a meganuclease.

Dr. Stoddard is highly optimistic about this new technology. "MegaTALs may possess the best combination of properties of any gene targeting protein," he explains. Lead author Dr. Sandrine Boissel, member of the Andrew Scharenberg Lab at Seattle Children's Hospital, states, "the major advantage of the megaTAL architecture is that it allows us to achieve high rates of DNA modification with greater target site specificity than any other available nuclease platform, making it particularly useful for gene therapy applications that require minimal off-target cleavage."

Boissel, Stoddard, and colleagues show the benefits of combining these two platforms in a series of *in vitro* experiments that detect DNA cleavage. The TAL effector binds with high affinity to a DNA sequence upstream of the targeted cleavage site and 'addresses' the meganuclease to its corresponding DNA sequence. Thus, both cleavage activity and specificity were highly enhanced over the meganuclease alone. Furthermore, by increasing the length of the DNA sequence recognized by megaTALs compared to meganucleases alone, off-target cleavage was seen to be substantially reduced. Finally, the addition of a meganuclease as the DNA cleavage domain avoids the requirement of two separate TAL effector protein chains to exert an effect, thus reducing the size of the enzyme platform and improving its potential to be adequately delivered, for example via viral vector delivery.

As a glimpse of the potential these new enzymes may have for therapeutic applications, the researchers designed a megaTAL to target the T-cell receptor alpha (TCRα) gene. Knockout of the TCRα gene in human T-cells could be used to improve the safety of some T cell-based therapies by preventing TCRs from generating graft-versus-host disease. The TCRα megaTAL was shown to exhibit a ~20-fold increase in activity over the meganuclease alone at targeting the TCRα gene in T-cells with extremely low off-target cleavage.

Despite these promising first results, naturally, as with any new development, there remains room for improvement with MegaTALs. Dr. Stoddard believes, "their ultimate value and application will depend on continued improvements in engineering the specificity of their nuclease domain." Similarly, Dr. Boissel sees the potential for "a redesigned interface between the TAL effector and meganuclease domains that would force these domains to work cooperatively and thus further reduce the level of cleavage activity at off-target sites."

Boissel S, Jarjour J, Astrakhan A, Adey A, Gouble A, Duchateau P, Shendure J, Stoddard BL, Certo MT, Baker D, Scharenberg AM. 2013. megaTALs: a rare-cleaving nuclease architecture for therapeutic genome engineering. *Nucleic Acids Res*. Epub ahead of print. doi: 10.1093/nar/gkt1224

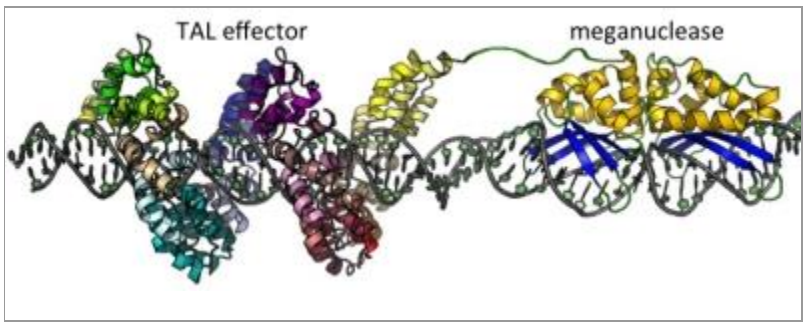


*Image provided by Dr. Barry Stoddard (Basic Sciences Division).*

A megaTAL, the fusion of a meganuclease with a TAL effector, is a new class of DNA targeting endonucleases with higher activity and specificity.