Identification of Zinc Fingers and Their DNA Targets

Category

Life Sciences/Biochemicals & Small Molecules

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Technology

NYU Langone Health researcher Marcus Noyes, PhD has developed a synthetic biology tool that allows screening billions of ZF-DNA interactions as they bind 64 possible nucleotide triplets. This new method takes into account the effect of adjacent ZF helices on tandem DNA binding. This allows the development of protein sequences that will bind a desired DNA sequence so that they can be used as tools for genomic or epigenomic manipulation. Overall, the method has been used to screen billions of unique ZFDNA interactions while changing adjacent finger influences, one at a time, to better understand ZF compatibility. This made it possible to offer a model for ZF-DNA binding that has eluded the field for a 30-years. Application of the method could improve the model for a better understanding of specificity.

Background

Cys2His2 Zinc fingers (ZFs) are short protein motifs that are the most common DNA-binding domains used by transcription factors to bind their target sequences of DNA. Zinc finger proteins bind DNA in tandem arrays from 2 to over 30 domains and this modularity allows for sequence specificity. It is known that the alpha-helix of the domain makes base-specifying contact with DNA, but other amino acids outside of the helix could be important as well. A unique characteristic of ZFs is that they mutate quickly in nature, to accommodate for new DNA targets. Further, decades of engineering ZFs have demonstrated their plasticity to take on novel specificities. However, it is not clear how ZFs are able to bind to such a wide array of targets as providing a ZF code has proven challenging. Here, a novel synthetic biology tool to screen for millions of ZF motifs was developed to better understand and engineer this binding specificity.

Applications

- Predicting sequence specificity for a given ZF-TF and DNA pair to understand how this TF (and similar ones) bind their nucleic acid counterpart.
- Engineering highly-efficient nucleases like Cas9 with specific DNA recognition domains
- By combining the specific binding of ZFs and nucleases such as the ones used in TALENs and CRISPR, a highly specific DNA-binding nuclease can be created, which is specific enough for therapeutic applications.

Advantages

Comprehensive screening capability Screen for all the binding modalities between ZF-containing proteins, such as TFs and DNA.

IP Status

Non-provisional patent pending