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ARTIFICIAL TRANSCRIPTION FACTOR- A TOOL FOR ENDOGENOUS TARGETED GENE REGULATION

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Introduction: A variety of biological processes including development, differentiation, and disease are regulated through gene expression. It may be due to upregulation or downregulation of a gene. Gene expression is mainly modulated by endogenous transcription factors. Transcription factors are produced in cytoplasm and eventually migrate in nucleus and interact with DNA and activate transcription. For RNA polymerase to successfully bind to eukaryotic promoter and initiate transcription a set of protein called transcription factor must 1st assemble on the promoter. These are multidomain proteins typically composed of a DNA binding domain (DBD), responsible for specific contacts with DNA bases, and an effector domain (ED). These can bind specifically or non- specifically to target molecule. Its property to bind specifically with target molecule is used to construct artificial transcription factor.

What is artificial transcription factor?

Artificial transcription factors (ATFs) are potentially a powerful molecular tool designed to target and modulate endogenous specific gene expression (Gommans *et al.*, 2005). In principle, these are chimeric protein, comprised of a DNA-binding domain, a transcriptional regulation domain or effector domain, and a nuclear localization signal (NLS). A DNA binding domain is required to bind to the promoter of a target gene, an effector domain to up- or down regulate the target gene, and an NLS to deliver an ATF into nuclei (because eukaryotic transcription occurs in nuclei). Therefore, if we can design and construct an artificial DNA-binding protein (or domain) that recognizes a target DNA specifically, we can create a desired ATF. DBD can be linked to a variety of effector domains such as Activator domains (the herpes simplex virus VP16), Repressor domains (kruppel-associated box) or DNA-modifying domains (such as the FokI). Thus, a distinctive feature of ATFs is their capability to up-regulate, down-regulate, or enzymatically modify DNA.

DNA binding domains for ATFs

There are presently three systems available for mediating site-specific DNA recognition of artificial TFs-

1. **zinc finger proteins (ZFPs)**
2. **Transcription activator-like effectors (TALEs)**
3. **Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) system.**

These proteins can serve as a scaffold for building enzymes that can modify DNA sequence, transcriptional regulation, or the epigenetic status at any site in the genome.

Each of these programmable DNA-binding proteins can be genetically fused to an effector domain to create custom enzymes that localize the effector function to the DNA target site. Various effectors domains have been widely used to create targeted changes to genome sequence, including nucleases, integrases, and recombinases. Alternatively, the fusion of these DNA-binding proteins to transcriptional activation and repression domains enables the control of gene regulation at targeted promoter or enhancer elements.

Applications of artificial transcription factors

1. **Regulation of the expression of specific endogenous genes-** By constructing specific type of DNA binding domain, expression of specific endogenous gene can be regulated. Dominant regulatory control of the expression of selected endogenous genes became possible with the emergence of zinc finger artificial TFs. The first study of gene regulation by altered transcription factors in living cells (yeast) was reported in 1992 by Thukal et al. They mutagenized the Cys2His2 zinc-finger domains of the yeast transcription factor ADR1 to alter the binding specificities and demonstrated that one of the ADR1 mutants recognized their target DNA and transiently activated a reporter gene harboring its target DNA in yeast. In 2007, Onori et al., reported activation of the dystrophin-related utrophin gene to complement the lack of dystrophin function in Duchenne muscular dystrophy. They generated a 4-finger ZFP that bound to a 12-bp target on the human utrophin promoter A and fused it to a VP16 activation domain. The resulting ATF increased activation of a reporter gene under the control of the utrophin promoter by approximately 7-fold. The ATF also increased the endogenous utrophin expression by 1.7-fold.

2. **Genome interrogation-** So far, genome interrogation has only been possible using zinc finger artificial transcription factors libraries. Genome interrogation is based upon the trans activity of an artificial TF-encoding gene. It is relatively easy to have essentially all members of a large library of different artificial TF-encoding gene constructs represented in single celled organisms or in cell cultures. The first proof of principle of genome interrogation in multicellular organisms was delivered for the model plant species Arabidopsis by using a relatively small collection of about 4000 GNN based three fingered artificial TF-encoding genes. In this study, a specific artificial TF inducing very high levels of somatic homologous DNA recombination was identified. Further experimental evidence indicated that this three finger ATF acts as an ectopic master switch orchestrating the timely expression of a set of endogenous genes. This then leads to enhanced somatic recombination. The resulting enhancement is much greater than the one that is accomplished by the over expression of each of the individual genes (Lindhout, 2006).

Conclusion

Artificial transcription factors are valuable tools for mediating the biological function of particular gene and cellular transcription regulatory network. These can be used effectively in gene therapy, tissue specific expression, genome interrogation etc where we can specifically enhance the expression of genes controlling the novel traits and phenotype.

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