E X P L A I N E R

TANGO

Targeted Augmentation of Nuclear Gene Output

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What is TANGO?

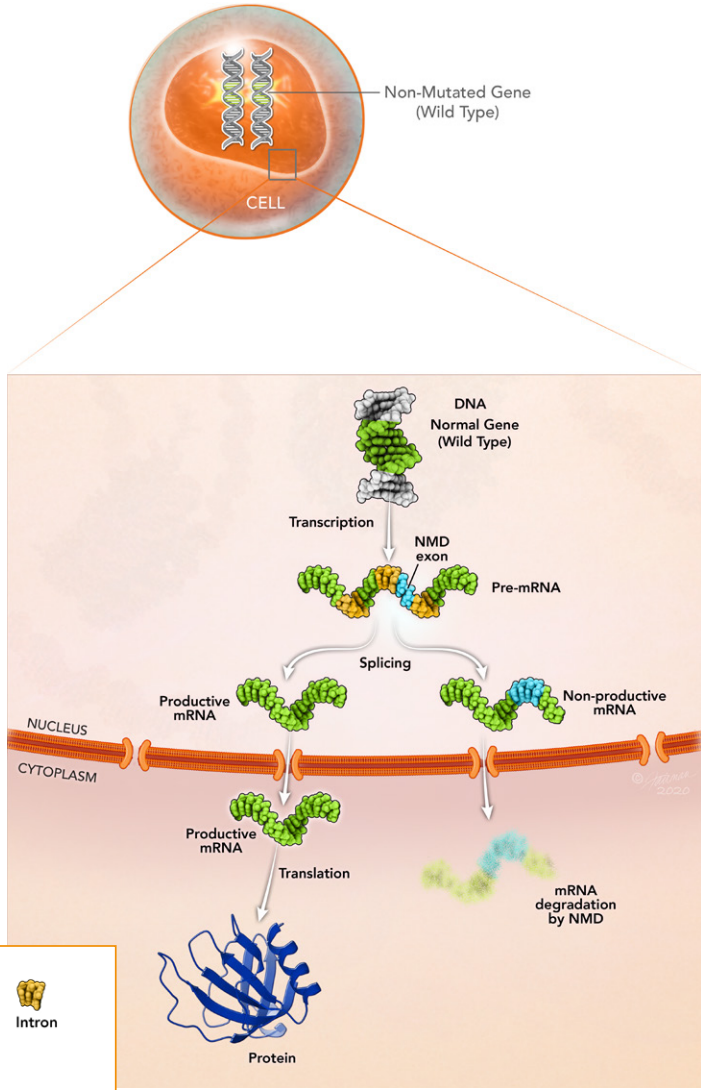
TANGO (Targeted Augmentation of Nuclear Gene Output) is Stoke's proprietary research platform. Stoke's initial application for this technology are diseases in which one copy of a gene functions normally and the other is mutated, also called haploinsufficiences. In these cases, the mutated gene does not produce its share of protein, resulting in disease. Using the TANGO approach and a deep understanding of RNA science, Stoke researchers design antisense oligonucleotides (ASOs) that bind to pre-mRNA and help the functional (normal, or wild-type) genes produce more protein. TANGO aims to restore missing proteins by increasing – or stoking – protein output from healthy genes, thus compensating for the non-functioning copy of the gene.

Protein Production

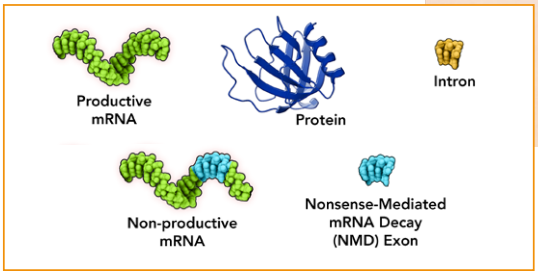
How the body makes proteins[1]

Proteins are critical to human health. They do most of the work in cells and are required for the structure, function and regulation of the body's tissues and organs.[2] Proteins are made through a highly regulated process. This process starts inside the nucleus with genes that contain DNA, the "instructions" for making the protein.

1. Cells read the information in a gene's DNA and make an identical copy called "precursor messenger (pre-mRNA)." In effect, the gene is making a transcript of itself, which is why it is called transcription.
2. Genes sometimes contain extra information that isn't needed to make a protein. The cell naturally edits, or removes, this unnecessary information from pre-mRNA using a process called splicing.
3. The edited copy is called mRNA and it moves outside of the nucleus into the cytoplasm.
4. The ribosomes in the cell read the mRNA and use those instructions to make a protein.



HOW THE BODY MAKES PROTEIN



1. https://ghr.nlm.nih.gov/primer/howgeneswork/makingprotein

2. https://ghr.nlm.nih.gov/primer/howgeneswork/protein

How the body regulates protein production

Between the creation of the pre-mRNA and the production of mRNA, a process called splicing occurs. During splicing, the cell gets rid of any unnecessary pieces of information in the pre-mRNA. One of these extra pieces of information that sometimes stays in the mRNA may lead to a "premature stop codon." These premature stop codons act as a signal to the cell during protein production. The signal tells the cell to destroy the mRNA before it makes a protein. This process happens constantly and naturally within the body to help it make the right form and amount of proteins needed for health.

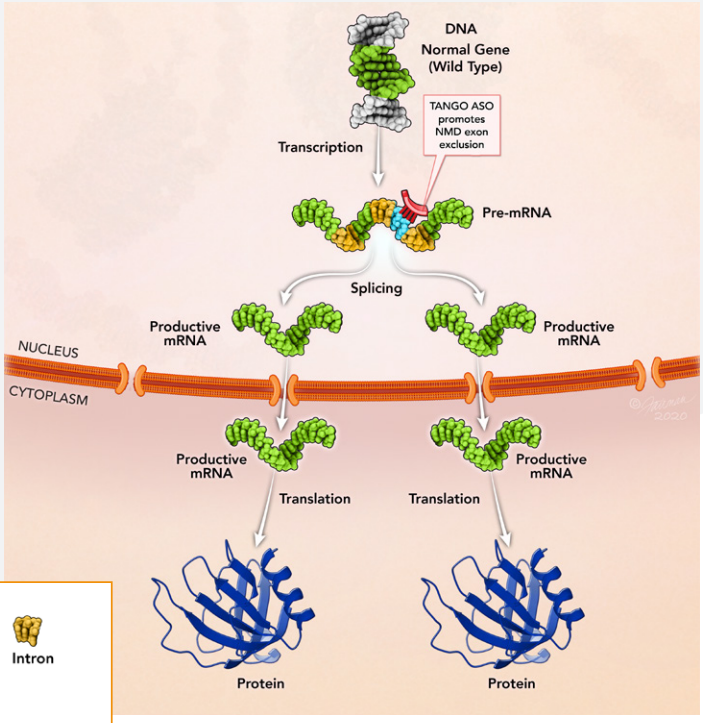
Haploinsufficiency: What it is and how it works

There are two copies of most genes. Together, they make 100% of a protein level necessary for a person to be healthy. Each copy contains the information to make half of the needed amount of a specific protein. In some diseases, a genetic mutation on one copy of the gene prevents it from producing its half of a protein, which can cause a disease that is classified as a haploinsufficiency. Dravet syndrome is one example of a haploinsufficiency.

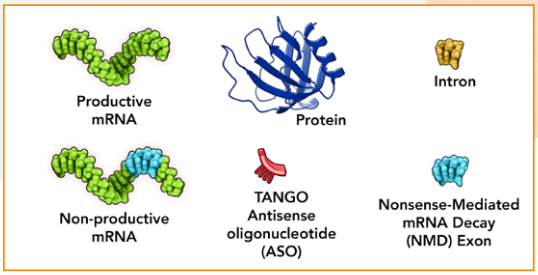
How does TANGO work?

Potential to restore missing proteins

Stoke's initial focus is on haploinsufficiencies. Based on a deep understanding of RNA science, Stoke is using its TANGO approach to make medicines called antisense oligonucleotides (ASOs) that bind to pre-mRNA to up-regulate – or stoke – protein production. These ASOs attach to regions that generate premature stop codons and prevent them from being included in the mRNA. Without this signal telling the cell to limit protein production, the mRNA goes on to make more protein than it otherwise would. While the ASOs bind to both the healthy copy and the mutated copy of the gene, the mutated copy will not produce any functional output. The healthy copy does the work of both, restoring protein production to near-normal levels.



PROTEIN UPREGULATION WITH TANGO ASO



What makes TANGO unique?

The difference between TANGO and other gene-based approaches

Gene editing is designed to insert, remove or otherwise directly edit a person's DNA. Gene editing targets a mutated copy of a gene and alters – or "corrects" – the sections that contain errors. In the process, the goal is to make the "broken" or mutated gene work properly.

Gene therapy is designed to introduce a new copy of the gene leaving the mutated copy/copies intact. It relies on the new copy to produce working protein.

Rather than address genetic diseases by replacing, repairing or editing faulty genes, Stoke's TANGO approach is being used to create potential new medicines that increase – or stoke – protein output from healthy genes. Unlike other gene-based approaches, TANGO ASOs do not alter DNA itself or add new genes.

There are several potential advantages to the TANGO approach:

- **May reduce the risk of unpredictable events.** TANGO does not manipulate DNA, and does not permanently alter genes. TANGO amplifies the body's natural protein creation. This may lower the chances of off-target effects.
- **Can create medicines to target a variety of genes.** Because TANGO ASOs do not require use of viruses, like viral vectors or invivo gene therapy, they can be used across gene targets of any size, type or function.
- **Ability to control dose level and duration.** The effect of TANGO ASOs can be titrated, or tuned, to produce more or less protein.
- **Simple and scalable manufacturing.** ASOs are manufactured using a highly scalable and established manufacturing process that does not require creating trillions of virus particles.

Ability to address underlying genetic cause of disease

Applicability to most loss-of-function mutations

Utility across small and large gene targets

No observed unwanted off-target effects

Ability to control dose level and duration

Utility across a wide array of diseases and tissue types

Simple and scalable manufacturing

Other Gene-based Methods of Treating Disease

Gene therapy uses synthetic DNA to treat disease. If a disease is caused by a faulty gene, gene therapy attempts to introduce a new gene copy.

Ex-vivo gene therapy introduces a new copy of the gene to cells in the lab before introducing the cells into the body.

In-vivo gene therapy introduces a copy of the gene directly into a person's own body, with the use of a virus to deliver it into the cells.

Cell therapy transplants new cells into a person's body to treat disease (a blood transfusion is a common type of cell therapy). In the case of ex-vivo gene therapy, those cells may contain new genes.

CAR-T cell therapy removes certain immune cells (T-cells) from a person's bloodstream, manipulates their genes in the lab, and re-introduces those modified cells back into the body. This technique is often used for immunotherapy cancer treatments.

Gene editing corrects, or "edits" specific sections of DNA in genes that contain errors, but doesn't replace entire genes. No gene editing treatments have been approved yet by the FDA.

CRISPR-Cas9 edits genes using a short piece of RNA. Once that molecule binds to a specific section of DNA, the process uses an enzyme called "cas9" to cut the DNA at a precise spot. Genetic material can then be added or deleted from the region and it's used in gene editing

Zinc Finger Nuclease cuts and manipulates specific DNA sequences with an artificial zinc-based enzyme.

Viral vectors are the most common delivery tool in gene editing and gene therapy. CRISPR-Cas9 or a new gene, respectively, are inserted into viral particles, which are then injected into a person's bloodstream. The virus then naturally injects the new genetic material into that person's cells.

Glossary of Genetic Terms

Antisense oligonucleotide (ASO): A short, synthetic oligonucleotide that can alter splicing and modify protein expression.

DNA (Deoxyribonucleic Acid): A helix-shaped molecule that acts as a master "cookbook" of recipes to make every structure and function in the body. DNA exists in every cell. Sections of DNA are called genes.

Gene: A section of DNA that holds the instructions for a protein or function in the body. Normally, there are two identical copies of a gene, one in each chromosome.

Haploinsufficiency: Occurs when only one of the two copies of a gene works and the other copy is mutated or inactive. As a result, the cell can only create half of the amount of a needed protein, which can lead to genetic diseases such as Dravet syndrome.

RNA (Ribonucleic Acid): A single-stranded molecule that takes on a number of functions in a cell, like transporting information to the cell's ribosome, and act as a template to make proteins.

Pre-mRNA (precursor-messenger RNA): A molecule that is made during the gene transcription process, pre-mRNA is a direct copy of a gene.

mRNA (messenger RNA): Results from editing or "processing" the pre-mRNA by a process called splicing. mRNAs are used to transfer genetic instructions to the cell's ribosome, where they serve as a template to create proteins. Pre-mRNA must be spliced to become an mRNA.

Ribosome: The machinery in a cell that is devoted to making proteins. They read mRNA and use the instructions to make proteins.

Protein: Proteins are large molecules inside every cell. They perform critical jobs related to the function and regulation of all the body's tissues and organs. Proteins are crucial to good health. Abnormal protein production can lead to various diseases and disorders.

Splicing: Pre-mRNA often contains extra information that isn't relevant to making a protein. That extra information needs to be edited out by the cell. This process is called splicing, and occurs constantly and naturally within the body.

Exon: A part of a gene that holds information that will eventually be used to make a protein. Non-coding exons or exons that don't hold information to make proteins can be removed during the splicing process, so only the information needed to make a protein remains on the mRNA.

Intron: A part of a gene that holds extraneous data, or data used for something other than making proteins. Introns are located in between exons and are edited out during splicing when pre-mRNA is processed into mature mRNA.

Premature stop codon: A signal that tells the cell to stop making a protein before that protein has been made into the full length that is necessary for it to function. If the cell's internal machinery sees this signal, it destroys the mRNA and stops it from making proteins. This is one way a cell regulates the amount of proteins it makes.

Nonsense-Mediated mRNA Decay (NMD): The process by which a cell destroys mRNA that contains premature stop codons. It acts as a "quality control" for the cell.