

Nanorobotic Approaches for Activation of Non-Coding DNA Using CRISPR-Cas9: A Conceptual Framework

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ABSTRACT

The human genome comprises approximately 3.2 billion base pairs, of which only 1-2% encode proteins, corresponding to around 20,000-25,000 functional genes. The remaining 98-99% of the genome, once regarded as "junk DNA," consists of introns, regulatory elements, repetitive sequences, non-coding RNAs, and other non-coding regions. Recent evidence suggests that while only 10-15% of these sequences are actively functional, the vast majority remain in an inactive state, potentially harboring remnants of ancestral and evolutionary genetic information. We propose a transformative concept that integrates nanotechnology with genome editing to explore this hidden genetic reservoir. Specifically, nanorobots could be employed to deliver CRISPR-Cas9 systems with high precision to non-coding regions of the genome. By inserting artificial promoter sequences to activate genes and termination sequences to regulate expression length, dormant or ancestral genetic elements could be reawakened in a controlled, stepwise manner.

Keywords- Non-Coding Regions, CRISPR- Cas9, Nanorobots.

I. INTRODUCTION

The human genome encompasses roughly 3.2 billion base pairs, but only a very small percentage (1-2%) directly code for proteins. The protein coding part amounts to approximately 20,000-25,000 genes¹, which is considerably fewer than earlier theorists expected. The remaining 98-99% of the genome does not code for proteins and referred to as junk DNA for a long time², simply because scientists did not know what it was there for. However, as is often the case with a better understanding of biological processes, the science has changed as we have compressed knowledge to improve understanding of non-coding genome. Non-coding DNA hosts introns, regulatory sequences, repetitive sequences, transposable elements, and several kinds of non-coding

RNAs³. Approximately 10-15% of these regions show robust evidence of biological function⁴, but they functionally mediate important heredity functions in supporting gene regulation, preserving genome stability and mediation of developmental features⁵. The vast majority of non-coding sequences are likely inactive, but that there are still questions as to why they are there⁶. They might be remnants of once functional genetic material, and markers of long-term evolution from other sequences, or they might represent sequences that used to have or still have functional significance.

Understanding these non-coding sequences is key to being able to have a more complete understanding of organization and evolution across the genome, and new evidence will likely keep coming that will challenge our understanding of the structure of the genome and its

history⁷. The difference between "useful" and "non-useful" DNA has grown increasingly complicated. This review summarizes what we know about the non-coding genome, the functional elements of the non-coding genome, and possible evolutionary origins, and highlights what is still uncertain.

II. DISCUSSION

Cotney colleagues performed Chip-seq for histone H3 lysine 27 acetylation (H3K27ac, a mark of active enhancers) in human, rhesus macaque, and mouse embryonic limb at four different developmental stages from bud to digit separation, defined as orthologous based on morphology and HOX gene expression. They found 2175 promoters and 2915 putative enhancers with significantly higher H3K27ac signal in human at one or more time points. Sixteen of these regions overlap with HARs, but most show no evidence of human accelerated modern human substitution rates or unusual diversity patterns across modern human⁸.

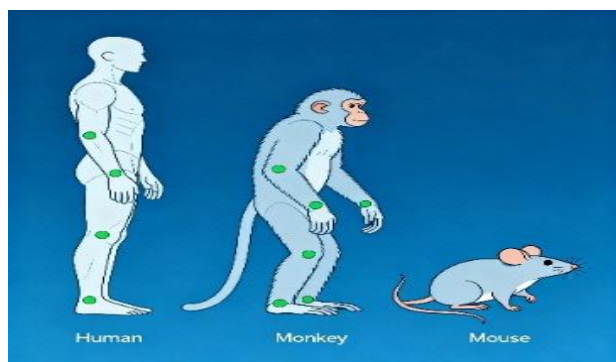


Figure 1. Difference in Limbs

Figure 1 shows the differences among the limbs of the three organisms, all of them have the same set of genes for limb development, but the differences lie in how those genes are regulated, such as when they are activated and how long certain genes remain active.

A comparison between human and chimpanzee brains highlights that the primary distinction lies in brain regulation and the timing of development between the two species.⁹ In short the pioneering research demonstrated that while the protein-coding segments of genes in humans and chimpanzees are nearly the same the patterns of gene expression, in the brain vary significantly^{10,11}. Particularly the human upregulations and alterations in expression were contrasted with those of chimpanzees of the downregulations and this speeding up is noticeably more pronounced in the brain compared to non-neural tissues like the liver and kidney^{11,12}. Therefore even a minor variation in their expression may result in a transformation of gene networks – hence it can be viewed as a potential explanation, for extensive developmental and physiological modifications occurring without the need to alter a vast quantity of

protein-coding genes. While scientists initially thought that adult gene expression patterns were vastly different, they subsequently found significant differences as well in the developmental stages of gene expression and their timing.^{12,13}

Hox genes largely perform the role across all animals in controlling development.¹⁴ What's fascinating though is observing how various animals employ them uniquely¹⁴. It boils down to modifications in the DNA segments that amplify gene expression (enhancers), the way DNA is organized (chromatin) and additional gene functions that don't alter the DNA sequence itself (epigenetics)^{15,16}. Consider fruit flies for instance which have their Antennapedia and Bithorax Hox genes. They possess borders that separate the head, chest and abdomen.¹⁵ This is due to enhancers unique to each species¹⁷ along with a mechanism known as Polycomb-mediated chromatin repression, which maintains order¹⁶. In vertebrates like humans, mice and birds the Hox clusters (A–D) function differently. They emerge in an order and at particular sites and moments¹⁴. The expression varies between species due to enhancers unique to each group, chromatin looping that connects distant regions and modifications in TADs (Topologically Associating Domains). TADs control the interactions between enhancers and promoters across distances¹⁸. Because of these factors, animals with identical Hox proteins can still exhibit diverse numbers of backbone segments, distinct limb configurations and facial shapes¹⁹.

Fish possess extra Hox groups because their ancestors experienced a whole-genome duplication event long ago²⁰. But how these Hox groups are used changed because the enhancers got rewired^{17,20} and the chromatin became easier to access¹⁸. That's why fish fins look nothing like our arms and legs. So, the important parts of Hox genes stay largely the same over evolutionary time. How they act differently in animals is because the control switches—not the protein sequence itself—have been tweaked as animals changed over eons²¹. Animal diversity in shape is mostly due to changes in regulatory control while using the same conserved developmental toolkit²¹.

III. NANOROBOTS CROSS BLOOD-BRAIN BARRIER

Mechanisms enabling nanorobots to successfully cross the Blood-Brain Barrier (BBB) are a prerequisite for their clinical use in CNS theragnostic. Purely for the purpose of efficiently crossing the cellular barrier, nanobots are inversely having to take over natural transport routes (pathways). The most prominent employed technique is receptor-mediated transcytosis (RMT). In this context, nanobots are covered with substances, which can selectively identify and bind to receptors on the surface of endothelial cells of the BBB. This connection activates the cells to wrap the nanobots

(internalization) thus the nanobots are transported into the cell and across the barrier via the transcytosis pathway, which is a route of transport that goes around the tight junctions linking the cells, therefore the nanobots reach the brain tissue. Moreover, the "Trojan Horse" strategy is a hypothetical one, which takes advantage of the migratory capacity of immune cells (e.g., neutrophils). Injecting nanobots into these immune cells, transporting them over the BBB, and discharging the payload in the target areas, for instance, tumors or loci of inflammation, in the brain parenchyma is the process of nanorobots application in therapy.²²

IV. NANOCARRIER-MEDIATED CRISPR-CAS9 DELIVERY

A pivotal experiment demonstrating nanotechnologist use in CRISPR-Cas9 delivery was conducted by Finn et al. (2018) In which lipid nanoparticles (LNPs) carrying Cas9 mRNA and single-guide RNA (sgRNA) were utilized to target the Angptl3 gene, in mice²³. Following administration these LNPs successfully transported the CRISPR elements directly

to liver cells leading to significant gene editing. The treatment caused a decline in the circulating ANGPTL3 protein concentration and as a result blood lipid levels such as LDL-cholesterol and triglycerides decreased. Furthermore, the gene-editing effect persisted for over 100 days following a single dose and the researchers observed no significant, off-target mutations or liver toxicity²³. What this work of Finn and coworkers achieves is to show that nanocarrier-based delivery systems can be the means by which safe, efficient, and tissue-specific CRISPR-mediated gene editing in vivo is accomplished, thus opening up the way to a therapeutic application of the technology for human diseases.

V. FUTURISTIC APPROACH

The non-coding region is normally not transcribed. We aim to activate it; for example, by selecting a segment of the non-coding region and adding a promoter and termination sequence, it may become transcriptionally active. We can use small DNA fragments, sometimes larger ones, and in some cases a combination of both, to observe the resulting effects.

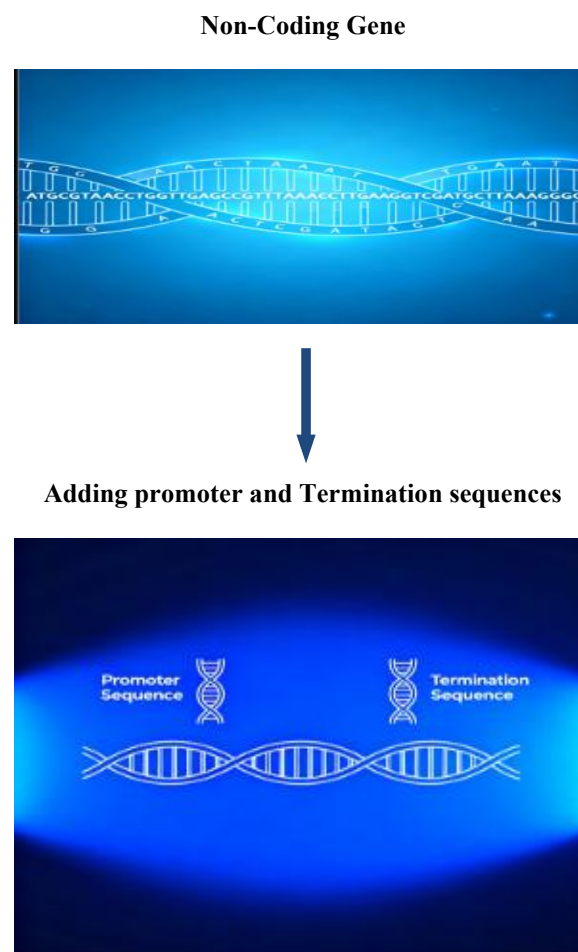
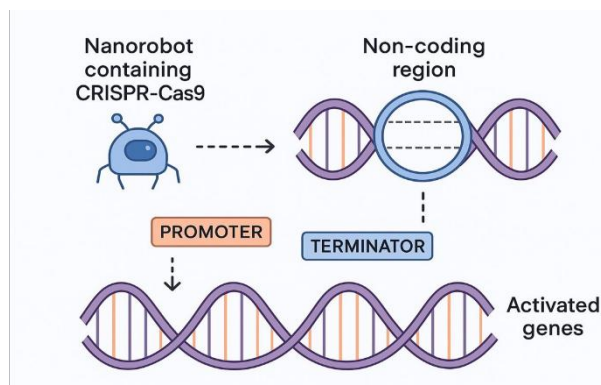


Figure 2 Adding Promoter and Termination Sequence

Suppose we discover a novel gene that provides benefits to a specific organ, such as the liver or pancreas. Then we design nanobots whose surfaces are coated with organ-specific receptors, enabling them to selectively target that organ. Once localized, the nanobots release Cas9. The Cas9 system is engineered to reach the intended genomic site and activate the target gene



Nanorobots containing CRISPR- Cas9

Human brains developed more than chimpanzee brains due to differences in gene expression pattern and gene activity duration. Longer activity might have led to more advancement of brain to maintain balance; it is crucial to avoid disrupting other function. We will use a similar approach: first, the nanorobots will be made to cross the blood–brain barrier by coating their surfaces with blood–brain-barrier–specific receptors, allowing them to pass through. After crossing, the nanorobots will release Cas9, which will then carry out its function at the target site

VI. HUMAN CAN ALSO FLY

Humans and bats have the same genes for limb development, but the difference lies in how those genes are regulated. If we could control this regulation, humans might also be able to develop wings, though this would likely require editing at the germline level. If this were achieved, something extraordinary might happen, similar to what is shown in the image.



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