DNA: More Than Just a Genetic Material*

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The article describes the non-genetic roles of DNA molecule and its applications in diagnostic and therapeutic fields such as in biosensors, barcodes, nanomachines, biochips, probes, and DNA scaffolds.

Introduction

Deoxyribonucleic acid (DNA) is an important biological molecule that was discovered as a hereditary material by Avery, Hershey and Chase. The three-dimensional structure of DNA was determined by Rosalind Franklin, James Watson and Francis Crick, for which the last two were awarded the Nobel Prize. The discovery of DNA as a carrier of hereditary material intrigued scientists, who dug further into the exact role of DNA in our lives. This started an era of wonderful discoveries about the roles of this powerful molecule. In addition to being a genetic material, DNA molecules may play roles outside the nucleus and the paradigm of replication and transcription. The various structures of DNA and its ability to recognize complementary pairs are responsible for ‘new’ roles and applications. DNA has been found to play significant roles in metabolism, biofilm formation and regulation, immune sensing, and more. These properties have also generated considerable interest in synthetic biology and chemistry and led to new applications in the fields of biosensors, medicine, and agriculture. We will describe a few such roles of DNA along with applications where DNA is used (Figure 1).

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**Figure 1.** The many roles and applications of DNA: the chemical and structural properties of DNA allow it to perform many different functions. In nature, extracellular DNA is utilized as a nutrient source by bacteria and also helps in biofilm formation. DNA can also function as an enzyme and can act as a ligand in different signalling cascades, most notably during the innate immune response. DNA has also been engineered for several synthetic applications, such as a probe for detection of specific sequences, as nanomachines for enhanced drug delivery, as barcodes for sample tagging and as biochips utilized in sequencing applications.

**DNA As a Nutrient Source**

Extracellular DNA (hereafter eDNA) is abundant in the environment. Bacteria regularly take up eDNA as part of their natural competency. Finkel and coworkers hypothesized that this eDNA could also be utilized by bacteria as a carbon source based on the observation that the natural competency of bacteria to uptake DNA, observed in many bacteria including *Haemophilus influenzae* and *Neisseria gonorrhoeae*, is greatly enhanced during starvation stress, possibly to allow uptake and consumption of eDNA [1, 2]. This hypothesis was tested with wild type as well as competency mutant *E. coli*, and it was observed that wild type cells reached a peak colony-forming unit (CFU) per mL, and then declined to 100-fold after three days during the death phase, and then remained relatively stable. It was proposed that the dead cells in the media released DNA which was utilized by the existing population as a food source. Even when filtered media from five-day-old cultures was used to inoculate the wild type and mutants, the mutants grew to only 1/10th of the wild type culture, presumably due to its incompetence to take up DNA. When the

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experiment was repeated with media treated with DNase I (an enzyme that cleaves DNA), both wild type and mutants showed similar growth rates, suggesting it was DNA as a carbon source that was assisting wild type *E. coli* to survive [2]. Since then, further studies have been conducted to decipher the mechanism of utilizing eDNA as a nutrient source, which has led to discoveries of extracellular deoxyribonucleases, further adding to the claim that eDNA can be used as a food source [3].

**DNA in Biofilm Formation**

Oftentimes, bacteria secrete a mixture of exopolysaccharides (EPS) along with other components to form a protective and adhesive layer called a biofilm. It has been found that a significant proportion of the biofilm matrix is eDNA. It was believed that the eDNA was merely a by-product of lysed cells in the biofilm. However, eDNA was shown to be released by living cells in substantial amounts by vesicles, prodding a deeper look into the role eDNA played in biofilms [4]. In the absence of eDNA (after treatment with DNase I), the formation of biofilms was severely inhibited in *Pseudomonas aeruginosa*, establishing that biofilms could be dissolved through DNase treatment [4, 5]. In *Caulobacter crescentus*, which produces two progeny, one of which is sessile and the other one motile, eDNA was found to limit the motility of the motile cells in the matrix [6]. These studies suggest that eDNA is important not only for biofilm formation but also for its maintenance. Understanding the mechanism behind biofilm formation and stability will help to design strategies to prevent the formation of adherent bacterial colonies in the context of diseases like cystic fibrosis, the formation of biofilms on pacemakers, and general disinfection.

**DNA As an Enzyme**

DNAzymes are oligomers, enzyme mimicking molecules. Such enzymes are extensively used in mRNA cleavage. Breaker and Joyce screened a library of over 1000 single-stranded nucleotide
sequences to uncover a DNAzyme that could perform a Pb²⁺ dependent cleavage of RNA with a high turnover rate [7]. Several RNA cleaving DNAzymes with different ion dependencies have since then been generated [8]. These enzymes are single-stranded molecules and exist in nature along with metal ions like Na⁺, K⁺, Mg²⁺, and Ca²⁺. Such enzymes deploy the rA (riboadenine) cleavage site for the cleavage of mRNA and are thought to downregulate its expression. DNAzymes have been implicated in various therapeutic applications like antiviral, anticancer, antibacterial, anti-inflammatory, and atherosclerosis [8]. Intracellular sensing and imaging of monovalent cations like sodium with high specificity is now possible with the aid of DNAzymes. Even though such DNA-based enzymes are highly stable compared to RNA enzymes like siRNA, their delivery inside the cell and low biological availability make it a tougher call for DNAzymes to be used as therapeutics.

DNA As a Ligand

DNA molecules often act as ligands for receptors, particularly in the case of immune sensing. A well-known example is the case of TLR9, a toll-like receptor family member, which senses CpG rich unmethylated bacterial DNA to trigger a downstream immune response, resulting in the release of cytokines [9]. DNA, in this case, acts as a pathogen-associated molecular pattern (PAMP). Studies have shown that CpG rich DNA can also inactivate the TLR9 response [10]. This ability of DNA to activate and inactivate the immune system is exploited in various therapeutic interventions where the goal is to modulate immunity. For example, in the case of allergies, endotoxic shock, and auto-immune diseases, inactivating ligands are used to suppress the immune response, whereas, in cases like cancer, activating ligands of TLR9 are utilized to jump-start the immune system into secreting tumor regressing factors such as IFN-α and TRAIL and activating natural killer cells [11–13]. The mammalian innate immune system has neutrophils patrolling incoming pathogens and forming extracellular DNA-neutrophils traps for engulfing such pathogens [14].
Similarly, plants have also evolved a mechanism to keep soil-borne pathogens at bay. Plant extracellular traps like NETs (nuclear extracellular traps) comprising DNA, H4 histone proteins, polysaccharides like xyloglucan, arabinoglucans, etc., have been shown to be involved in trapping pathogens at the root tips. Plant pathogenic bacterium *Ralstonia solanacearum* escapes these root extracellular DNA traps by secreting nucleases that act as molecular scissors and degrade extracellular DNA [15]. Interestingly, the presence of self DNA in the extracellular matrix was reported to trigger an immune response in plants and the activation of immunity to subsequent pathogen attacks [16].

**DNA Based Applications**

DNA has become a powerful material, making its way in various applications in the last several years like biosensors, nanomachines, biochips, probes, DNA scaffolds, etc. Here, we describe various applications of DNA nanostructures.

**DNA As Biosensors**

One of the most well-known examples of DNA-based sensors are ‘molecular beacons’. Molecular beacons show limited to no fluorescence when the quencher and fluorophore attached to opposite ends of DNA are brought together by the DNA’s hairpin structure. However, in the presence of a complementary sequence, the hairpin loop is broken, and a fluorescent signal can be seen. DNA probes, which are 100–1000 base long sequences, are widely used in biomedical detection and clinical diagnostics as biosensors for optically visualizing adherent cell traction forces, cell migration, and movements [17–19]. DNA-electrochemical biosensors are employed in various applications such as investigating DNA-drug interaction mechanisms, identifying proteins and metals, and pollutant detection [20]. These sensors use electrodes along with DNA probes to sense the electrochemistry of the subject molecule.
DNA As Nanomachines

DNA can also be used as pH and ion sensing molecular machines and as therapeutics nanodevices. Many of these DNA machines undergo structure switches to indicate changes in their environment. For example, one of these early machines developed undergoes a transition from the B-form of the double helix to the Z-form of the double helix depending on the concentration of [Co(NH₃)₆]³⁺ ions [21]. These transitions can be measured using fluorescence resonance energy transfer (FRET) experiments. In recent times, many DNA-based sensors have come up that can detect mercury, lead, silver, and hydrogen ion concentration, etc., along with the development of many other nanomachines such as DNA walkers, hybridization driven devices, and various different kinds of switches [22–24]. One of the main reasons responsible for these applications is the different secondary structures that DNA can adopt, such as Z-DNA, G-quadruplexes, i-motifs, etc., in the presence of different ions, as well the various associations it can form based on its complementarity.

DNA As Biochips

Biochips comprise an array of DNA fragments located over a chip smaller than a stamp size that can capture target DNA resulting in the generation of a detectable signal.

DNA For Barcoding

Another breakthrough in DNA-based applications is the utilization of DNA as barcodes. A barcode is a machine-readable representation of the data, where the data describes something about the object that carries the barcode. Using a specific sequence of DNA as a barcode, it is possible to identify individual samples in
a mixture of many samples. Barcoding has made it easier to perform multiplexing in DNA sequencing reactions covering a large number of samples at a time [28]. DNA barcoding uses internal transcribed spacers (ITS) and many conserved genes like plastid and mitochondrial genes and retrotransposons across species for phylogenetic analysis to identify an unknown sequence [29]. Metabarcoding is another approach wherein environmental DNA (eDNA) is used for distinguishing a wide variety of species in the study of a community [30].

DNA As Scaffolds

The ability of DNA aptamers to bind to target molecules with high affinity and internalized by endocytosis makes DNA an excellent material to be used as scaffolds for targeted drug delivery with good biodegradability. DNA nanostructures could be assembled into different shapes like cubes, tetrahedrons, triangles, or linear nanoparticles, either using small DNA tiles with sticky ends or using long single-stranded DNA using staples [31–33]. These DNA nanostructures can be used as carriers for small molecule drugs like doxorubicin, photosensitizer TMPyP4, siRNAs, antisense RNA, CpG, and proteins (Fab antibodies, etc.).

All in all, DNA is involved in several other functions apart from its most prolific role as the codifier of genetic information (Figure 1). It performs many biological functions, such as acting as a nutrient source, contributing to biofilm formation, functioning as an enzyme, and acting as a ligand in many signaling cascades. It is also extensively used in many synthetic applications, like acting as a biosensor and nanomachine to relay information about important interactions and environmental conditions, being used in DNA barcoding and biochips, as well as, acting as a carrier for several important molecules. This versatility allows us to utilize DNA in several applications, in fields ranging from medicine to agriculture and more, and underscores why this incredible biomolecule is the cradle of life for more reasons than one.
Suggested Reading


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