Nanotechnology for Future Molecular Systems using DNA

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With the progress in molecular biology and nanobiotechnology, biological molecules attract people's attention as materials for next-generation devices that can realize molecular smart systems. Here focusing on DNA as a bio-molecular material that contribute to the sustainable development of future ICT societies, researches on new transformation of matters or information transport systems using biological functions will be introduced.

1 Introduction

Molecular devices that use biomolecules are energy efficient and capable of miniaturization and integration. They have been attracting much attention as the future high-speed information processing devices to replace silicon devices. Focusing on DNA, which is a highly functional biomolecule, we report researches on developing systems for substance transformation and information transport using biological functions here. Aiming to establish advanced technology that is not just an extension of the current information and communications technology (ICT), our Bio ICT laboratory is carrying out bio-ICT research and development to create a new paradigm in ICT using biological functions. Our goal is to improve the performance and functions of information and communications systems in accordance with the objectives of the mid-term plans of NICT; to contribute to the finding of solutions to a variety of issues ranging from those affecting our daily lives to the global environment, such as healthcare, education and global warming, through research and development in ICT. It is expected that our results will contribute to the stimulation of related academic fields and industries, and development of the future ICT society.

In the following sections, we will first explain the fundamental properties of DNA, and their potential applications. Then we will report on the current research at NICT and the prospects for the future.

2 The structure, functions and characteristics of DNA

2.1 The structure of DNA

2.1.1 The structural materials of DNA and its double helix structure

DNA is a type of nucleic acid called deoxyribonucleic acid. Nucleic acid is a biopolymer made of nucleotides, consisting of the three components, sugars, phosphoric acids, and nucleic acid bases, joined through organophosphate bonds.

The sugars found in nucleotides are five-carbon sugars containing five atoms of carbon each. The carbon atoms are numbered from 1' to 5' with Carbon 1' having an aldehyde group (CHO group) attached to it. To distinguish them from the carbon atoms in the bases, those contained in the sugars are marked with prime marks after the carbon. The sugar component of DNA is deoxyribose with a hydrogen group (H group) at the 2' position. Compared to ribose, which is a component of ribonucleic acid (RNA), with a hydroxyl group (OH group) at the 2' position, it is not easily hydrolyzed and it is thermodynamically stable. As can be seen in the example of the ancient Oga lotus fruit that germinated and flowered after being dug out of ruins from the Jomon period over 2,000 years ago, in a peat layer around 6 m underground, DNA is an extremely stable substance that makes it suitable for preserving genetic information. It is also believed to have the durability to make it suitable for use in materials for molecular devices.

Nucleic acid bases consist of purine bases with six-membered and five-membered purine rings made of carbon and nitrogen, and pyrimidine bases with six-membered pyrimidine rings made of benzine with the

carbon atoms at positions 1 and 3 replaced with nitrogen. Nucleotides form long chains through the repetitive formation of organophosphate bonds in which the hydroxyl group (-OH group) of phosphoric acid bound to Carbon 5', and the hydroxyl group (-OH group) at the 3' position on the neighboring sugar bond to give off water. The four types of nucleic acid bases bound to Carbon 1' on the deoxyribose are the purine bases, adenine (A) and guanine (G), and the pyrimidine bases, cytosine (C) and thymine (T). The genetic information in the amino acid sequence of the protein is coded into this nucleotide sequence. The only differences between nucleotides are these nucleic acid bases, so DNA sequences are also known as base sequences.

The purine base, A, and pyrimidine base, T form complementary base pairs, as do the purine base, G, and pyrimidine base, C. The DNA chain with its double stranded (duplex) structure is made of these complementary bases pairs, which form hydrogen bonds between them. AT base pairs form two hydrogen bonds between them, compared to GC base pairs, which form three. This results in a difference in their stability. The base pairs are stacked on top of one another, and the inner spaces between them are hydrophobic resulting in van der Waals forces (intermolecular forces that act to attract electrically neutral molecules) that lead to stacking interactions bringing base pairs closer together. This causes the sugar-phosphoric acid backbone to twist, resulting in a helical structure.

2.1.2 The characteristics of the double helix structure (Watson-Crick base pairs): Major grooves and minor grooves

Two types of grooves of different width are formed on the DNA helix because the connecting line of base pairs joining the double strand (sugar-phosphoric acid backbone) of the main chain does not pass through the center of the helix. This shift is caused by the base pair stacking and the torsion of the sugar-phosphoric acid backbone. The wider grooves are known as major grooves, and the narrower, shallower grooves are known as minor grooves. On the outer side of the helix, ionized hydrophilic hydroxyl groups (-OH groups) –O- of organophosphate bonds are exposed to the solvent, while hydrophobic parts of bases are exposed to the solvent in major grooves, and hydrophilic parts in minor grooves. The number of chemical side chains of proteins that act like modulators in transcription and replication, and the number of nitrogen and oxygen molecules that can form hydrogen bonds differ between the major and minor grooves, having four and three respectively. Hydrophobic interactions, dipolar interactions, hydrogen bonds, electrostatic interactions, etc., are what stabilize the three-dimensional structure of DNA. These factors are in turn affected by the external environment, so that environmental changes may change the shape of DNA. The minor grooves in particular, where hydrophilic parts are exposed to the solvent, change their double helix shape dramatically in response to changes in its hydration state. For example, the DNA found in cells are mostly of a form known as B-DNA (right-handed helix with 10.5 bases per turn of the helix, distance between base pairs of 3.4Å, helix diameter of 20Å, and 92% humidity) in which the differences between the major and minor grooves are clear. The shape of B-DNA is stabilized by water, which flows into the minor grooves. On the other hand, in a dehydrated state as in a spore, the DNA assumes the shape of what is known as A-DNA (right-handed helix with 11 bases per turn of the helix, distance between base pairs of 2.6Å, helix diameter of 23Å, and 75% humidity) in which there is little difference between the major and minor grooves. Other DNAs are known to assume other double helix structures such as C-, D- and E-. Moreover, repeated sequences of G and C bases under high salt concentrations assume the shape of what is called a Z type in which the helix is left-handed (with 12 bases per turn of the helix, distance between base pairs of 3.7Å, and a helix diameter of 18Å). It is not properly understood why DNA assumes this shape. Z-DNA was believed to be a special form attainable under artificial conditions, but it is now known that there are numerous sequences on the genome DNA which could assume the Z shape, and supercoils (superhelices) formed during genetic transcription and DNA replication are believed to form Z-DNAs. It is also known that the methylation of cytosine of DNA easily transforms B-DNA to Z-DNA.

There are numerous sequence-specific proteins that interact with DNA, and their recognition mechanisms are poorly understood. But some proteins are known to recognize base sequences by detecting distortions in the double helix structure of DNA caused by biases in base sequences. For example, it has been reported that arginine residues of proteins are able to recognize concentrations of negative electric charges caused by the narrowing of the minor groove as the result of repeated A or T bases in sequences[1]. In this way, the major and minor grooves are believed to play vital roles in the functions of DNA. Other

than that, we are beginning to understand that DNA also responds to changes in the environment to assume a variety of different three-dimensional forms with a wide range of functions.

2.1.3 Multihelical structures: Hoogsteen base pairs

It was in 1963, ten years after Watson and Crick put forth their double helix model for the structure of DNA[2], that Hoogsteen reported the existence of crystalline structures formed by hydrogen bonds that were different from Watson-Crick base pairs[3]. These structures formed by hydrogen bonds were named Hoogsteen base pairs, and they are formed by two nucleic acid bases that are held together by two hydrogen bonds in the major groove. In Watson-Crick base pairs, the hydrogen bond acceptor formed by the nitrogen atom at position 3 on the pyrimidine base is also the nitrogen atom at position 1 on the purine base, but in Hoogsteen base pairs this hydrogen bond acceptor is the nitrogen atom at position 7. DNA can use these Hoogsteen base pairs to assume structures other than that of the double helix. For example, when another base pair binds to a Watson-Crick base pair through a Hoogsteen conformation, the DNA assumes a triplex (triple stranded) structure known as H-DNA[4]. The triplex structure is formed when one strand of the DNA is made of purine bases, while the other strand is made of pyrimidine bases, with symmetrical sequencing between the two strands. In a triplex structure, one half of this area unravels, and the pyrimidine chain becomes the third strand, forming new base pairs with purine bases. The combinations of base pairs under these circumstances are C-G-C or T-A-T, so a symmetrical base sequence is a necessity. When the solvent is mildly acidic, the pyrimidine chain becomes the third strand, but if the solvent is neutral and there is magnesium, the purine chain becomes the third strand. Reversed Hoogsteen pairing occurs when the purine chain becomes the third strand. Triplex DNA has been found in regions regulating genetic expression in nature, and it is believed to play some kind of role in genetic recombination and DNA restoration[5][6].

Moreover, DNA and RNA rich in G bases are known to be formed purely by Hoosteen base pairs called guanine quadruplexes (G-quadruplexes). The guanine quadruplex requires four guanine triplets (a combination of three bases) separated by short spacers, and four G bases joined by Hoogsteen bonds in a planar structure to form the quadruplex. Sequences like these are often found in telomere and promoter regions, and guanine quadruplexes have also been shown to possibly exist in living organisms[7][8]. Telomeres are structures found at the end of DNA strands of eukaryotes with characteristic repetitive nucleotide sequences, and various localized proteins making characteristic structures, which are believed to protect the DNA terminals. The guanine quadruplex in telomeres is believed to control the activity of telomerase (an enzyme that adds telomeric DNA sequences to telomeric DNA terminals), but in the promoter region it blocks the bonding of transcription factors, or on the contrary it can cause and maintain supercoiling during transcription, which is believed to contribute to the enhancement of gene expression. Other than that, there have been reports of cruciform structures[9] that play an important role in DNA recombination and restoration, slipped DNA[10] formed by slippage during replication when the same base sequences are repeated in the same direction, DNA nodules[11] in which single strand sections of triplex structures become the third chain of a different triplex structure, as well as the formation of octuple helix structures[12] through slippage when the same sequence is repeated eight times. It is still a mystery as to what roles such structures play in living organisms, but DNA as with proteins, is believed to assume a variety of three-dimensional structures, which play vital roles in realizing advanced biological activities such as transcription control.

2.1.4 Superhelical structures (supercoils)

Apart from helical structures formed by Watson-Crick and Hoogsteen base pairs, structures known as supercoils (super helices) can appear on DNA resulting from its over- or under-winding. When DNA is transcribed or replicated, the double helix must be unwound to form single strands. When a double helix polymer like DNA is unwound, other areas are also rotated around the axis of the helix creating heavy twisting. This torsion is not a problem if the DNA is an artificially synthesized short, straight chain, but in the kind of long, straight chains of DNA found in eukaryotes, or circular DNA found in prokaryotes, the DNA strand cannot rotate freely. The twisting created by this unwinding creates a major helical structure, known as the supercoil. DNA is a right-handed helix, so the superhelix formed in the direction of the progress of the replication or transcription bubble is also a right-handed helix (positive superhelix). When these positive superhelices grow long, they resist the unwinding process, which can stop the progress of transcription and replication. To overcome this problem and facilitate

smooth transcription and replication, an enzyme known as topoisomerase catalyzes a reaction in which it cuts the unwound portion of the DNA during replication, allowing the other portion of the DNA to be untangled before they are rejoined.

There are theories proposing that the same mechanism is used to overcome twisting during transcription, or that RNA polymerase, which is a transcriptase, rotates in accordance with the twisting of the double stranded DNA to unwind it without creating the problem of over-twisting. The stress placed on a double helix as the result of a right-handed superhelix can be relieved by winding the DNA around its axis in a left-handed twist. The DNA inside the nucleus of a eukaryote is wound in a left-handed turn around histones, so it does not get stressed as the result of superhelix formation. The right-handed superhelix formed in duplex DNA generally function to promote the formation of homologous pairs (a reaction in which single strands of DNA originating from the separation of a double helix pair up with complementary strands from double helices of other double stranded DNA with similar base sequences) in homologous recombination (recombination of sequences between DNA strands with structures of similar base sequences) during meiotic divisions (cell division in reproductive cells such as sperm and ova) and somatic divisions, or promote the formation of structures such as cruciforms and Z-DNA. The supercoil is considered to be one of the many varieties of three-dimensional structures that plays a vital role in the DNA.

2.1.5 The chromatin structure

The chromatin structure is another important structure assumed by eukaryotic DNA. The long-chain genome DNA of eukaryotes is wrapped around a histone octamer to make a structure known as a nucleosome. Several of these nucleosomes join together to form chromatin structures, which makes DNA compact enough to fit inside the nucleus. Until now, the role of the nucleosome was believed to be to turn the long DNA into a compact package for storage within the nucleus. But it has come to light that the control sequence involved in transcription is folded precisely around the histone in the nucleosome, to control access to the chromatin by the transcription control elements, and control the transcription process itself. Only a very limited portion of the eukaryote genome DNA is transcribed, and most of it is in an inert state. Because histone is rich in positively charged amino acids, it is strongly alkaline, and it binds with acidic DNA to form the electrically balanced, stable chromatin structure, which is believed to support the inactivity of genes. On the other hand, it is known that in genes that are actively transcribed, the histone is chemically modified and becomes electrically unstable loosening the chromatin structure. Histone is chemically modified by acetylation, methylation, phosphoric acidification, and ubiquitylation, through which it is involved in controlling the functions of chromatin, such as genetic expression, replication, and controlling of DNA functions. A detailed explanation of this phenomenon will be given in the next Subsection.

2.2 The functions of the DNA

In the past, the main functions of DNA were considered to be the accurate recording, preservation, and replication of genetic information, and it was believed to be proteins that can form complicated three-dimensional structures that were involved in regulating genetic expression. It has gradually come to reveal that DNA is able to organize various three-dimensional structures similarly to proteins to control genetic expression. The human genome was completely elucidated in 2003, and it came to be known that protein coding sequences make up less than 2% of the overall genetic material, and that more than 50% is filled with various repeated sequences[13]-[16]. It is now believed that these kinds of non-coding regions are responsible for actively regulating genetic expression. In this paper we will report on the functions of DNA as an outstanding biological information device that can even regulate genetic expression, in addition to accurately recording, preserving, and replicating genetic information.

2.2.1 Recording and preserving genetic information

A gene generally refers to a structure with a coding region with amino acid sequences coding for DNA proteins, a region that codes for functional RNA sequences, and a region that is not transcribed but functions to control transcription. Genes in their entirety including regions whose functions have not yet been elucidated are called genomes in the broad sense of the word. In base sequences, 20 varieties of amino acids are each coded for by a combination of three bases (codons). When the DNA sequence is transcribed onto the RNA, it is translated into amino acids by the ribosome (composed of ribosomal proteins and ribosomal RNA). These amino acids then join into chains to form proteins. The concept of "genetic information of organisms (information contained in the base sequencing of DNA) undergoing transcription and

translation to realize functional expression" is known as the central dogma, and it was proposed by Crick in 1958[17]. Reverse transcription was later discovered, and a part of this theory was corrected, but it remains a central principal in molecular biology even today.

The double helix is a fundamental structure of DNA, and due to this complementary double stranded structure, it is able to use one strand for storage (sense strand), and the other as a template for RNA in transcription (antisense chain). The sugar that makes up the DNA is deoxyribose, and it is not easily hydrolyzed, making it stable. But RNA is made of ribose, a sugar that is easily hydrolyzed, and therefore unstable. This means that when DNA transcription stops and RNA production ceases, the RNA breaks down over time and proteins do not continue being synthesized. Carrying out protein synthesis in this way through RNA, enables the building of a multiplex gene expression regulating system that makes use of the different properties of DNA and RNA.

Moreover, the replication is a semiconservative process in which the parent chain is used as one of the double helices of the daughter chain. Should an error occur during replication resulting in a mismatch in the double helix, the spiral structure becomes warped allowing the error to be detected and corrected. For this reason, it is believed that the basic double helix structure of DNA is a convenient and practical structure for carrying out accurate DNA replication.

2.2.2 The DNA function for regulating genetic expression

Mechanisms used by organisms to regulate genetic expression are diverse. What is meant by genetic expression here is the transcription of DNA information onto RNA, which is then translated into protein synthesizing processes. Biological activities require necessary genes to be expressed at the right time, in the right cells. Transcription and translation also consumes a considerable amount of energy, so the synthesis of proteins that are no longer required must be stopped. The purpose of genetic expression regulation is to control the timing and amount of protein synthesis. Fundamental regulation of genetic expression in eukaryotes involves the following:

(1) Regulation that makes use of DNA regulatory sequences and gene expression regulatory factors
(2) Regulation of the chromatin structure such as the acetylation and deacetylation of histone
(3) Regulation through the chemical modification of DNA such as its methylation
(4) Regulation of transcription and translation by non-coding RNA such as riboswitches or through RNA interference
(5) Regulation of transcription by, e.g., triplex or quadruplex non-coding DNA

The mechanisms for gene expression can be divided into those that are genetic (based on base sequences that code for amino acids) and epigenetic. In this paper we will focus on epigenetic regulatory mechanisms in our explanations.

Transcription control through the chromatin structure is a typical regulatory mechanism along with RNA interference and DNA methylation. The DNA base sequences cannot be transcribed onto the RNA if the DNA is wound onto the histone. The chromatin structure must be loosened to expose the DNA. If this release is not done, the general transcription factor necessary for transcription cannot get close enough, resulting in little genetic expression of tightly compacted areas (heterochromatin). The reason why one of the two X chromosomes of a female mammal becomes inactivated is because it assumes a heterochromatin structure along almost its entire length. The structure of chromatin is stable, with the acidic DNA and the alkaline histone balancing each other out electrically. Therefore, when histone becomes acetylated for example, weakening its positive charge, its bond with the negatively charged DNA is also weakened. This loosens the chromatin structure to expose the DNA, enabling its transcription.

DNA methylation is a reaction involving the addition of a methy group (CH3 group) to the Carbon 5' atom of the pyrimidine ring of cytosine, or to the nitrogen atom at position 6 of the purine ring of adenine. DNA methylation physically prevents the bonding of the transcription factor to the DNA, and the methylated DNA binds with chromatin remodeling proteins to form heterochromatin (compacted, inactive chromatin), which regulates transcription. The methylation of DNA is inherited by daughter cells after cell division, and it not only plays a vital role in the proper generation and differentiation of cells in higher organisms, but it has also been reported to play a variety of other roles including the suppression of genetic expression of detrimental factors such as those of viruses taken into the genome of the host. In using DNA as material for molecular devices, it will become possible to make use of this kind of chemical modification to control systems.

Non-coding RNA is a general term for RNA that is not

translated into proteins, and it makes up around 68% of the genome[16]. Non-coding RNA is involved in the regulation of transcription and translation, and it is also involved in riboswitches (a protein found in a region that is not translated, but has sequences that function to regulate transcription termination and translation within a messenger RNA molecule (mRNA), which has base sequence information and a structure that may be translated into proteins), RNA interference (a phenomenon in which a double stranded RNA regulates the expression of a specific gene), and the formation of heterochromatins, as well as epigenetic regulation of genes through a variety of processes such as RNA-directed DNA methylation. Ribonucleic acid (RNA), which functions as a ribozyme catalyst, is also a type of non-coding RNA. It used to be believed that biological reactions were catalyzed by enzyme proteins, but upon entering the 1980s, Cech et al. discovered that RNA functions as a catalyst to promote splicing reactions (reactions for cutting, pasting, inserting and transplanting), which they named ribozyme[18][19]. In 2004, a deoxyribozyme was discovered that functions as a DNA ligase to join DNA molecules together[20].

Non-coding DNA that is not transcribed onto RNA makes up around 30% of the non-coding region[13]–[15]. This region also includes promoter regions and enhancers for regulating genetic expression, as well as various types of repeated sequences that are involved in regulating genetic expression through active modification of its three-dimensional structure such as in quadruplexes.

In these kinds of ways, biological systems make use of a diverse range of mechanisms to regulate genetic expression and realize advanced biological activity. A lot remains unknown about the functions of non-coding DNA regions. It is expected that the creation of new functions advance further in the future through elucidation of the unknown structural motifs of DNA and their functions to reveal the relationship between three-dimensional structures and their functions.

2.3 Other important characteristics

2.3.1 Electrical conductivity

Organic compounds were generally thought of as being insulators, but in the case of molecules with regions of overlapping p orbitals such as in cyclic compounds made of a series of double and triple bonds with intramolecular π conjugated systems, electrons are able to move through the π electron cloud to show narrow-band-gap-insulator or semiconductor-like properties. DNA has regions where the p orbitals overlap as the result of stacking interactions between base pairs, and it is known to be an organic semiconductor.

2.3.2 Magnetic properties

DNA was believed not to have any magnetic properties, but in 2005 it was reported for the first time that the genome DNA of λ-phage showed paramagnetism at low temperatures[21][22]. Because A-DNA in a dry state does not show magnetism, it is believed to become a source of spin magnetism created by the pairing of free π electrons, generated through interaction between water molecules and base pairs, with electrons with antiparallel or parallel spins. As an organic semiconductor with both magnetic and semiconductor properties, it is anticipated that DNA realizes devices with new functions and properties.

2.3.3 Optical properties

Liquid crystals are matter in a state with orientational order, discovered in a group of organic substances, and they include a variety of substances such as proteins, amphiphiles such as surfactants, and viruses. Liquid crystals formed by inorganic substances are almost unheard of, but in recent years it has come to light that stratified inorganic crystals such as graphite can form liquid crystals[23]. Liquid crystals may enter a liquid crystalline phase, and be differentiated into thermotropic (reacts to changes in temperature) liquid crystals or lyotropic (reacts to changes in concentration) liquid crystals. Thermotropic liquid crystals exhibit phase changes only in reaction to temperature or pressure, and thermoplastic resin is an example of this. Lyotropic liquid crystals are made of numerous substances, and they exhibit phase changes in reaction to temperature and their constituents. They are often found in biological tissue such as cell membranes. The main liquid crystalline phases are the smectic phase in which rod-like molecules are not arranged regularly within the stratified layers, the nematic phase in which molecules are aligned in one direction, the cholesteric phase which exhibits an aspect of molecules being aligned in one direction, but they are aligned in a spiral formation, the cholesteric blue phase in which the molecules assume a complex structure with unique properties, the cubic phase in which the ordered structure assumes a three-dimensional periodic structure with cubic symmetry, the columnar phase in which disk-shaped molecules are layered on top of one another, among others. Thermotropic liquid crystals are formed by excluded

volume effects arising from shape anisotropy of the molecules. Compared to this, lyotropic liquid crystals are formed through micro-phase segregation due to disparities in intermolecular interactions. When amphiphilic molecules form aggregates in a solvent, lyotropic liquid crystals can assume different shapes including a hexagonal shape arranged like the crystalline structure of a snowflake in which six hydrophilic groups become arranged on the outside of a cylindrical shape with hydrophobic groups on the inside, or a reverse-hexagonal structure in which hydrophilic groups face the inside with hydrophobic groups on the outside radiating out from the center, or a lamellar structure in which bilayers form (in which two molecules arrange themselves back-to-back with their hydrophobic groups on the inside) to become layered on top of one another.

Numerous liquid crystal phases exist in the living body, and lamellar structures formed by sphingolipids between the cells of the human stratum corneum not only function to prevent water from transpiring from the body, but they also function in a variety of other ways such as in the expression of structural color through the cholesteric phase formed by optically active chitinous substances, or the expression of an insulating effect from neural pulses in the smectic phase formed by amphiphilic phospholipids. DNA solutions are also known to exhibit characteristic optical properties above a certain concentration in the cholesteric phase[24]. A characteristic of substances that can take the form of liquid crystals is that their molecular shapes depart from spherical symmetry, and helical macromolecules that have rod-shaped helical structures fulfill the requirements of this characteristic. The helical structure is formed in the living body through hydrophobic interactions in which the hydrophobic parts try to shy away from water. The helical structure separates the hydrophobic parts from the hydrophilic parts, resulting in an amphiphilic molecule. In the case of the DNA double helix, the stability of the helical structure is attained through hydrogen bonding between the complementary base pairs. If the helical macromolecule solution is dilute, it does not exhibit anisotropy, but once the concentration passes a certain threshold level it triggers phase separation, resulting in the two phases of a solution and a solvent, with the molecules becoming aligned and exhibiting anisotropy. This orientation is thought to be because it is disadvantageous for long rod-like molecules to remain in a completely random arrangement, even when not taking the interactions between rod-like molecules into account[25][26].

When molecules enter a liquid crystal state, their molecular orientation can be made use of to generate advanced functions that supersede the functions of individual molecules. For example, laser oscillation requires an accurate resonator with a periodic structure on a nanoscale, but cholesteric liquid crystals reflect circularly polarized light of a specific wavelength, or they can spontaneously form periodic helical structures that are about the same size as the wavelength of light, and that can be trapped. For this reason this helical structure can be used as a distributed feedback laser oscillator that does not require micromachining. Furthermore, flexible liquid crystal polymers can be used to make film laser that can be rolled. The development of cholesteric liquid crystals that electricity can be passed through, and lowering the energy threshold to make the liquid-crystalline laser oscillate are the issues that need to be resolved before practical applications can be found for devices that operate on electrical excitation, but DNA is believed to be a promising material for these kinds of organic semiconductor lasers.

2.3.4 Terahertz spectral characteristics

Terahertz spectroscopy for examining the properties of materials using terahertz waves has been attracting substantial attention in recent years for its ability to detect weak interactions between molecules and things that cannot be seen using X-rays. Electromagnetic frequency in the range of around 1 THz (wavelength of 300 μm), known as terahertz waves, are difficult to create and detect, which was the reason not much progress had been made in research into developing light sources and finding applications for them. Since researchers at Harvard University succeeded in generating terahertz waves at room temperature in 2008[27], research in this unexplored area took off. The frequency range of terahertz waves includes absorption frequencies characteristic of molecular bonds such as hydrogen bonds and van der Waals bonds. Therefore, there are expectations it will allow us to obtain information on molecular networks in bioorganic molecular crystals dissolved in aqueous solutions, proteins with high-order structures, and DNA with double helix structures. In the living body, terahertz waves are generated by the activities of membranes, and there is debate on whether solitons (solitary waves) known as breather waves are generated in resonance with the terahertz waves on DNA[28][29]. It is believed that bubble structures found on DNA during transcription and replication are also formed by breather waves. There are even reports of terahertz

waves regulating sequence-specific genetic expression[30], and it is believed to be possible to control genetic expression such as cell differentiation by making use of this response characteristic of DNA to terahertz waves.

3 Applications using DNA

In this section, we will first discuss applications using DNA that make use of its properties reported in the previous section, in organic electronics and near field communication. After that, we will report on actual research being carried out at the Bio ICT Laboratory.

3.1 Organic electronics

3.1.1 DNA as organic semiconductor material

Organic electronics is a field of electronics in which organic materials are used. Traditional electronic devices were based on artificial arrangements of inorganic materials such as silicon, and they required large amounts of energy to manufacture. On the other hand, organic electronic devices based on organic molecules with innate functions, compared to electronic devices based on inorganic materials, not only place less burden on the environment and are more energy efficient, but they also have the advantage of being flexible and they can be made into thin films. For example, the self discharge rate of thin foil cells made of organic materials is virtually zero, they are long-lasting, and they can be made into small, lightweight, thin and high-capacity batteries. Other organic electronic devices such as organic EL lighting, organic thin film logic memories, organic solar cells, and organic semiconductor lasers attract expectations for practical realization. Light-emitting diodes (LED) made of organic compounds that emit light when a voltage is applied to them, have already been made use of in organic EL displays. Furthermore, as a next-generation technology, it is expected that electronic device manufacturing technology (printable electronics technology) using printing technology will realize lightweight, thin and flexible devices that will not break even when dropped.

Although organic electronic devices using DNA have yet to overcome issues in sensitivity, stability, duplicability, durability, etc., they have been put to practical use in certain sensor-type devices because we can expect future developments. Examples include the eSensor produced by GenMark Diagnostics (USA) combining microfluids technology with electrochemical detection, the microarray of CombiMatrix Diagnostics (USA) that enables high sensitivity detection by measuring electric current in DNA using a biocatalyst, and the DNA base differentiation system of Oxford Nanopore Technologies (UK) that uses nanoapertures. Other than that, there are DNA chips with new functions and lab-on-a-chip devices that are being developed[31]. It is generally difficult to obtain organic semiconductors of high purity due to difficulties in refining them, and they are easily affected by impurities or oxygen in the atmosphere leading to deterioration of their electrical properties. On the other hand, DNA is easy to refine making it possible to obtain highly pure materials. It is also possible to design three-dimensional structures and therefore develop new functions through sequence programming. They can overcome problems in the organic electronics field and contribute to its development as a material for organic semiconductors with the kind of advantageous properties mentioned in the previous section.

3.1.2 The possibility of DNA as a DNA spintronics material

Spintronics is an engineering field in which both the electric charge and spin of electrons are made use of to realize devices with functions and performance not achievable in electronics. Until now, mainly inorganic materials such as metals and silicon had been used in spintronics, but using DNA, a biomolecular material made of light elements such as hydrogen and carbon, will allow new devices to be developed that suffer little spin-polarization loss.

3.1.3 The possibility of DNA as material for liquid crystal semiconductors

As it was mentioned in the previous Section, molecular material such as DNA with a large π electron conjugated system has the right properties for use as a semiconductor. The carrier (a free particle that transports electric charges) transport process differs between this type of semiconductor and a silicon semiconductor, and rapid charge transfer is achieved through the hopping of charges between molecules. Furthermore, liquid crystal semiconductors in liquid crystal states regulate the orientation of their molecules through characteristic autonomous orientation. This enables the carrier mobility (carrier transport speed divided by the field intensity) to be increased relatively easily to amplify the response speed by raising the electrical current.

In general, organic semiconductors are highly soluble in a wide variety of organic solvents due to the

characteristics of their chemical structure. DNA is hydrophilic, and the nucleic acid bonded to polyethylene glycol (PEG) has been reported as being soluble in almost all organic solvents (acetonitrile, benzene, alcohols, halogens)[32]. This has enabled DNA devices to be used with not only water, but also organic solvents. For example, DNA with PEG dissolves well in ink made of organic solvents, and this enables the production of liquid-crystalline organic semiconductors that are ideal for use in printable electronics technology suitable for machining devices at ordinary temperature and pressure.

Moreover, in printable electronics a higher performance in organic semiconductors can be obtained the higher the crystallinity of the material of low molecular weight. DNA makes use of the sequence programming to control its three-dimensional structure, enabling this kind of molecular design. What needs to be solved is how to control separation of the semiconductor from the solvent, a problem that occurs as the result of convection currents and random crystallization inside droplets. There is a need to create homogenous semiconductor layers, but due to the geometric design of DNA it is possible to control their crystalline state, and regulate their molecular orientation through electric fields, magnetic fields and other external fields. DNA is therefore also believed to be suitable for use in printable electronics materials that will allow the creation of lightweight, flexible devices.

3.2 Near field communication

Cells that make up organisms communicate with their surrounding environment and other cells, while controlling their genetic expression to sustain life. Communication between cells is carried out in a variety of ways such as through biologically active substances produced within the living body, electric signals such as neural pulses, and gap junctions through which the cytoplasm of cells becomes joined together directly.

In this paper, we focused on substance transformation and information processing through near field terahertz waves and molecular orientation in crystalline states, and we will report on applications for these. In using DNA as material for nano devices, it is believed to be advantageous to make active use of the properties of DNA molecules in communication.

3.2.1 Terahertz communication within cells

Terahertz waves in the frequency range of 1 THz (wavelength of 300 μm) are halfway between the frequencies of radio waves and light waves, and they can penetrate a variety of materials like radio waves, while also allowing control using lenses and mirrors like optical waves. Terahertz waves have shorter wavelengths than millimeter waves, and they have spatial resolutions that allow them to be used for a variety of imaging purposes. They can penetrate all kinds of substances enabling visualization of things that could not be seen with X-rays, making them suitable for non-destructive imaging. Furthermore, in regard to band structure control and exciton control in semiconductors using terahertz waves, research is being carried out into finding practical applications for these in high-speed communication of information. Terahertz waves are absorbed well by water molecules in the atmosphere so they have limitations such as a short range (a maximum of around 1 km in the 0.1 THz range), but they allow large volumes of information to be sent at once, for which there are large expectations for their application in high-speed communication technology such as for downloading movie files within near fields.

Moreover, as it was mentioned in the previous Section, terahertz waves include absorption frequencies characteristic of molecular bonds such as hydrogen bonds and van der Waals bonds, and there are expectations they will allow us to obtain information on molecular network structures. From the fact that terahertz waves are believed to resonate with interactions between biomolecules, the physicist, Fröhlich in the 1960s presented a hypothesis (Fröhlich's hypothesis) that "cells vibrate in resonance somewhere between the terahertz and millimeter wave range, and that plays a vital role in communication of information within and between cells"[33]. He believed that terahertz waves were generated within cells by the activities of the membrane. In eukaryotes, chromosomal DNA is enveloped in the nuclear membrane, which is known to form a dynamic membranous structure providing a place for genetic expression. There is a possibility that terahertz waves generated by the activities of this nuclear membrane are involved in the regulation of this genetic expression. We are on the verge of discovering that the localization of chromosomal DNA within the nuclear membrane is involved not only in genetic expression, but also in maintaining the structure of the chromosome, and repairing DNA, among a variety of other functions[34]. In addition, irradiation with terahertz waves has been reported to regulate and promote sequence-specific genetic expression, and it is hoped that it will become possible to reprogram cells or control their differentiation using

terahertz waves[30].

3.2.2 Liquid crystal fields

A liquid crystalline state is a state which exhibits the anisotropy of crystals while simultaneously retaining the fluidity of a liquid state. The characteristics of liquid crystals, which are liquid yet exhibit a regular molecular arrangement as in crystals, are that they have the inherent structural flexibility of liquids while the molecules arrange themselves autonomously through self-organization. In addition, the molecular orientation and functions of substances that exhibit liquid crystal properties can be controlled through external fields such as the shape of the surface or electric and magnetic fields. For example, controlling the orientational order with an eternal field can artificially create disarray in the molecular orientation to allow targeted molecules to be moved freely within the liquid crystal, or allow communication of information by making use of the spread of orientational order.

Many liquid crystal states exist within the living body. As a helical polymer, DNA itself can enter a liquid crystal state as it was mentioned in a previous section. The biggest characteristic of DNA is that its orientational order can be controlled by the designing of artificial motifs with an even larger variety of three-dimensional structures through sequence programming, and use of the molecular shape[35]. Here we will report on research into finding practical applications for liquid crystals that take advantage of these characteristics of the DNA.

3.3 Application of biomolecules in substance transformation systems

Here we will examine initiatives to build substance transformation systems that make use of biomolecules in next-generation molecular systems that use DNA. Biomolecules taken out of the living body are removed from their complex support systems fostered in the process of evolution, resulting in a massive deterioration of their reaction efficiency or stability, and this hinders their application. The molecular smart system we report below makes use of the liquid crystal field in an effort to overcome these problems.

3.3.1 Substance transformation fields using biological functions

The substance transformation system using biological functions we report here is a non-cellular transcription and translation system. Synthesizing proteins using cells has many problems, namely, "synthesis is time-consuming," "synthesizable proteins are limited," "only small quantities of proteins can be synthesized," and "the time and cost required for synthesis make it inefficient." For these reasons, protein synthesis through non-cellular transcription and translation systems is indispensable in research into biological functions and drug discovery. At the Bio ICT Laboratory, we are aiming to apply the technology to environmental sensors that function outside cells, and artificial organelles that function inside cells. In the process we are developing an RNA aptamer synthesizing system that uses an artificial DNA motif known as a triple crossover (TX) tile with a molecular switch function[36][37]. As part of an idea for a ubiquitous sensor network (a network that carries out appropriate operations based on its own sensory information), this will become the fundamental technology for building an ad hoc network (self-distributing wireless network) through environmental intelligence (ambient intelligence). In addition, this technology can be applied to controlling cellular functions, enabling it to be used in a variety of fields. For example, if it is introduced into a stem cell, it can control cell differentiation, or if it is introduced into a microorganism, it can increase their productivity in making antibiotics or improve the productivity of bioenergy using photosynthetic bacteria. It can also raise the durability of plants to diseases and the environment in endophyte agriculture making use of symbiosis between plants and bacteria.

Aptamers incorporated into systems are nucleic acid molecules or peptide (a compound consisting of two or more amino acids joined by peptide bonds through dehydration synthesis) molecules that bind to a specific target molecule to change its level of activity. This system is designed to produce preprogrammed RNA aptamer molecules only when target molecules are present in the environment.

This reaction process can be divided into the two stages of target sensing using branch migration, and production of functional molecules through the transcription reaction. Modularization of the system with the aim of attaining higher functions is known to lower the efficiency of branch migration progress, while efforts to decrease the background noise of transcription lowers the efficiency of transcription. In order to overcome these problems we are currently working on promoting branch migration through the use of terahertz waves, and improving the efficiency of transcription through the use of liquid crystal fields. We will give a detailed explanation of this system below, and

report on efforts to overcome these problems.

3.3.2 The promotion of reactions using terahertz waves

TX tiles that make up our system are artificial motifs designed so that a triple layer structure is formed by the exchange of complementary strands at four crossover points by the four short, single strands of DNA (Fig. 1[38]). Incorporated into the tiles are sequences coding for RNA aptamers, promoter sequences needed for transcription, and sequences for recognizing targets. The tiles function as molecular switches (TX switch) that control the turning on and off of transcription. Targets inside the living body, which act as triggers for transcription, include biomarkers such as mRNA in saliva that reflect the entire DNA, and pieces of nucleic acids such as DNA in the natural environmental genetic pool. When the four single stranded DNA molecules are combined into a TX structure, the transcriptase (RNA polymerase) cannot access the promoter sequence, so the transcription switch is turned off. When the TX switch senses the target, the TX structure is programmed to undergo structural change to unravel itself through the exchange of complementary strands known as branch migration. This enables the transcriptase to access the promoter sequence, and turns on the transcription switch. In the model system, a malachite green aptamer was used for the RNA aptamer sequence incorporated into the TX switch. In the malachite green aptamer, the aptamer bonds to the organic pigment, malachite green, boosting its fluorescence a thousand fold [39], allowing the switching on of transcription to be confirmed by its fluorescence. RNA aptamers with the sought after functions can be selected from a random pool through molecular evolution known as in vitro selection. Aptamers have a variety of functions, from promoting to obstructing the activity of the target, and the targets that they bond to are also varied, ranging from biomolecules to metals. RNA aptamers are easy to design and synthesize compared to proteins, and because they bond specifically with their targets to change their level of activity, extensive research is being carried out into their use in drug discovery.

By combining the TX switch with an RNA aptamer to suit the purpose of its use, it is possible to create an environmental intelligence sensor that senses and gathers extracellular nucleic acids[40], which are thought to be a source of genetic evolution for microorganisms in the environment. Using the TX switch combined with a functional aptamer will enable the creation of a system in

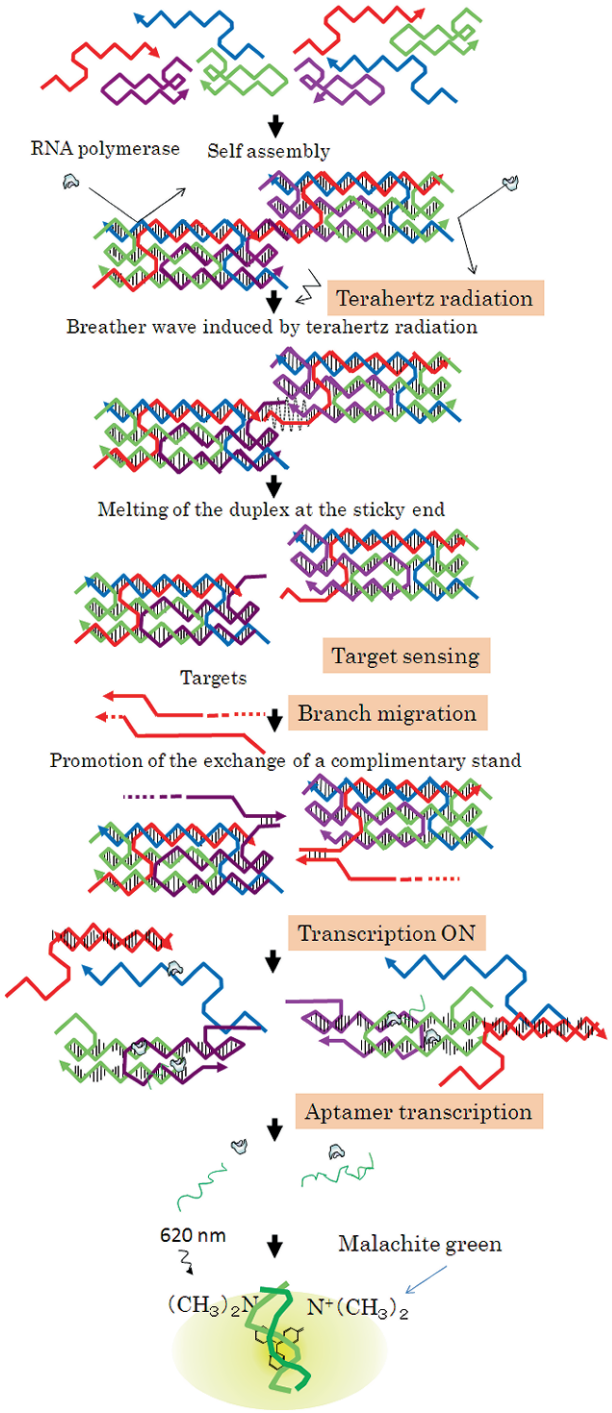

Fig.1 TX sensor

which it is introduced into a stem cell to gather information and trigger its differentiation into the target cell. It can also be used to develop an artificial organelle that can be incorporated into a photosynthetic bacteria to allow it to track the state of the cell, inhibit cell division, and ensure efficient production of hydrogen which can be used as bioenergy. In this way, functional aptamers incorporated into TX switches can provide vital systems on which the building of new devices can be based.

Augmenting the functions of systems with the aim of

developing such devices brings us face to face with issues that need to be resolved. For example, when an AND circuit that turns itself on when it recognizes multiple targets is incorporated into the system to enable more accurate control, it is necessary to link together modularized DNA motifs with different functions, but this makes it difficult for the target molecules to access the modules due to steric hindrance by the motifs, leading to limitation of branch migration which is a problem. Nevertheless, there are hopes that this problem can be overcome. Branch migration can be promoted by inducing a solitary wave (soliton) known as a breather wave on the DNA with terahertz waves (Fig. 1). According to theory, solitons cannot be generated when the proportion of G and C bases is high. We are therefore trying to build a simulation model that will enable testing of breather wave induction on DNA, as well as looking into sequences that induce solitons, irradiation energy, optimal frequency, irradiation time, optimization of the irradiation chamber, etc.

3.3.3 Molecular manipulation through reaction field designs

When the TX switch is turned on, the RNA aptamer sequence incorporated in advance is transcribed. In our model we used a fluorescent aptamer, allowing transcription to detect through its fluorescence, but it cannot eliminate fluorescence when the switch is turned off. This fluorescence is brought by the continuous transcription (so-called "transcription leak") in areas where the TX structure is loose. The accuracy of molecular switches is improved by preventing the continuation of transcription in the OFF state. When the sequence design of the TX switch is altered to lower the noise from transcription while the switch is turned off, it lowers the level of noise, but also leads to the problem of lowered transcription efficiency.

To improve transcription efficiency, it is necessary to increase the turnover efficiency of transcriptase. For example, in a non-cellular protein production system using X-DNA, or in other words an X-shaped artificial DNA motif, as the reaction field, it has been reported that reaction efficiency increases 300 times compared to a non-cellular system in a normal solvent[41]. The reason for the promotion of production efficiency is believed to be due to the higher concentration of DNA during transcription than in the normal solvent-based system, bringing the enzyme and target genes closer together to increase the enzyme turnover rate. Moreover, because during translation, the size of the protein complex of the translation system including the ribosome is bigger compared to the size of the network formed by X-DNA, it is trapped by the X-DNA network and prevented from diffusing, which is believed to be the reason of the turnover rate increase.

Here X-DNA is functioning as a biocatalyst that converts substances by making use of the functions of biomaterials and organisms. Isolated enzyme catalysts, microorganisms, plants, and animal cells are generally used as biocatalysts, but their advantages lie in the fact that "unlike rare metals such as platinum, used as complex catalysts, they are not a limited resource," "reactions proceed simply by mixing the biocatalysts with substrates," "compared to chemical catalysts, the conditions required for reactions to occur are milder, with reactions proceeding at around room temperature," and so on. When using artificial DNA motifs as biocatalysts, their advantage lies in the ease with which their detailed structures can be designed to enable them to function effectively as catalysts.

As is demonstrated in the example of X-DNA, building a reaction field by using an artificial motif that will allow the designing of finer spatial structures than in X-DNA motifs is hoped to increase turnover efficiency through regulation of the mobility of the small transcriptase, and overcome the TX switch problem of "lowered transcription efficiency brought by improvement in transcription leak." Furthermore, if this space could be created in the liquid crystalline phase, it will become possible to concentrate molecules in a predetermined reaction space using external fields such as electric or magnetic fields. In designing a liquid crystal molecule with artificial DNA motifs, we can control the polarity and magnetism of the l molecule by sequence programming. Organic compounds such as DNA that are able to form liquid crystals have aromatic rings and double bonds within their molecules, and they include polar groups or groups that show a large tendency toward polarization. A bias in the electric charge of the molecule is created depending on the positions and numbers of these polar groups, the symmetry of molecules, the extent of their conductivity indicating the extent of their polarity when placed in an electric field, etc. Because it is possible with DNA to design three-dimensional structures such as artificial motifs through sequence programming, it is also possible to control its polarity. This makes it easy to control the orientation of the molecule in an electric field.

In this way, designing artificial motifs enables the realization of a variety of functions that cannot be realized

with ordinary DNA molecules alone. For example, it has been reported that when molecules smaller than the liquid crystals are mixed into the liquid crystals, they autonomously gather around defective areas (areas of disarray in their orientation) of the liquid crystalline phase[42]. Making use of this characteristic, it is expected that it will become possible to increase reaction efficiency by mixing, e.g., transcriptase into the liquid crystalline phase made of artificial DNA motifs, and allowing it to concentrate autonomously in disorderly areas by controlling the distribution of orderly areas within the liquid crystal using electric or magnetic fields.

The main advantage of DNA is that it is easy to design three-dimensional molecular structures with polarity or different functions through sequence programming. DNA allows fine spatial designing of its structures, and it also has outstanding functions as a tool for designing reaction fields because it can be controlled through electric fields, magnetic fields, and terahertz waves.

3.3.4 Future issues

In this paper we have explained that as a biomaterial, DNA is not only a substance with unique functions and properties, but it also has outstanding features as material for molecular devices. In the development of next-generation molecular devices using biomaterials, our challenges include the development of organic semiconductor lasers and thin-film organic-solar cells. Compared to inorganic semiconductors such as silicon, the level of carrier mobility in transporting electrons and holes is small in organic substances, and it is not possible to pass large electric currents through them. This problem is one of the issues that needs to be resolved in the development of new devices.

For example, semiconductor lasers are generally made to emit light by passing a large current through a light-emitting diode (a semiconductor device that emits light when a voltage is applied in the forward direction) and trapping this light in a resonator to magnify it. It hardly emits light unless the electric current exceeds a certain threshold level. If it were possible to create a layer of regularly aligned molecules, for example, by dissolving them in a solvent and painting them onto a substrate, this would increase carrier mobility and enable a large current to be passed through it. It is expected that DNA overcome these problems using its characteristics as a liquid crystalline organic magnetic semiconductor material whose molecular orientation can be controlled by external fields, and the ease with which its three-dimensional structure can be designed by making use of its self-organization ability. On the other hand, it is believed to be possible to create an organic semiconductor laser by improving the efficiency of the laser resonator and lowering the electric current needed for oscillation. Adding a fluorescent pigment to liquid crystals with a helical periodic structure is known to induce laser oscillations as in distributed feedback resonators. This kind of precision structure can be created through DNA self-organization.

In this way, the characteristics of DNA are that it is a liquid crystalline organic magnetic semiconductor material, and it allows easy designing of three dimensional structures through self-organization. By using this DNA in combination with other organic semiconductors, there are large expectations for it to enable the realization of new functions that could not be realized by previous organic semiconductors.

The knowledge obtained from increasing the functionality of TX switches could also be used as fundamental technology for organic semiconductor lasers. Moreover, although it was not discussed in this paper, DNA is an integrated software-hardware information processing system. This feature means that during encryption, it is possible to supply true random numbers using a random process generated during calculations, with no need for an external random number source. Only pseudorandom numbers can be generated with a mathematical algorithm, so an essentially random physical process is necessary to generate true random numbers, but there were problems associated with such physical processes such as the fact that they cannot be generated in adequate quantities, or they were difficult to observe. On the other hand, using DNA enables the generation of physical random numbers when it is needed, in the required quantities. The random process can also be made visible allowing it to be observed directly, and this technology will lead to the development of a next-generation random number generator.

In these ways, DNA is expected to enable the building of innovative devices in a variety of fields as a superb molecular material. When using biomolecules as material for molecular devices, it is often difficult to build systems as robust and reliable as those of the living body, but to realize sufficient stability and reliability outside the living body, we will be able to build such systems by making full use of the characteristics of DNA.

4 Conclusions

Faced with the limit in miniaturization and integration of silicon devices, there is an urgent need to develop smart devices based on new materials and new processes that suppress energy consumption while enabling fast information processing. Here we described that DNA is a promising material for such devices, and we introduced the seeds of next-generation devices. All organisms make full use of DNA functions to realize extremely advanced systems. A characteristic of such biomolecules is that they are multi-functional. DNA has numerous outstanding properties. It is a material for molecular devices that will enable the realization of a variety of new functions to build next-generation molecular smart devices. Development is also being carried out into artificial nucleic acids, LNA (Locked Nucleic Acid)[43] and PNA (Peptide Nucleic Acid)[44], with bolstered functions, to improve the capability of DNA and RNA molecules themselves. Will artificial systems ever be able to exceed natural systems with their advanced functions created through evolution? Can we build smart systems based on new concepts that are not simply an extension of natural systems using the outstanding capabilities of DNA as functional molecules? At the Bio ICT Laboratory we aim to create a new information communication paradigm making full use of DNA. Toward this objective, we establish advanced technology for designing and controlling molecules and challenge developments in new-generation molecular devices through research of unknown DNA structural motifs and functions.

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