

Oligonucleotide Technology – A Research Platform for Chemical Biology

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Abstract: During the past three decades, considerable progress has been made in the field of oligonucleotide synthesis. Today, oligonucleotides and their derivatives can be prepared by highly optimised, automated procedures. They are used as sophisticated tools in fundamental research, as diagnostic reagents and as pharmaceutical compounds. In this paper, some applications of oligonucleotides are highlighted.

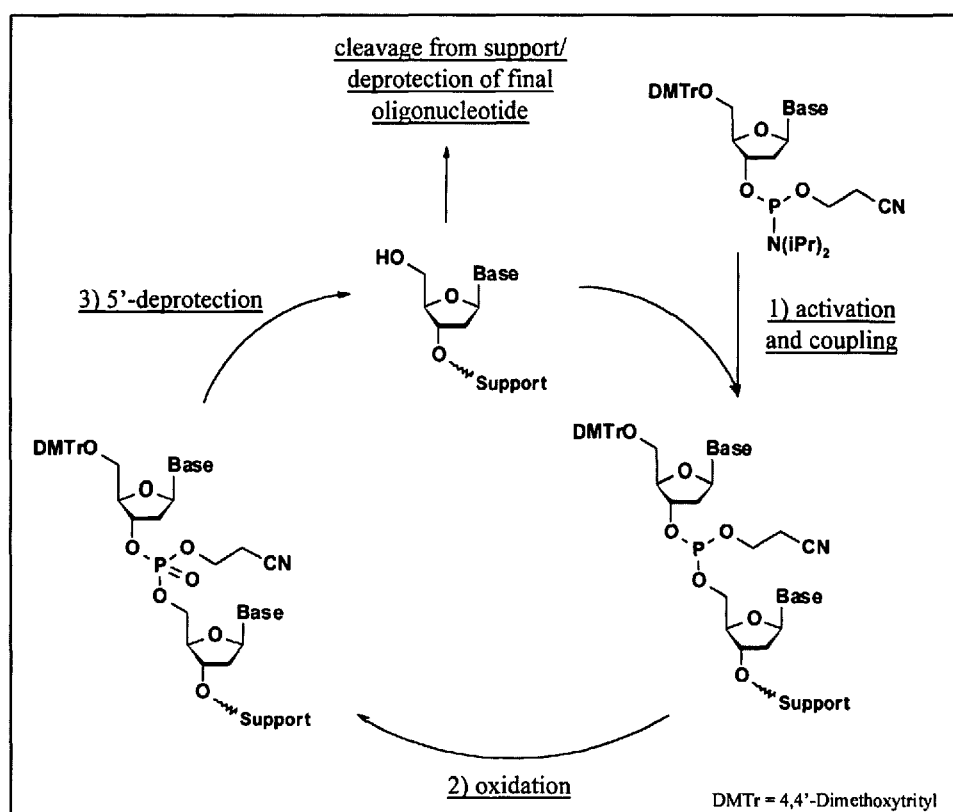
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Introduction

Soon after the discovery of the chemical structure of deoxyribonucleic acid (DNA) in 1953 [1], chemists started searching for methods for the *de novo* synthesis of nucleic acids. In the late 1960s and early 1970s, Khorana and co-workers developed procedures for the synthesis of oligodeoxynucleotides. These efforts resulted in the total synthesis of an *E. coli* tyrosine tRNA suppressor gene [2]. The synthesis, which was carried out in solution by the phosphodiester method, required several years of demanding work. Many features of the chemistry used for the synthesis of this 207 base pair long gene are still evident in today's highly developed protocols for oligonucleotide synthesis. In the ensuing years, solution synthesis was replaced by a solid support based method, and the original phosphodiester method was superseded, first by the phosphotriester and later by the phosphite triester method. In the early 1980s, phosphoramidite chemistry, introduced by Caruthers and coworkers [3] and later improved by Köster and coworkers [4], opened the way for automa-

tion [5]. Today, oligonucleotide synthesis is a high yielding, fully automated process (Scheme 1). Once the necessary building blocks are available, almost any type of oligonucleotide can be synthe-

sized in very short time, usually less than 24 h. In addition, the extremely high coupling efficiency (>98%) allows for the synthesis of oligonucleotides up to a length of 100 residues [6].



Scheme 1. Solid support synthesis of oligonucleotides by the phosphoramidite approach. The repetitive steps are: 1) activation and condensation of a 3'-phosphoramidite building block to the 5'-terminal residue of the support bound, growing chain; 2) oxidation of the intermediate phosphite triester to the phosphate triester; and 3) 5'-deprotection of the newly added building block. A further step that improves the quality of the synthesis consists in capping of deprotected, unreacted oligonucleotide (omitted here for clarity). After completion of the synthesis, the oligonucleotide is cleaved from the solid support and base protecting groups are removed.

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Antisense Oligonucleotides

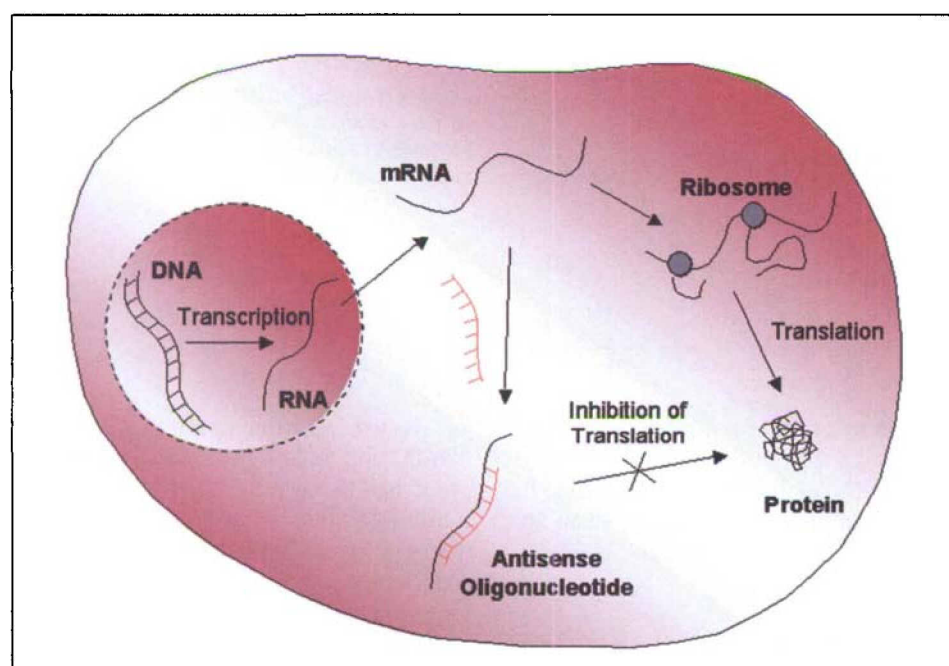
The discovery that short oligonucleotides can specifically inhibit cellular gene expression [7] triggered a decade of intensive research aimed at the design and synthesis of many different types of oligonucleotides (for review see [8–11]). As illustrated in Scheme 2, an oligonucleotide that is complementary to a part of a gene can bind to the cellular messenger RNA. Binding is highly specific and follows the Watson-Crick base-pairing scheme. By binding to its target, the antisense oligonucleotide prevents the trans-

lation of the messenger RNA into the encoded protein by the ribosomes.

Due to the existence of cellular nucleases, however, oligodeoxynucleotides are rapidly degraded in a biological environment and, therefore, of limited use. Chemical modification leads to resistance towards nucleolytic degradation and, hence, increased biological activity of antisense compounds. This finding resulted in the generation of a large number of oligonucleotide analogues modified at sugar, base or backbone (Fig. 1A). Besides conferring nucleolytic stability, the chemical modification of choice should

possess a number of other features, such as good hybridisation properties, good synthetic accessibility, cellular uptake and induction of RNase H, a cellular enzyme that cleaves RNA bound to the antisense oligonucleotide [12]. Some prominent types of modification fulfilling several of these aspects are shown in Fig. 2.

The goal of developing oligonucleotides into a new class of pharmaceutical compounds has substantially driven the field and resulted in the introduction of Vitravene™ in 1999 as the first antisense compound on the market (Fig. 3). Vitravene™ was developed for topical treat-



Scheme 2. Inhibition of gene expression by antisense oligonucleotide. Translation of the mRNA into protein is inhibited by binding of an oligonucleotide complementary to a region of the mRNA.

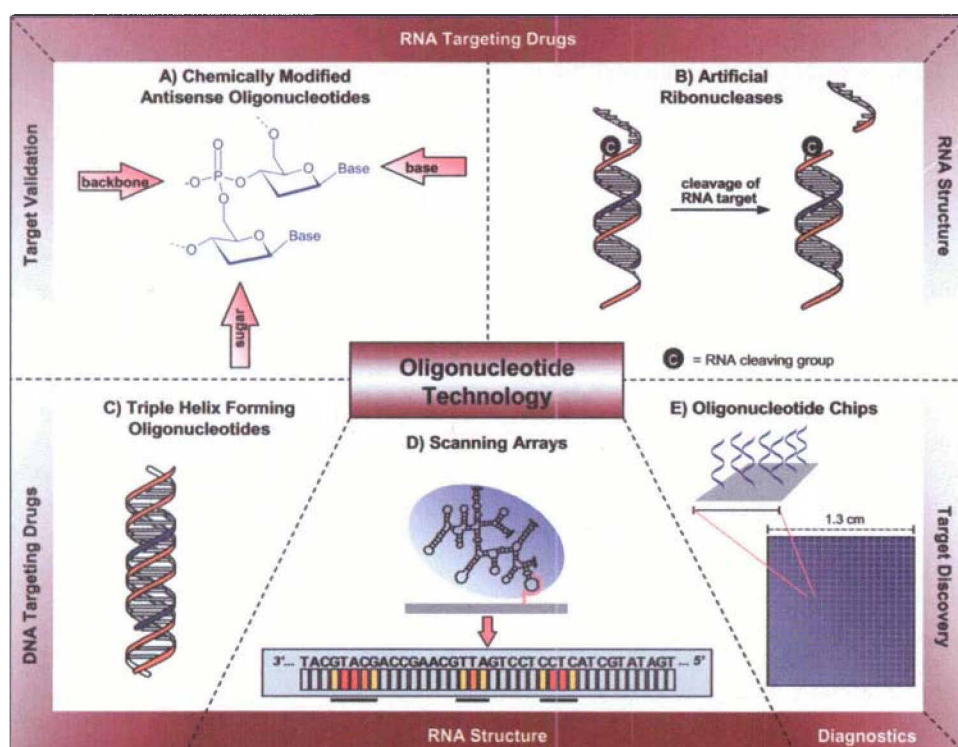


Fig. 1. Oligonucleotide technology as a research platform. Oligonucleotides find widespread use, e.g. as antisense compounds (A), as artificial ribonucleases (B), as triple helix forming oligonucleotides (C), for the preparation of scanning arrays (D) and in high-density oligonucleotide chips (E).

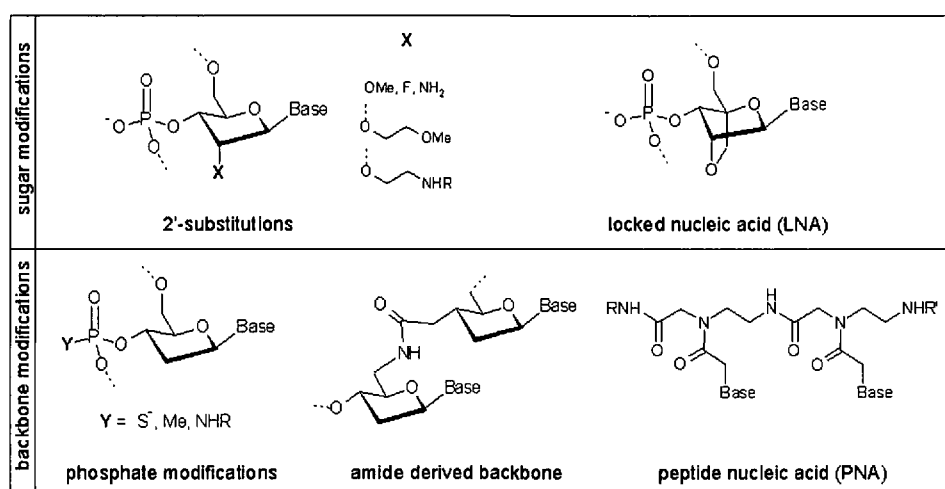


Fig. 2. Selected chemical modifications of oligonucleotides. Top: modifications of the ribose; for more detailed information see [13][14] (2'-methoxyethyl ribose), [15] (2'-aminoethyl ribose), and locked nucleic acid (LNA) [16]. Bottom: modifications of the backbone; amide backbone [17] and peptide nucleic acids (PNAs) [18]

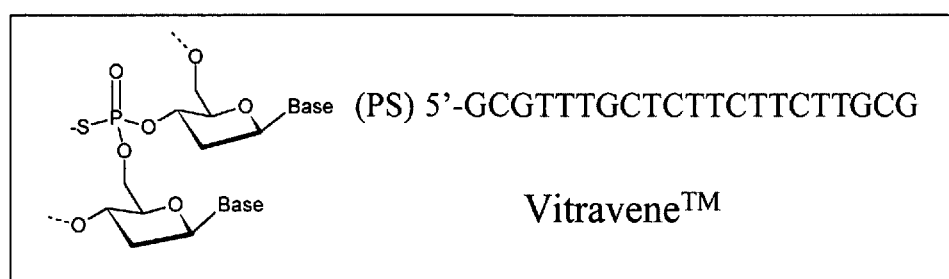


Fig. 3. Vitravene™, a 21mer phosphorothioate oligonucleotide is used for topical treatment of cytomegalovirus induced retinitis (Vitravene™ is a trademark of Novartis AG).

ment of cytomegalovirus (CMV) retinitis [19][20]. The phosphorothioate-modified 21-mer oligonucleotide is complementary to CMV mRNA. The mechanism of viral inhibition is consistent with an antisense mechanism, though other mechanisms may contribute to the antiviral activity [20].

Apart from their pharmaceutical potential, antisense oligonucleotides are powerful research tools in academia as well as in industry. As a method for specifically interfering with the expression of a single gene in a biological system, antisense can be of invaluable help in identifying and validating therapeutically relevant targets that emerge from genome-based research [21]. While problems due to non-specific interactions of modified oligonucleotides with molecules other than the targeted mRNA limit – to a certain extent – the generality of the approach, the simplicity and speed of the method are, indisputably, substantial advantages. Not surprisingly, antisense technology belongs to today's standard repertoire of genomics-oriented companies [21–23].

It needs to be mentioned that, in addition to being tools and drugs targeting genetic information, sugar modified oligonucleotides have been used to address the fundamental question of the origin of nucleic acid structure. For example,

Eschenmoser and co-workers have synthesised an array of different pyranosyl-derived RNA congeners to determine the rules dictating the structure of nucleic acids (for a recent review see [24]).

Triple Helix Forming Oligonucleotides

Parallel to the work aimed at developing antisense (or RNA targeting) oligonucleotides, an intensive research effort has also been devoted to the synthesis and evaluation of triple helix forming (or DNA targeting) oligonucleotides (Fig. 1C). After the first demonstration of sequence specific binding of oligonucleotides to homopurine stretches of DNA by formation of *Hoogsteen* hydrogen bonds [25–27], several modifications of oligonucleotides were described which bind to double stranded DNA with high specificity and affinity [15][28]. DNA may offer some fundamental advantages over RNA as a biological target. In particular, DNA exists in a lower number of copies per cell and generally has a longer life time than RNA. Consequently, the inhibition of gene expression at the level of DNA should have a higher biological impact than inhibition of the same gene at the RNA level. In addition, DNA binding can interfere with several processes, such

as replication, transcription, recombination, and repair mechanisms. On the other hand, there are still a number of limitations that have to be overcome (recognition of mixed sequences; DNA accessibility and binding under physiological conditions) [29–31]. Therefore, application of the triple helix approach in biological systems is currently less advanced than the use of antisense oligonucleotides.

Artificial Nucleases

While the number of different oligonucleotide building blocks is already high, the variety can be further increased by the addition of chemical and biological functionalities onto the oligonucleotides. The properties of oligonucleotides can be altered by covalently attaching lipophilic or polar moieties, different types of peptides, enzymes, dyes or intercalators [32][33]. In particular, reactive groups have been linked to oligonucleotides to cleave nucleic acids in a sequence specific way. The latter group has been exploited especially for the purpose of sequence-specific cleavage of nucleic acids. Such conjugates have been described as artificial nucleases and find applications as rare cutters of large genomic DNAs [34] or as tools for structur-

al mapping of nucleic acids (see *e.g.* [35]). In addition, they also have potential as drugs aimed at destroying DNA and RNA targets [36][37]. Cleavage of DNA targets commonly involves the use of redox-based systems (transition metal derivatives) [38], resulting in oxidative damage and cleavage of the DNA backbone. RNA cleavage, on the other hand, is achieved using either Lewis acids (lanthanides, transition metals) [39–43] or acid-base catalysts (oligoamines, imidazoles) [44] (see Fig. 1B for illustration). This type of cleavage follows a hydrolytic pathway. RNA is intrinsically more susceptible towards phosphodiester hydrolysis than DNA. This is due to the presence of the 2'-hydroxyl group in RNA, which acts as an internal nucleophile in the transesterification reaction. Recent efforts in this field resulted the development of artificial ribonucleases acting with multiple turnover [45] as well as a successful application in a cellular system [46].

Oligonucleotide Arrays

The combination of specificity (Watson-Crick base pairing) and simplicity (only four bases) renders oligonucleotides highly attractive as diagnostic and research tools. Long before pharmaceutical interest arose, oligonucleotides had been used as gene specific probes. Their use as probes includes applications such as *in situ* hybridisation, the polymerase chain reaction (PCR) or, lately, gene chips. The latter are composed of large arrays of oligonucleotides covalently attached to an

appropriate substrate [47][48]. Such gene chips allow the identification and quantification of specific nucleotide sequences. Since their production is highly automated, these chips are destined to become mass ware and will find their place in every life science laboratory [49]. Hundreds of thousands of different oligonucleotide probes can be either synthesized or post synthetically placed on an area as small as a fingernail in a spatially defined manner (Fig. 1E). Since the technology is amenable to high-throughput genomic applications, it will facilitate techniques such as differential expression profiling [50–52] or single nucleotide polymorphism (SNP) mapping [53]. Today, high-density oligonucleotide chips are mostly still made of unmodified oligonucleotides, but future needs may require analogues. First steps in this direction have already been taken and chips composed of peptide nucleic acids (PNAs) rather than oligodeoxynucleotides have been reported [54].

A special type of array is represented by oligonucleotide scanning arrays. Scanning array technology is an innovative approach to study RNA structure and was introduced by E. Southern and co-workers [55]. This method enables, in a very straightforward way, the analysis of hybridisation-accessible sites of the RNA molecule of interest. The construction of such 'scanning arrays' is surprisingly simple and has been described in detail [55]. Briefly, classical oligonucleotide synthesis is carried out on a carrier surface (such as glass or polypropylene) using *e.g.* a rectangle-shaped reaction cell (see Fig. 4). After covalent attachment of

the first nucleotide to the surface, the reaction cell is shifted along one of its sides by a given increment, followed by attachment of the second nucleotide. Repetition of this procedure (shift of the reaction cell, nucleotide attachment) gives rise to an oligonucleotide array as shown in Fig. 4, each of the bands representing a different oligonucleotide. If the synthetic sequence is complementary to a given RNA target, the final array will contain all possible oligonucleotides which are complementary to said target. Two neighbouring oligonucleotides are called shiftmers, since they are complementary to two overlapping sites of the RNA target, which are shifted relative to each other by one single nucleotide. By hybridisation of the RNA target to the scanning array, a binding pattern is obtained which is dependent on the structure of the target (Fig. 1D).

The method does not allow the determination of structural details at high resolution, but it does allow rapid identification of accessible sites on the RNA in a very reliable, reproducible and straightforward manner. The identification of accessible regions of an RNA molecule is of importance for the design of RNA targeting drugs [56]. It also allows detection of structural changes after binding of a small molecule, an oligonucleotide or even a larger biomolecule to an RNA target, thereby providing a simple and efficient method to gain insight in drug-RNA binding interactions. The understanding of such interactions will yield valuable information for the design of new drugs targeting bacterial or viral RNAs.

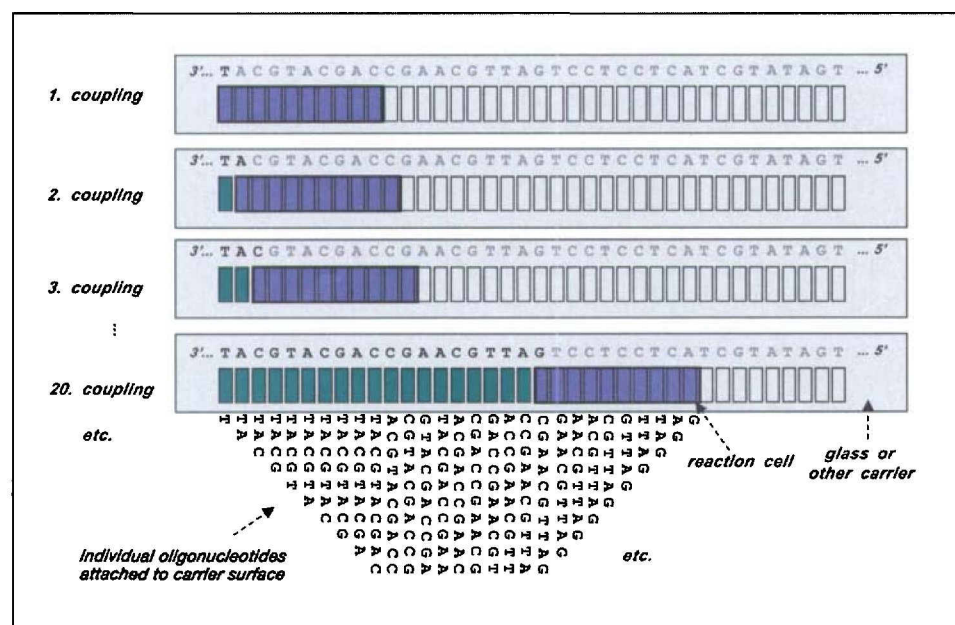


Fig. 4. Preparation of an oligonucleotide scanning array. After each monomer-coupling cycle, the reaction cell is displaced by a certain increment. In this way, all possible complementary oligonucleotide (here arbitrarily 10-mers) against a given RNA target are prepared in a sequential, combinatorial way on a solid surface.

Conclusions

Oligonucleotides are essential tools for research in chemical biology. The range of applications varies from their use as standard primers to highly sophisticated gene probes; even pharmaceutical usefulness has been demonstrated. The combination of oligonucleotide chemistry with other high technology sectors, such as nanotechnology, automation and information technology, will create further applications in the future. In particular, due to their ability to recognize genetic information, oligonucleotides will be indispensable tools for genomic research.

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