Synthesis of Long Double Stranded Concatemer Molecules by Self-Association of Short Single Stranded DNA Sequences

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In this work we reported synthesis of long (from hundreds to thousands base pairs) linear and circular double stranded (ds) concatemers composed of tandem oligonucleotide repeats. The concatemers were produced following a method published [1] almost 40 years ago from 5'-end phosphorylated half-complementary decameric or hexameric oligonucleotides (Fig. 1, left panel) as well from the oligonucleotides containing modified nucleic bases or mismatched ones. Using the above method we synthesized various uniform linear DNA polymers, including telomeric DNA, as well as long (kbp) repeating single stranded DNA

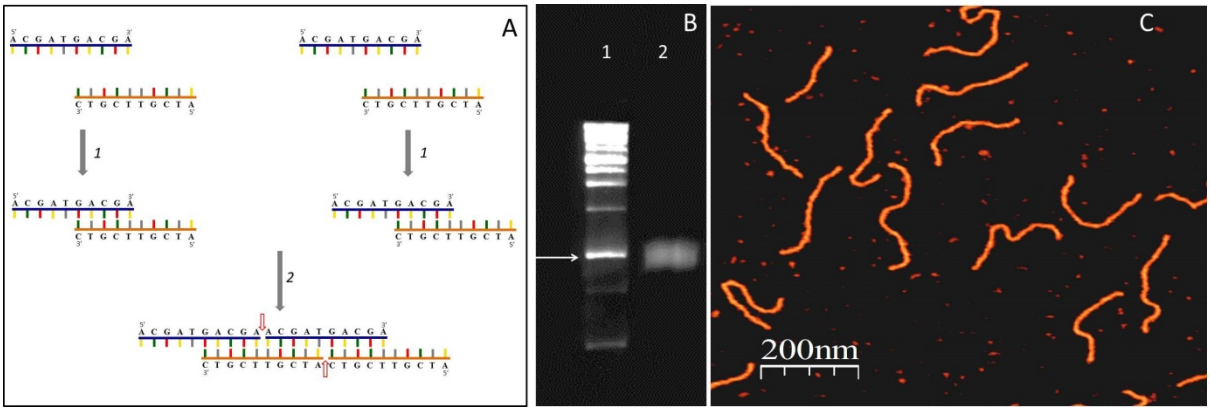

Figure 1. A. - Schematic illustration of assembling of two single stranded decameric half-complementary oligonucleotides into a ds nucleic acid polymer. 1 – Hybridization of complementary fragments at 3'-ends of the oligonucleotides. 2 – Binding of the oligonucleotide dimers (step 1) to each other. Association of dimers with tetramers and structures composed of many oligonucleotide fragments being formed yields long concatemeric molecules. The oligonucleotides composing the polymer are covalently connected by DNA ligase (red arrows). Gel Electrophoresis (B, lane 2) and AFM imaging of DNA concatemers, assembled from a pair of half-complementary decameric 5'-end phosphorylated deoxyribonucleotides, ACGATGACGA and ATCGTTCGTC. The gel was stained with ethidium bromide. Lane 1 is a 1 kb DNA-ladder (the band corresponding to 1000 bp DNA is indicated by the white arrow). AFM was performed on molecules adsorbed on muscovite mica in a semicontact (tapping) mode.

molecules. The advantage of the method is that it enables incorporation of artificial nucleotides along the whole length of the DNA. We have synthesized long (hundreds of nanometers) concatemeric dsDNA molecules containing: methylated cytosine nucleotides, fluorescent dyes-labeled nucleotides and mismatched base pairs (Fig. 2). The modified nucleotides can be introduced into one or both strands composing the double helical polymer. The frequency of occurrence of the modified nucleotides in the DNA can be varied. We have prepared concatemers comprising a mismatched pair of bases per every 5 or 10 base pairs in the sequence. The latter molecules cannot be synthesized either by chemical methods (due to

the length limitation) or by DNA polymerase. The DNA molecules comprising reactive groups capable for anchoring metal particles or/and other redox active elements along the nucleic acid polymer can be used as wires or transistors in future nanoelectronic devices and circuits.

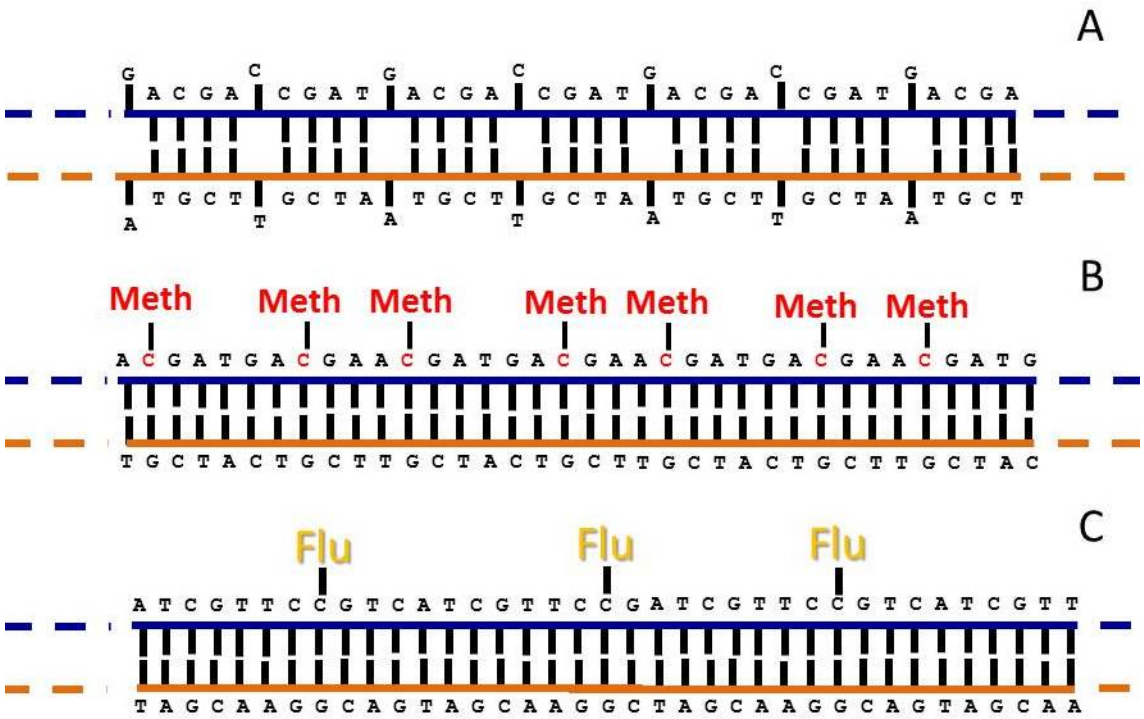

Figure 2. A. - Schematic drawing of ds concatemers prepared as illustrated in Fig. 1A from decameric oligonucleotides containing mismatches (A), 5-methilcytosine nucleotides (B) and a Fluorescein-modified nucleotides.

References.

[1] Z.A. Shabarova, N.G. Dolinnaya, S.I. Turkin, E.S. Gromova., *Nucleic Acids Res*., **8**, 2413-2429 (1980).