

THE USE OF TOPOLOGICAL PROPERTIES OF DNA

A New Weapon Against Pathogen Agents

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The Bertin Company is one France's flagships in research and development. Recognition is owed to it for its experiments with the famous hover train which links Paris to Orléans. New and now well-known innovations were born in its research and development unit. Among them one counts applications in the nuclear, space aeronautics and defense fields.

About ten years ago, in close cooperation with the famous Genethon, the Bertin Company set up a subsidiary dedicated to biotechnologies. This subsidiary is called Labimap. Engineers at Labimap have successfully automated the key techniques of the laboratory of molecular biology. These techniques include: DNA electrophoresis and membrane transfer, DNA extraction, automated sequencing and polymerase chain reaction. When they first came out about ten years ago these automation advances knew no competition on the market. Thanks to the tireless performance of such robots, Genethon was able to score several points in its fight against genetic diseases. (Figure 6).

Polymerase Chain Reaction

Recent news alerts us to the urgent need for rapid and powerful techniques to identify pathogen agents. Among the methods used by laboratories Polymerase Chain Reaction (PCR) is in a leading position. Following its invention in 1986, Kary Mullis, a young US researcher was awarded a Nobel Prize. PCR allows for the duplication of a strand of DNA (deoxyribonucleic acid) up to millions of times so that it can be easily detected. As each fragment of DNA is specific to the organism which produced it, the PCR method makes it possible to precisely identify very low quantities of bacteria or viruses.

The PCR method, however, requires costly reagents. Specific enzymes must be used. They are extracted from heat resistant bacteria found in hot springs. Such bacteria are brought back from the bottom of oceans, using bathyscaphes during geophysical explorations. Worthwhile molecules were then cloned in order to produce genetically modified bacteria. To implement PCR one also needs complex apparatus, which being patented, is sold at high prices. As a result, the PCR technique is rarely used in developing countries though it would be most useful there.

Palindromic DNA

Researchers at Labimap have managed to devise a DNA duplication technique somewhat similar to PCR but which can take place at body temperature. Unexpectedly the classical poetic form known as palindroma inspired Labimap's scientists. A palindroma is a verse which can be read in any direction as in the following examples:

"A man, a plan, a canal, Panama."

"Madam, I'm Adam."

The inventor of Palindroma was Sotadès of Maronéa who lived in Ptolemaic Egypt. He was a mathematician and a satirical poet, a sort of Ancient Rabelais. He paid dearly for his daring speech. The Pharaoh Philadelphus had him rolled into a lead sheet and thrown into the sea. Only a few minor works of his have lived through to our times.

Palindromas do fascinate mathematicians. For instance the date of 20 February 2002 is a number palindroma (20022002). The next date palindroma will be 11 February 2011(1102211). Classical musicians were also keen on palindromas. Johan Sebastian Bach's two-voice canon entitled *Offrande Musicale* and known as *Cancrizans*, i.e. the crayfish is made up of two

symmetrical parts: a melodic line and then the same melodic line repeated backwards. Thus it forms a musical palindroma.

The sequence of a DNA strand is made up of a series of four nucleic bases: A (Adenine), T (Thymine), G (Guanine) and C (Cytosine). These bases complement one another on a two by two basis. A links with T and G links with C. These four bases can be arranged in a palindroma-like sequence. When such is the case, the DNA strand spontaneously bends into a 'hairpin' shape. (Figure 1).

The topological specificity of the molecule of deoxyribonucleic acid can thus be successfully used. Polymerase enzymes can use the 'hairpin-shaped' DNA strand as primers which are required to duplicate the DNA. (Figure 2)

Once primed, the DNA duplication reaction can carry on until reagents (triphosphate nucleotides) have been exhausted. In this manner ten thousands of daughter molecules can be made from one DNA molecule. Needless to say that this reaction is a specific one: the DNA goes through polymerase and duplication only in so far as it is complementary to part of one of the two palindromic primers. This works much in the same way as a signal from a radio broadcasting station, which is amplified only if the frequency of the resonating circuits is tuned to that of the carrier wave. This reaction takes place at a constant temperature. Within a few hours, starting from micro-quantities of DNA equivalent to a few molecules, one can observe the appearance of duplicated DNA which is visible to the human eye in the presence of fluorescent stains. (Figure 3)

Thus one can detect DNA which was duplicated through specific hybridation thanks to a tracer identified by a radioelement. (Figure 4).

'In situ' duplication is a most interesting application of this technique. One refers to 'in situ' duplication when a polymerase chain reaction occurs in cells which have been placed onto a microscope slide. This technique is used to bring out cells which have been infected by viruses.

Unfortunately this technique can be tricky to implement. Cells are heated and cooled down at 95°C and 0°C in alternate fashion several tens of time. Then these fragile cells are placed onto slides where they have a tendency to float and detach themselves in the buffer solution. When adhesion to the slide is maintained, these delicate cellular structures often lose their shape when undergoing a process which is more akin to steam cooking than classic microscopic coloring. This is to be regretted for *in situ* duplication is, in theory, a promising technique. To be able to specifically color virus-infected cells is an age-old dream of microscopic observers. That is why many teams are working eagerly to improve results in this area.

Isothermal duplication brings a sizeable improvement. Operations are carried out at constant temperature. The temperature can be regulated by acting on the length and the sequence of the DNA fragments. Moreover, the highly reacting products are strands of DNA whose molecular weight is high and thus they do not escape out of the positive cells PCR products as often do. Rather than pale-colored cells surrounded by a halo, one can expect to observe clearly contrasted cells thanks to fluorescence and this, in a reproducible fashion.

In practice the method used is as follows: after fixation and possibly a first staining a drop from the buffer solution containing the four triphosphate nucleotides (dATP, dTTP, dGTP, dCCPT), a thermo stable DNA polymerase enzyme, ions required to guarantee the reaction and possibly a few additives. A fluorescent molecule marks one out of the four nucleotides. Small plastic lenses the size of an eye lenses are placed on the drop so as to

