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The Unusual Origin of the Polymerase Chain Reaction

A surprisingly simple method for making unlimited copies of DNA fragments was conceived under unlikely circumstances—during a moonlit drive through the mountains of California

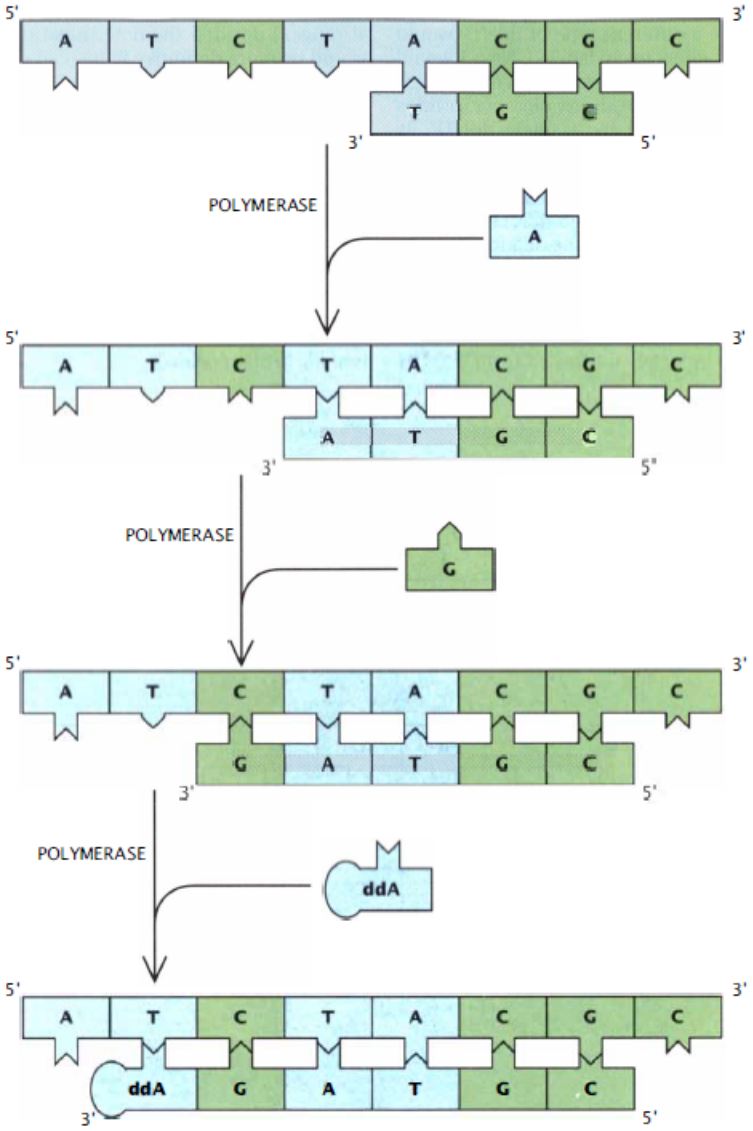
by Kary B. Mullis

Sometimes a good idea comes to you when you are not looking for it. Through an improbable combination of coincidences, naiveté and lucky mistakes, such a revelation came to me one Friday night in April, 1983, as I gripped the steering wheel of my car and snaked along a moonlit mountain road into northern California's redwood country. That was how I stumbled across a process that could make unlimited numbers of copies of genes, a process now known as the polymerase chain reaction (PCR).

Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute: it requires no more than a test tube, a few simple reagents and a source of heat. The DNA sample that one wishes to copy can be pure, or it can be a minute part of an extremely complex mixture of biological materials. The DNA may come from a hospital tissue specimen, from a single human hair, from a drop of dried blood at the scene of a crime, from the tissues of a mummified brain or from a 40,000-year-old woolly mammoth frozen in a glacier.

In the seven years since that night, applications for the PCR have spread throughout the biological sciences: more than 1,000 reports of its use have been published. Given the impact of the PCR on biological research and its conceptual simplicity, the fact that it lay unrecognized for more than 15 years after all the elements for its implementation were available strikes many observers as uncanny.

The polymerase chain reaction makes life much easier for molecular biologists: it gives them as much of a particular DNA as they want. Casual discussions of DNA molecules sometimes make them sound like easily obtained objects. The truth is that in practice it is difficult to get a



DNA POLYMERASE, an enzyme, can lengthen a short strand of DNA, called an oligonucleotide primer, if the strand is bound to a longer "template" strand of DNA. The polymerase does this by adding the appropriate complementary nucleotide to the three-prime end of the bound primer. If a dideoxynucleotide triphosphate (ddNTP) such as dideoxyadenine (ddA) is added, however, no further extension is possible, because the three-prime end of the ddA will not link to other nucleotides.

What I did not realize at the time was that there were many good reasons why my sequencing idea could not work. The problem was that oligonucleotides sometimes hybridize with DNA sequences other than those intended; these unavoidable pairings would have made my results ambiguous. Even in the hands of those skilled in the art of careful hybridization, it was impossible to bind oligonucleotides to whole human DNA with sufficient specificity to get anything even approaching a meaningful result.

were somewhere near the cutting edge of DNA technology.

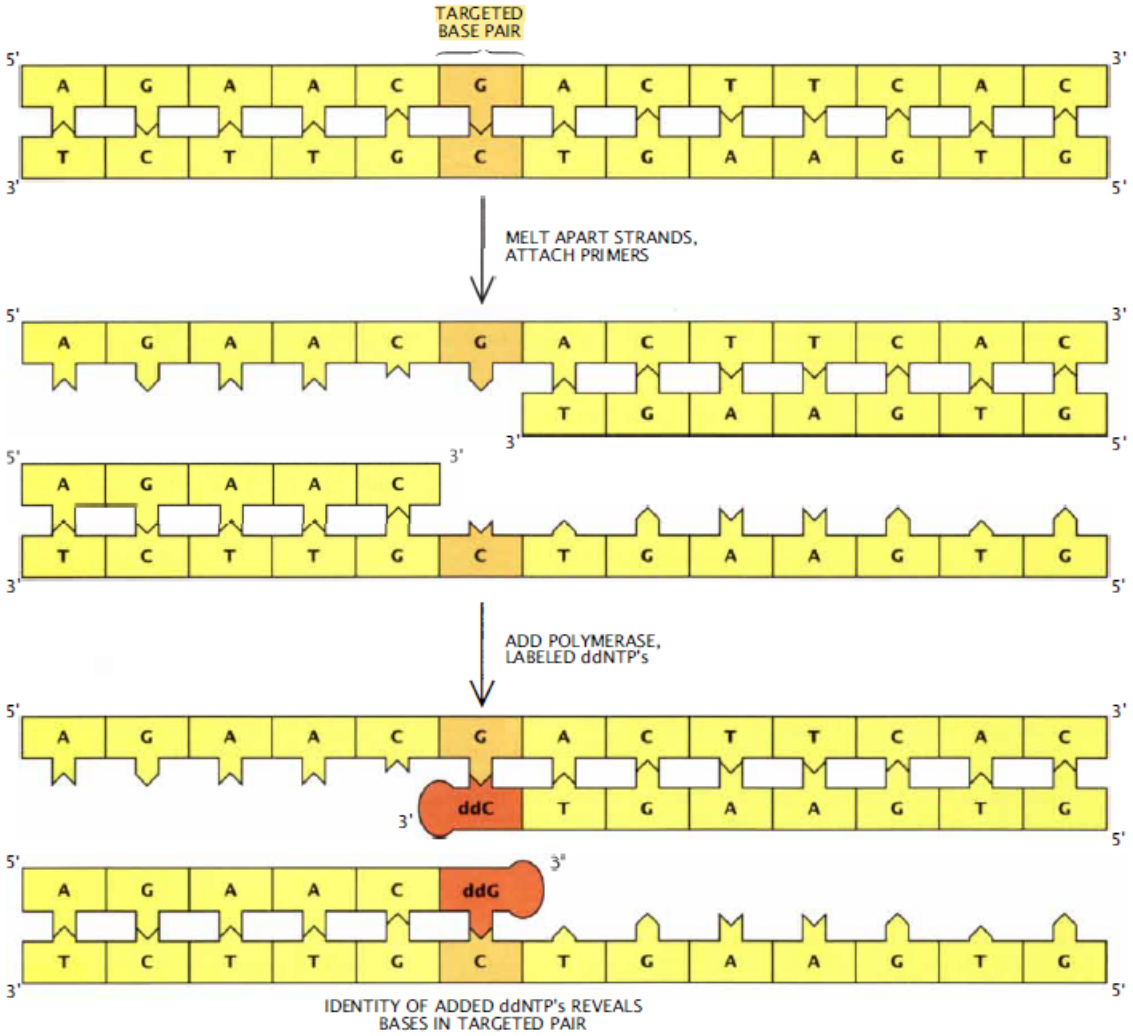
One Friday evening late in the spring I was driving to Mendocino County with a chemist friend. She was asleep. U.S. 101 was undemanding. I liked night driving; every weekend I went north to my cabin and sat still for three hours in the car, my hands occupied, my mind free. On that particular night I was thinking about my proposed DNA-sequencing experiment.

My plans were straightforward. First I would separate a DNA target into single strands by heating it. Then I would hybridize an oligonucleotide to a complementary sequence on one of the strands. I would place portions of this DNA mixture into four different tubes. Each tube would contain all four types of ddNTP's, but in each tube a different type of ddNTP would be radioactively labeled. Next I would add DNA polymerase, which would extend the hybridized oligonucleotides in each tube by a single ddNTP. By electrophoresis I could separate the extended oligonucleotides from the residual ddNTP's; by identifying which radioactively labeled ddNTP had been incorporated into the oligonucleotide, I could determine the corresponding complementary base in the target strand. Simple.

Around Cloverdale, where California 128 branches northwest from U.S. 101 and winds upward through the coastal range, I decided the determination would be more definitive if, instead of just one oligonucleotide, I used two. The two primers would bracket the targeted base pair I hoped to identify. By making the oligonucleotides of different sizes, I would be able to distinguish them from each other. By directing one oligonucleotide to each strand of the sample DNA target, I could get complementary sequencing information about both strands. The experiment would thereby contain an internal control at no extra inconvenience [*see illustration below*].

Although I did not realize it at that moment, with the two oligonucleotides poised in my mind, their three-prime ends pointing at each other on opposite strands of the gene target, I was on the edge of discovering the polymerase chain reaction. Yet what I most felt on the edge of was the mountain road.

That night the air was saturated with moisture and the scent of flowering buckeye. The reckless white stalks poked from the roadside into the glare of my headlights. I was thinking about the new ponds that



TO DETERMINE THE IDENTITY of a targeted base pair in a piece of DNA, the author hoped to apply a variation on a technique called dideoxy sequencing. First two primers would be bound to the opposing strands in the DNA at sites flanking the targeted pair. DNA polymerase and dideoxynucleotide triphosphates (ddNTP's) would then be added to the mixture, which would allow each of the primers to be extended by only one base. The identity of the added ddNTP bases would reveal what the complementary targeted bases were. The technique could work with only one primer, but the use of two would provide a control for checking the results. Planning this experiment led the author to the polymerase chain reaction.

From my postdoctoral days in Wolfgang Sadee's laboratory at the University of California at San Francisco, where John Maybaum was devising clinical assays for nucleotides, I remembered that my DNA samples might contain stray traces of nucleotide triphosphates. It would complicate the interpretation of the gel, I figured, if stray nucleotides introduced with the sample added themselves to the three-prime end of the primers before the planned addition of the labeled ddNTP's.

One thought I had was to destroy any loose nucleotide triphosphates in the sample with alkaline phosphatase, a bacterial enzyme. This enzyme would chew the reactive phosphate groups off any nucleotide triphosphates, thereby rendering them inert to a polymerase reaction. Yet I would then somehow have to eliminate the phosphatase from the sample, or else it would also destroy the ddNTP's when I added them. Normally one can deactivate unwanted enzymes by heating them and altering their essential shape; I believed, however, bacterial alkaline phosphatase could refold itself into its original form. I therefore rejected alkaline phosphatase as an answer to the problem.

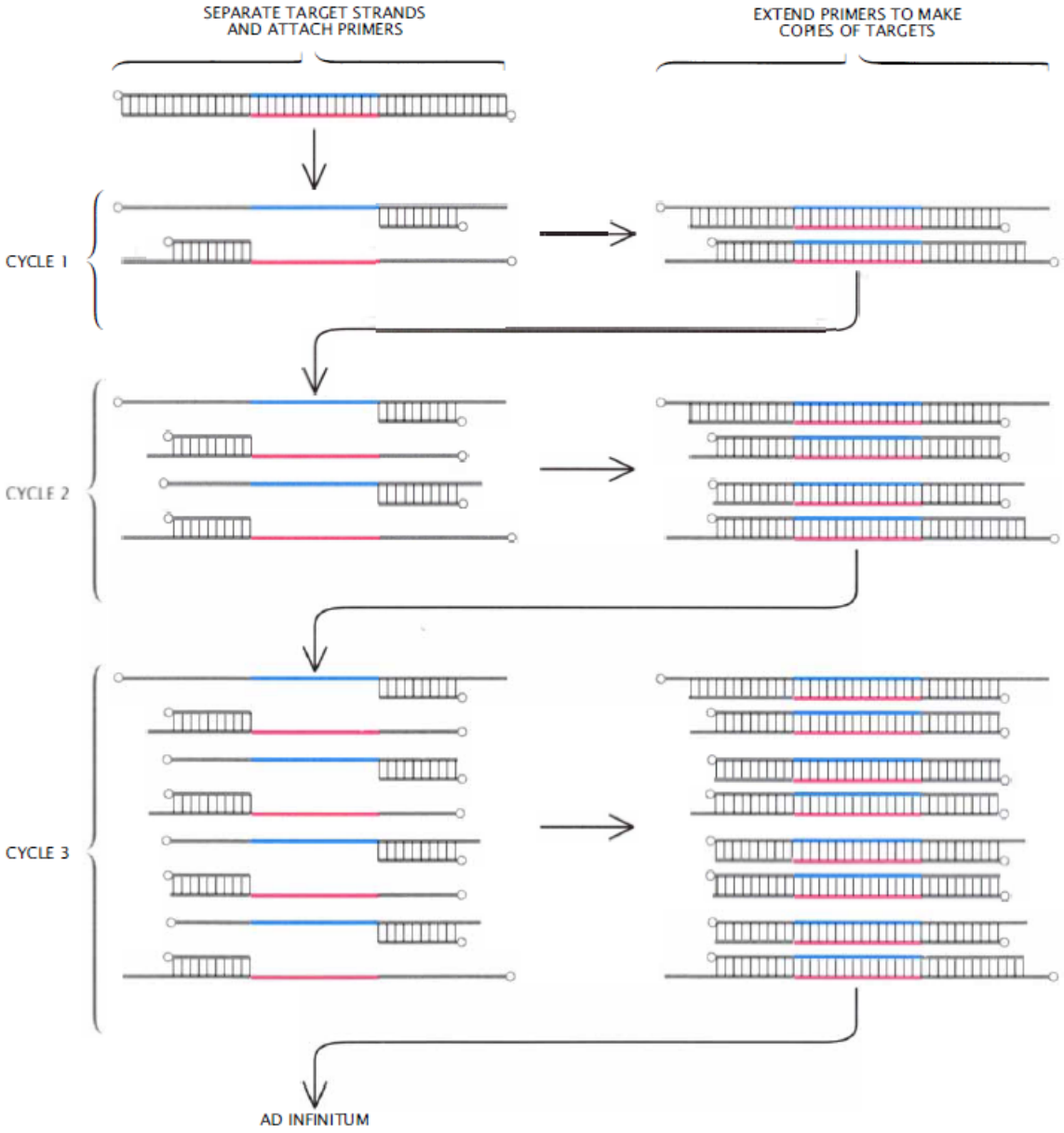
I was, in fact, mistaken. Much later I learned that alkaline phosphatase can be irreversibly denatured by heating if no zinc is present in the solution. As it turned out, my mistake was extraordinarily fortunate: had I known better, I would have stopped searching for alternatives.

I reasoned that if there were enough nucleotides in the sample to interfere with the experiment, there would also be enough for the DNA polymerase to act on. By running the sample through a kind of preliminary mock reaction with oligonucleotide primers and polymerase but without ddNTP's, I could easily deplete any nucleotides in the mixture by incorporating them into the extending oligonucleotides. Then, by raising the temperature of the sample, I could separate the extended oligonucleotides from the DNA targets. True, the extended oligonucleotides would still be in the sample; but because there would be far more unextended primers than extended ones in the mixture, the DNA targets would probably hybridize with unextended primers when the mixture cooled. I could then add ddNTP's and more polymerase to perform my sequencing experiment.

Yet some questions still nagged at me. Would the oligonucleotides extended by the mock reaction interfere with the subsequent reactions? What if they had been extended by many bases, instead of just one or two? What if they had been extended enough to create a sequence that included a binding site for the other primer molecule? Surely that would cause trouble....

No, far from it! I was suddenly jolted by a realization: the strands of DNA in the target and the extended oligonucleotides would have the same base sequences. In effect, the mock reaction would have doubled the number of DNA targets in the sample!

Suddenly, for me, the fragrance of the flowering buckeye dropped off exponentially.



POLYMERASE CHAIN REACTION is a cyclic process; with each cycle, the number of DNA targets doubles. The strands in each targeted DNA duplex are separated by heating and then cooled to allow primers to bind to them. Next DNA polymerases extend the primers by adding nucleotides to them. In this way, duplicates of the original DNA-strand targets are produced.

Under other circumstances, I might not have recognized the importance of this duplication so quickly. Indeed, the idea of repeating a procedure over and over again might have seemed unacceptably dreary. I had been spending a lot of time writing computer programs, however, and had become familiar with reiterative loops—procedures in which a mathematical operation is repeatedly applied to the products of earlier iterations. That experience had taught me how powerful reiterative exponential growth processes are. The DNA replication procedure I had imagined would be just such a process.

Excited, I started running powers of two in my head: two, four, eight, 16, 32.... I remembered vaguely that two to the tenth power was about 1,000 and that therefore two to the twentieth was around a million. I stopped the car at a turnout overlooking Anderson Valley. From the glove compartment I pulled a pencil and paper—I needed to check my calculations. Jennifer, my sleepy passenger, objected groggily to the delay and the light, but I exclaimed that I had discovered something fantastic. Nonplussed, she went back to sleep. I confirmed that two to the twentieth power really was over a million and drove on.

For the next few months I continued to study and refine the PCR with the help of Fred A. Faloona, a young mathematics wizard whom I had met through my daughter. Fred had helped me with the first PCR experiment by cycling the DNA mixture—in fact, that had been his very first biochemistry experiment, and he and I celebrated on the night of its success with a few beers.

In the following months we confirmed that the PCR would work on larger and larger fragments of plasmid DNA. Eventually we obtained some human DNA from Henry Erlich's laboratory and produced evidence for the amplification of a fragment from a single-copy gene.

Today many of the initial hitches or inefficiencies of the PCR have been worked out. Several slightly different protocols are now in use. I usually recommend that the DNA samples be

BACKGROUND

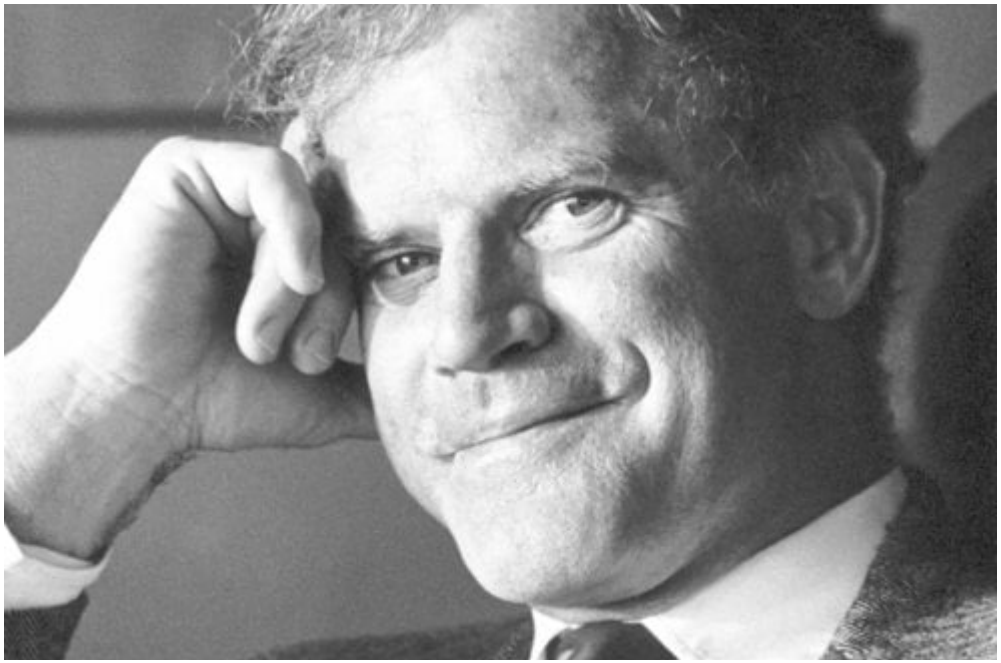


Photo is taken Nobel Foundation archive

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THANK YOU