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The Polymerase Chain Reaction (PCR) as a model for the origin of life

De Boer, L.E.M., 1999 - The Polymerase Chain Reaction (PCR) as a model for the origin of life - in: Reumer, J.W.F. & De Vos, J. (eds.) - ELEPHANTS HAVE A SNORKEL! PAPERS IN HONOUR OF PAUL Y. SONDAAR - DEINSEA 7: 91-94 [ISSN 0923-9308]. Published 10 December 1999

The Polymerase Chain Reaction (PCR) is discussed as a possible model for the origin of life. Innumerable cycles of abiotic DNA replication, involving recurrent mutations at a rate of $1 : 2.10^4$ could have led to an enormously varied population of DNAs. Competition among these DNAs for efficient replication could have resulted in selection and eventually in co-evolution of DNAs and proteins which is a prerequisite for the origin of life.

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Keywords: PCR, origin of life

INTRODUCTION

Numerous studies over the past decades have demonstrated that many kinds of organic molecules that are essential for life may have originated under anorganic conditions on the primeval earth or even extraterrestrially. Molecules such as amino acids, nucleotides, oligopeptides, oligonucleotides and even longer polymeres of these may be synthesised under specific circumstances that may also have occurred on earth, ranging from high temperature zones near oceanic vulcanos to drying out lagoons on ocean shores (Imai *et al.* 1999, Bernstein *et al.* 1999, Keefe *et al.* 1995, Keller *et al.* 1994, Ferris *et al.* 1996, Robertson & Miller 1995).

However important such organic molecules must have been to the origin of life, life itself did not start before a system of self-replication evolved. Self-replication, or reproduction is the major characteristic distinguishing the living from the non-living world. Self-replication is the driving force of life. Once a mechanism of self-replication arose, all structures that originated during subsequent organic evolution, ranging from the simplest organelles in primitive bacteria to complex structures in plants and animals served only one goal: to enable the self-replication mechanism to continue to function and to become more and more efficient.

In spite of all advances in research into the origin of organic molecules, still little or no progress has been made in answering the question of how the self-replication mechanism evolved on a molecular level. Basic to self-replication in all living organisms are polynucleotide chains (DNA/RNA) and polypeptide chains (proteins). Neither of these two is able to replicate by itself, but together they can: the polypeptides serve as catalyzers (enzymes) for polynucleotide replication, whereas the polynucleotides function as templates that guarantee exact replication of their own chemical structure as well as the production of polypeptides with consistent amino acid sequences. In the search for how such a

mechanism might have arisen on the primeval earth, the Polymerase Chain Reaction (PCR) may serve as a model.

THE PCR TECHNIQUE

The PCR technique is widely used in a variety of genetic and biochemical research projects (Watson *et al.* 1992). Basically, the technique involves the production of large numbers of copies of a single DNA molecule in the absence of any organisms or organelles. The enzyme polymerase is used to catalyse DNA replication in this process. Polymerase is present in all living organisms, but as the PCR technique involves high temperatures (see below), use of the heat resistant polymerase from *Thermus aquaticus*, a bacterium living in hot springs, is the method of choice. The reaction mixture also includes the four different nucleotides needed for DNA replication and specific primers, which indicate where on the DNA molecule replication should start and end. These primers are short DNA sequences (up to 20 nucleotides) that are complementary to the section of the DNA where the replication should begin. The double stranded DNA molecule which is to be multiplied is added to this mixture. The temperature is increased to 94^{o}C which causes the DNA strands to separate. This is followed by cooling of the mixture to 40^{o}C, at which temperature the primers bind to the two single strands. Subsequently the polymerase replicates the strands, so that two double strands of DNA are formed. The mixture is heated then to 94^{o}C again causing separation of the two double stands into four single strands. Subsequently, renewed cooling results in binding of the primers and a new round of replication by polymerase. This cycle of heating and cooling with subsequent replication can be repeated time and time again, and with each cycle the number of DNA copies is doubled. Thus, after 10 cycles there are 2^{10} (=1024) copies, after 20 cycles 2^{20} (1,048,576) copies and after 30 cycles over one billion. In the laboratory one cycle can be completed in less than 15 minutes. Thus, 30 cycles to produce a billion copies could be completed in less than half a day. An interesting aspect of the PCR technique is that occasionally 'mistakes' are made in the replication. It has been shown that one incorrect nucleotide is incorporated for about every 20,000 ($2x10^4$) nucleotides. Such incorrect nucleotides are 'inherited' over the subsequent cycles, and thus may be considered as 'mutations'. A mutation occurring in the 15th cycle (when there are over 30,000 copies of the original DNA molecule), will lead to 20,000 copies of the mutated strand in the 30th cycle. With a mutation rate of 1: 20,000 the probability is then high for a second mutation in one of the mutated strands to occur, leading to a double mutation strand. By the 45th cycle, with over 30,000 double mutation strands, a third mutation is likely to occur, etc. If this is extrapolated over hundreds or thousands of cycles it is obvious that mutations will accumulate, giving rise to an enormous variability of strands, some of which are very different from the original one. It is even possible that strands would arise with sequences in their chain resembling the code for the synthesis of the polymerase chain involved in the reaction.

PCR AND THE ORIGIN OF LIFE

If, in the light of the above model, we now consider primeval earth during the times when life originated, we see that many of the chemical ingredients for a PCR may have been present: nucleotide precursors for DNA/RNA, numerous kinds of oligonucleotides that might serve as primers for the DNA/RNA replication process (such primers may have been very simple repetitious sequences), longer DNA/RNA sequences (50 or more nucleotides) that may have served as templates in the process, and many varied kinds of oligo- and polypeptide chains. Surely, the last category will not have included polypeptide chains identical to the polymerases used in the PCR technique. However, it has been demonstrated that pantetheine, a precursor to coenzyme A (a crucial

enzyme for metabolism in living organisms) could have been synthesised under prebiotic conditions (Keefe *et al.* 1995). Similarly, precursors may have been present on primeval earth with a potential of some kind of polymerase-like activity; perhaps much less effective than the current day polymerases, possibly acting in combination with anorganic catalysors; perhaps acting under different conditions; but in any case with a possible primitive polymerase-like effect.

Fluctuating temperatures, essential for PCR, may also have existed on primeval earth. One of the possible causes of these may have been considerable differences between day and night temperatures due to atmospheric conditions during those times. These would have led to 30 cycles of premordial polynucleotide replication every month, over 1000 cycles every 3 years and millions of cycles in the course of 10 millenia. Thus, even if a primitive polymerase-like precursor would have been very inefficient compared to the polymerase used in the PCR, very extensive accumulation of mutations in polynucleotides could have occurred over a (geologically speaking) very short period of time. An absolutely incredible variety of polynucleotides could have arisen over millions of years (that is billions of cycles). Prerequisite to this would be the continued presence of the polymerase precursors, the simple oligonucleotide precursors and the various nucleotides.

However extensive the accumulation of polynucleotide mutations may have been, at this anorganic stage the random mutation process would have only led to an ever increasing, random variety among the polynucleotide population, unless there would have only been selective forces lending certain polynucleotides a better chance to replicate than others. One possible selective force could have been a scarcity of nucleotides. This would result in competition between the polynucleotides for nucleotides. Under such circumstances polynucleotides being in close proximity of the primitive polymerase-like precursors would have an advantage over those having a random distance to the enzyme molecules in the reaction mixture. The polynucleotides in close proximity to enzyme molecules would start (after the drop of temperature to the required level) and complete replication earlier than the others and thus would use the available nucleotides before the others would have a chance. The result would be an evolution directed towards polynucleotides with some kind of (chemical?) affinity (and thus prox-imity) with the primitive polymerase precursors. Evolution would then lead to an ever increasing affinity in certain polynucleotide lineages. The same reasoning would apply for circumstances with a scarcity of polymerases. Similarly, there would be competition among the polynucleotides for the most effective polymerase-like enzymes, which would lead to an evolution towards the most optimal polynucleotide/polymerase combination.

The author challenges scientists with much more biochemical expertise to study the question whether an evolution towards polynucleotides with an increasing affinity to polymerase-like polypeptide enzymes might result in an increasing number of sequences in these polynucleotides that are similar to sequences that in a living organism would code for polymerase production. In the evolutionary process postulated above, the most efficient polymerase-like molecules would direct the evolution of polynucleotides. A molecular evolutionary process leading to the origin of life, however, cannot only have involved an influence of polypeptides on the changes in polynucleotides, but also requires an influence of the latter on changes in polypeptides. This would be the case if indeed the evolution in the polynucleotide population would be in the direction of sequences that would be more and more similar to the code for the polypeptide involved. This would provide a basis for co-evolution of polynucleotides and polypeptides as a basis for mutual replication, the essential characteristic of life.

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received 12 July 1999

DEINSEA - ANNUAL OF THE NATURAL HISTORY MUSEUM ROTTERDAM
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