LIFE IN A TEST TUBE?

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Kornberg and Goulian succeeded in transferring from the living cell to the "test tube" chemicals necessary for reproduction of viral DNA. Scientists recognize the accomplishment not as the creation of a living organism, but rather as one enabling a DNA template to make a "copy of itself" in a test tube by a reproductive process normally occurring only within a bacterial cell. This process and the experimental procedures used are described in detail. Up until the present time, the closest approaches to "making of life" have been limited to establishment of proper conditions in which DNA or RNA polynucleotides from viruses or in living cells could manifest their potentialities.

Introduction

While many were discussing the first of the human heart transplants in mid December, 1967, suddenly the popular presses made the electrifying announcement that life had been created in a test tube. In a speech the night before, President Johnson had made the statement that we were about to receive one of the "most important news stories you ever read."

Reports in the popular news media preceded by some weeks the formal written scientific report. Even though certain of the early articles possessed an extravagant character–especially in the large print–they were on the whole remarkably accurate, probably because scientists themselves held a news conference to clarify what had been accomplished.

At the press conference, Nobel laureate Arthur Kornberg, presently of the Stanford University School of Medicine in California, and his associate, Mehran Goulian, a Stanford postdoctoral fellow, explained their research done in cooperation with Robert L. Sinsheimer of California Institute of Technology.

Essentially the announcement was that biologically active DNA (deoxyribonucleic acid) from viruses had been synthesized in the laboratory. Actually they did not manufacture viral DNA starting only with simpler non-viral chemicals, since as will be shown later, viral DNA was an essential part of their reaction mixture. Rather they succeeded in transferring from the living cell to the "test tube" the chemicals necessary for reproduction of viral DNA.

This scientific advance is considered to be important because DNA constitutes the hereditary material or genes which give to living things those characteristics earned from generation to generation. DNA characteristically is found in all organisms with the exception of certain viruses whose genes consist of RNA (ribonucleic acid). A synthesis using infectious RNA had been performed two years previously by Sol Spiegelman and coworkers at the University of Illinois; and so this recent work which made use of DNA viruses was to some extent an extension of those prior results.

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Important Factors of DNA Research

Investigator Kornberg has been engaged actively in molecular research with DNA synthesis for over a decade. In 1959 he shared a Nobel prize in physiology and medicine for discovering DNA polymerase, an enzyme which catalyzes the production of DNA. For this production to occur there must be a DNA template, an energy source, and the necessary building blocks (nucleotide forerunners or precursors). However, the resulting polynucleotide (DNA) would not exhibit biological activity like the parent template molecule.

The current success in producing DNA molecules which would manifest biological reproductive activity is based on several important factors. These are (1) purification of DNA polymerase, (2) selection of an ideal DNA template, and (3) utilization of a new polynucleotide-joining enzyme.

The DNA polymerase used by the Kornberg team in early experiments contained contaminating enzymes (nucleases). After the synthetic DNA was produced in incubation mixtures containing the polymerase, the contaminating enzymes would cause breakages in the new DNA. Thus the reproductive activity of DNA was destroyed. When the degrading nucleases were removed from the mixture, the purified polymerase led to production of DNA capable of reproduction.

DNA Organization

Exactly how DNA in the chromosomes of plant and animal cells is organized and controlled is not yet well understood. However, studies have indicated that DNA in the cell nucleus acts as a template for production of RNA which moves from the nucleus to the surrounding region (cytoplasm) of the cell. Here the RNA operates with the ribosomes and dictates the conformation of various proteins including en-

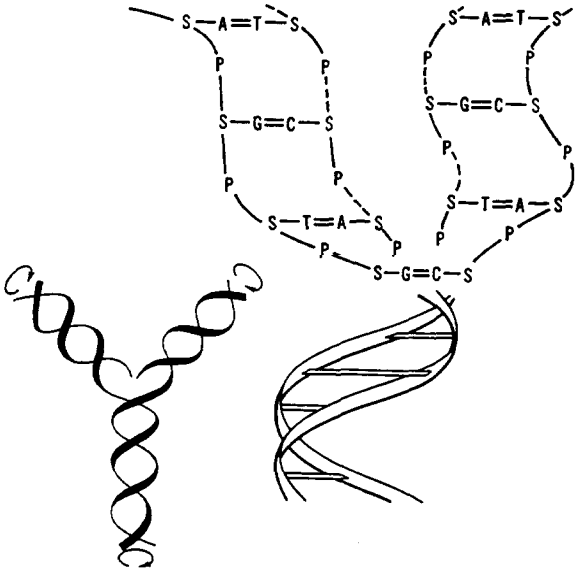

Figure 1. At left and bottom are diagrammatic views of the DNA helix. Above is a more detailed structure of DNA polynucleotide as it is reproducing. Each nucleotide is composed of Sugar, Phosphate, and a base, A, T, C, or G. (Courtesy Wiley & Sons, Inc.)

zymes which are essential for life of the cell and the organism of which the cell is a part.

The type of DNA widely discovered in various animal, plant, and human cells consists of two strands twisted about each other to form a helical structure. DNA resembles a ladder which has been twisted so that the two sides are spiral-shaped. The strands are joined together at the region of the rungs by hydrogen bonds. Each strand is called a polymer (poly, many; mer, parts) because it is composed of many repeating structural units. Each of these units is a nucleotide and so each polymeric DNA strand popularly is termed a polynucleotide.

DNA contains four types of nucleotides. All four contain a phosphate group, sugar, and a base. The difference resides with the bases, which are named adenine, thymine, cytosine, and guanine.

The structures of polynucleotides have been determined by physical and chemical means because they are too small to be visible. An electron microscope has been used for photographing strands of DNA, but cannot reveal the nucleotide sequence. The width of a DNA helix is 2 millimicrons, and it would take more than 12 million of these helixes side by side to equal one inch.

DNA and Proteins

In addition to functioning as a template for RNA production which leads to formation of vital proteins, DNA becomes a pattern for its own reproduction at appropriate times. When this happens, the two polynucleotide strands separate. The linear sequence of bases in each strand will determine which nucleotides will be required to reconstruct the double-stranded condition. Where there is an adenine base, a thymine-containing unit moves in so that thymine joins with the adenine. Where the template strand contains thymine, an adenine unit will join.

When reproduction is complete, adenine (A) will be joined to thymine (T), and cytosine (C) to guanine (G). (See Figure 1) These specific combinations constitute a four-letter genetic alphabet which is A-T, T-A, C-G, and G-C. The sequence of these "letters" distinguishes one organism genotypically from all others. Proteins will be constructed according to the "orders" given by the arrangements of letters.

Proteins are composed of units known as amino acids, and they range in size from about 50 to 3,000 amino acids. It takes a sequence of three nucleotides to determine one amino acid. For production of a relatively small chain of 150 amino acids, a nucleotide sequence of 450 would be required. This sequence would constitute what popularly is termed a gene. It is necessary that the nucleotide sequence be preserved exactly in order that correct proteins be produced for maintenance of the life which is characteristic of each organism.

Minute Complexity Involved

Each organism contains within its genome not just a few genes, but in most cases thousands or millions. In the human body there are some 10 trillion cells, and each cell normally contains 46 chromosomes in its nucleus. Within the set of chromosomes of each cell, there are perhaps 3 million genes composed of nucleotide pairs numbering in the vicinity of 5 billion.

Cells of cows, mice, or corn each have a similar number. A single colon bacterium of *Escherichia coli*, which is about ½ micron ($^1/_{50,000}$ of an inch) wide and 2 microns long, has a single chromosome containing a single DNA molecule. The extended DNA molecule is about 1 millimeter long (or about 500 times the length of the *Escherichia coli* cell).

Each of these DNA molecules contains about 10 million nucleotide pairs, which constitute the thousands of genes giving the organism its structural and functional characteristics. It should be pointed out that not all genes are operating at one time, but each functions at appropriate times during an organism's life history. The proteins called *histones* seem to have an important role in *regulating* gene action.

Does all this minute complexity, with the coordination of operation necessary, point to a supernatural designer and sustainer? Those who

accept the argument that design implies a designer say yes. Others say they believe that life was self-creating and is self-sustaining. Both of these positions are based on an individual's philosophical ideas which are not subject to rigorous proof. We shall say more of this later on.

Comparison of DNA and RNA

In most organisms DNA is a double-stranded helical structure, but there is considerable variation found among viruses. The best known DNA viruses, like smallpox, polyoma, T2, T4, and T6 have double-stranded DNA. Several groups of bacterial viruses carry their DNA in a single-stranded condition. Among RNA viruses, influenza, poliomyelitis and bacterial virus F2 possess a single RNA strand, whereas, in the Reo viruses, RNA has been found in the double-helical form.

Even if the genetic information is carried normally on a single nucleotide sequence rather than a double one, the complementary replica strand can be produced at the appropriate time. So it appears that the important fact is that some type of nucleotide is present whether it be RNA or DNA.

The structure of RNA is similar to DNA and differs from DNA in that (a) it normally possesses the base uracil instead of thymine, (b) carries an extra oxygen atom on each sugar portion, and (c) is usually single-stranded (whereas DNA is usually double-stranded).

The question whether nucleic acid (DNA or RNA) is required in all types of reproductive activity associated with living things still may be open; for there is suggestive evidence that certain disease conditions may be due to some type of "subviral" transmitting agent which is capable of reproduction. Scrapie, a disease of the nervous system, may be caused by such an agent.

Circular Viruses

The recent research of Goulian, Kornberg, and Sinsheimer involved use of one of the smallest of the viruses. It is called ØX174 and carries a single strand of DNA in circular form.

Original evidence, which came primarily from electron microscopy, was interpreted to mean that DNA molecules were linear with two free ends. Now it appears that some DNA's can exist in either circular or linear form. The polyoma virus contains a circular double DNA, whereas ØX174 has the single circular strand, which remains circular during the time the complementary second strand is formed.

DNA circles are not confined to viruses as once thought, for even the *Escherichia coli* DNA molecule has been found in a circle. It has been suggested that the circular condition could have an important part to play in reproduction.

In order to understand the significance of the research of Kornberg's team in relation to life in general and the creation of "test tube" life in particular, it is necessary to understand reproductive processes, especially of the material they utilized.

Reproduction in Complex Forms

Most plants and animals with which we are familiar begin as a single cell which divides and distributes its DNA equally to all daughter cells. The quantity of DNA in the sex cells (sperm and eggs) would be one half the amount in body cells. This is true for organisms such as birds, frogs, reptiles, mammals, and insects.

When speaking about creating life in a test tube, scientists usually are not thinking of anything as structurally complex as these. It is, however, possible to raise bird eggs in a glass incubator, frog eggs in a glass dish, and fruit flies in a bottle. Doing this involves the providing of an environment in which DNA can manifest its potentialities.

Creation of just a tiny fruit fly with its wings, eyes, legs, digestive tract, nervous system, reproductive system, muscular system, etc., involved proper alignment of some 10 millions of polynucleotide pairs in the DNA. Of course, not all forms of life are this complex, but even so up to the present time, the closest approaches to the "making of life" of any kind have been limited to establishment of proper conditions in which DNA nucleotides could manifest their potentialities.

Bacteria and Viruses Considered

When we consider bacteria we find a group which largely is invisible without magnifying equipment. *Escherichia coli* is a good example. About 10,000 of them together on one plane would be required to form a spot large enough to be within the range of visibility of the unaided eye.

Bacteria reproduce asexually. When conditions are right for their multiplication each cell splits to form two cells. Here again the nucleic acid is distributed equally to the resulting cells so that each has the same genetic material. As a result all cells so produced will manifest a similar phenotype-looking and acting like others of their species.

When we consider viruses, we find doubt about their being alive at all; for one of the characteristics of life involves the ability to reproduce. Viruses are not known to live outside of living cells except in the sense of surviving passage from cell to cell, for their activities go on only within cells.

It has been suggested that viruses, which are relatively simple structures, compose a link be-

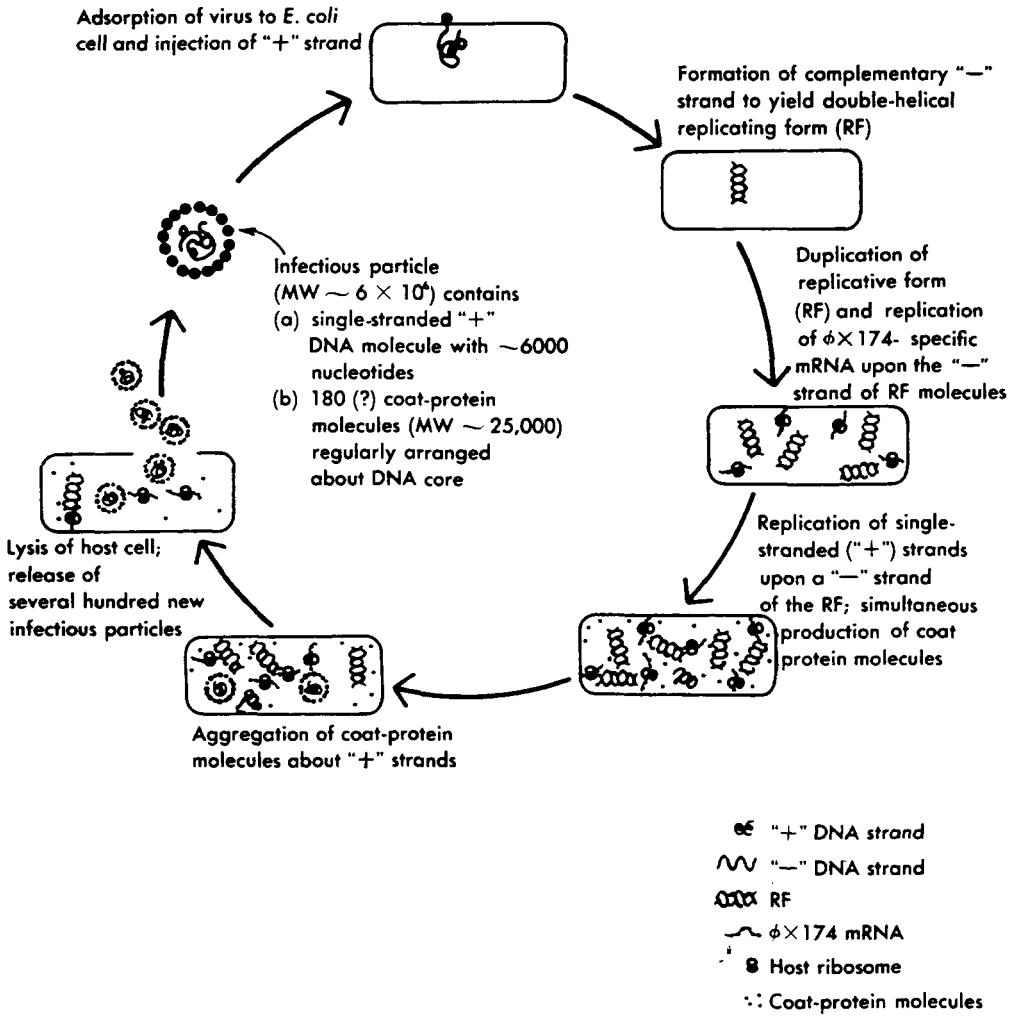

Figure 2. Life cycle of the ØX174 virus. This virus employs a circular DNA. The diagram shows only a small portion of the (+), (-), and (RF) circles. MW, relative Weight of a single particle or molecule, the hydrogen atom being approximately one. (from Watson)

tween nonliving and living substances. This does not appear likely because a virus lacks reproductive ability. Outside a living cell it is subject to forces which eventually will destroy it. Inside a cell its only function appears to be the supplying of information necessary for its own multiplication **at the cell's expense.**

So a virus may represent what was at one time a normal cellular constituent. Maybe as a result of some change it "escaped" from the cell's control while still requiring the cell for reproduction. Whether or not this is true, it does not seem likely that viruses existed prior to cells since viruses need cells for their perpetuation.

Variation, Reproduction of Viruses

Viruses show great variation in size and structural complexity; the largest virus approaches the size of very small bacteria. All viruses differ from cells in containing only DNA or RNA, whereas all cells have both DNA and RNA as discussed previously. The nucleic acid of viruses is in the center and is surrounded by a protein coat.

In order to reproduce, the virus must transfer at least its nucleic acid to the appropriate host cell. If the core is DNA, then the DNA will serve as a template for RNA, which works with the cellular machinery such as ribosomes and enzymes for producing the viral proteins. Also the DNA will serve as a template for its own replication.

If the viral core is RNA it will function directly in producing viral proteins and replicating more RNA. In both cases the specific nucleic acid core material and proper viral protein shell will be produced. These then join to form mature viral particles which will escape from the cell and attack more cells.

In addition to production of nucleic acid and coat protein, often one or many enzymes will be formed as well. In one way or another, these will be necessary for successful viral multiplication. For instance, if the cell has a heavy wall around it, the viruses could be trapped. Therefore many viruses have a gene coding for a cell-wall destroying enzyme which will cause the bacterial cell to lyse (or split open) at the appropriate time.

Life Cycle of a Virus

Before considering in some detail what Goulian, Kornberg, and Sinsheimer reported, it might be wise to discuss the normal life cycle of the virus they used, namely ØX174. (See Figure 2.) The designation Ø indicates that this virus is a phage or bacteriophage (phage refers to eating or consuming). Phages destroy bacteria.

The phage, ØX174, is one the the smallest viruses and has a particle weight of only 6 million which is the size of some very large protein molecules. The viral core consists of a single circular strand of DNA consisting of 5,500 nucleotide residues. This is 5 or 6 genes. Surrounding this DNA core is a coat of protein molecules.

The virus is adsorbed to the cell wall of a bacterium of *Escherichia coli.* The DNA, which is called a (+) strand is injected and the protein coat remains outside the bacterial cell. Once the single (+) strand of DNA is inside the cell, a complementary (–) strand of DNA is formed, thus producing a double-stranded helix. This double-stranded DNA is called a replicative form (RF).

The RF itself is duplicated forming more RF. Also there is replication of specific ØX174 RNA molecules upon the RF molecules.

Then there is a replication of single-stranded (+) strands upon the (–) strands of the RF. At the same time, protein coat molecules are produced.

The protein coat molecules then aggregate around the (+) strands. Thus mature virus particles are formed.

The cell wall of the bacterium lyses and several hundred new infectious viral particles are released.

The new particles each in turn can infect a cell of *Escherichia coli,* and cause it to produce more viral DNA and viral protein and then to release several hundred more viruses. Thus the process continues.

Experiments Duplicated Viral Activity

The recent experiments were set up to duplicate outside the cell what normally happens inside. To prepare for the experiment, the researchers treated intact viruses with phenol in order to remove protein coats, thus leaving the pure DNA or what would serve as (+) strands or circles.

From *Escherichia coli,* DNA polymerase and polynucleotide-joining enzyme were obtained and purified. More *Escherichia coli* cells were broken (by a sonic method); the fluid was centrifuged and the supernatant boiled. This supernatant solution, therefore, contained the soluble heat-stable materials from the bacterial cell.

The incubation mixture in which the first DNA reproduction occurred was composed of the following: ØX DNA, four nucleotide precursors (deoxynucleoside triphosphates, purchased from an outside source), DNA polymerase, joining enzyme, DPN (a hydrogen carrier), boiled *Escherichia coli* extract, magnesium chloride, potassium phosphate buffer, mercaptoethanol, and albumin. Incubation time was 180 minutes at 25° C.

During incubation the natural ØX DNA (+) circles served as templates for formation of complementary (–) chains, the DNA polymerase catalyzing polymerization of the (–) chain. As the nucleotide precursors joined to become units in the new (—) polynucleotide chain, they underwent certain changes including loss of some phosphate groups. The joining enzyme catalyzed the joining of opposite ends of the newly formed DNA (–) chain so that a duplex circle (RF) was complete (Figures 3 and 4). These circles were like RF isolated from infected *Escherichia coli* cells, except that the partly synthetic RF lacked supercooling found in natural RF.

Duplex DNA Circles Produced

The new duplex circles were exposed to a pancreatic DN ase which broke one of them and led to their separation. The synthetic (–) strands could be separated from the (+) strands because in the incubation mixture thymine had been replaced by the unnatural, yet biologically active and heavier, bromouracil. When the material was centrifuged the bromouracil-containing (–) strands could be separated because of their greater density.

It was demonstrated by radioactive tests that the new (–) strands were not contaminated by (+) template strands. The (+) template material had been prepared with radioactive hydrogen (tritium, H'), and nucleotide precursors used for synthesis of the new (—) strands contained radioactive phosphorus.

The new (—) circles infected *Escherichia coli* cells. This had to be done using specially prepared *E. coli* since the DNA, which lacked the usual protein coat, was unable to penetrate normal bacterial cells. The specially prepared *E. coli* were without their cells walls and when they

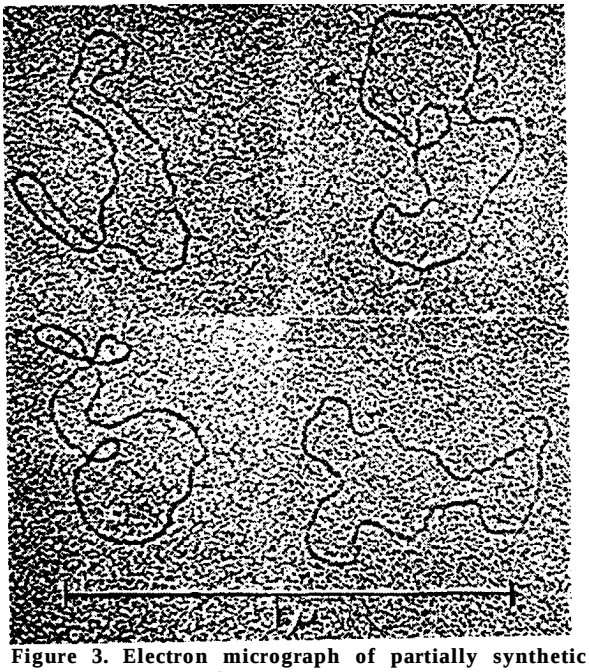

Figure 3. Electron micrograph of partially synthetic duplex circles. (from Goulian and Kornberg)

were thus altered DNA could enter. Under the laboratory conditions used in this research, bacterial cells without their walls are globular in shape and known as spheroplasts. The newly-formed DNA (—) circles infected spheroplasts and produced new intact viruses.

DNA Circles Infected Bacteria

The investigators also produced completely synthetic duplex circles by setting up an incubation mixture analogous to the one used previously. When the positive circles were separated, they could act as templates leading to the formation of RF within the *Escherichia coli* spheoplasts. Thus the researchers were able to perform an *in vitro* production both of infective (–) and (+) circles (Figure 5).

These experiments have demonstrated what might be occurring within the cells of *E. coli* after invasion by the ØX174 viral DNA. The investigators say it appears likely that the same enzymes utilized in their experiments are the ones used by infected *E. coli* cells to carry out the DNA polymerization and ligation of the ends of DNA strands. The enzymes they used were extracted from the *E. coli* bacteria, and the experimental environment with the boiled *E. coli* extract, etc., approximated conditions found inside *E. coli* cells.

Interpretation Evaluation of Research

To refer to this recent research development as "production of life in a test tube" is plainly a dramatization of the research story. Scientists

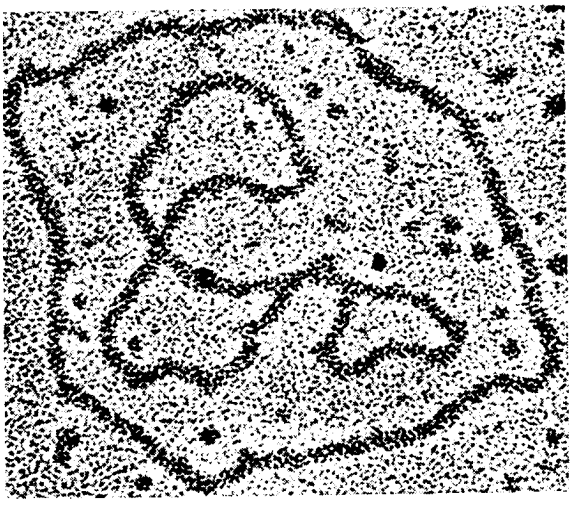

Figure 4. Two duplex circles of ØX174 DNA. One is extended, and the other is folded twice giving the appearance of 3 joined loops. Width of picture is approximately 500 millimicrons. Photo by Ron Davis of California Institute of Technology.

recognize the accomplishment not as the creation of a living organism, but rather as one enabling a DNA template to make a *copy of itself* in a "test tube" by a reproductive process normally occurring only within the bacterial cell.

However, the work probably does constitute the closest approach yet to what most of us think of when we say, "life in a test tube." The research establishes an important step forward in our understanding and control of life, and could lead to improved treatment of various diseases including cancer as well as man's gaining control over some hereditary conditions.

It probably is unwise to condemn the investigators involved in this recent project as recognition seekers. Even though their research may have been reported in extravagant terms, the men themselves are careful researchers. Their utilization of public media rather than only the technical press (in this case the December, 1967, issue of the *Proceedings of the National Academy of Sciences of the U.S.A.)* can be justified.

National funds channeled through government granting agencies over the past seven years in support of Kornberg's research have amounted to about $2,000,000. Because these were public funds, the taxpayer who understands what is being done will be more willing to see his money used to support such basic research. Thus Kornberg decided that the cause of science would be advanced by general publicity. He said,

> I felt this work could be more easily interpreted for the public than some other things we have done. Lately I have become aware of the need for science to be better understood by the public, and I've had the feeling that we

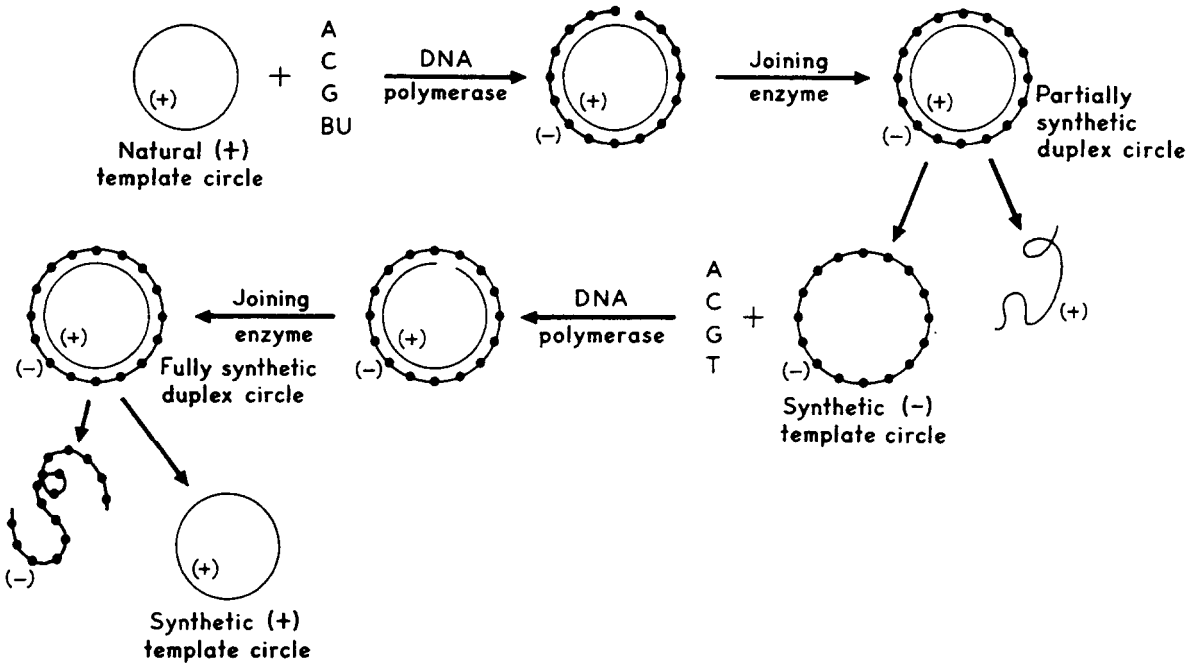

Figure 5. Enzymatic synthesis of viral ØX174 DNA. Both the synthetic (—) template circles and the synthetic (+) circles would infect bacterial cells, causing them to produce the appropriate complementary strands as well as the surrounding viral protein. Both duplex circles are considered replicative forms (RF). The letters A, C, G, BU, and T stand for the nucleotide precursors containing respectively adenine, cytosine, guanine, bromouracil, and thymine. (after Goulian, Kornberg and Sinsheimer).

haven't always exploited our opportunities for gathering public support.

Most Investigators Cautious

Most scientific investigators dealing with phenomena associated with living things realize the complexity of life, and they realize the small part they actually are playing in clarifying some mechanistic details of how life maintains itself. We are increasing steadily our knowledge of the structure and function of DNA in reproducing itself and in producing RNA and the proteins necessary for life. But it should be pointed out that **all** living things, including the tiny ameba, possess hundreds and thousands of enzymes and almost none of the structure and stereo arrangement of these proteins even are known.

We know the amino acid sequences of a few proteins–these being various members of probably less than thirty protein families. We are not able, yet, even to read off the nucleotide sequences of DNA molecules. A few relatively simple substances like amino acids, polypeptides (or strings of amino acids), sugars, nucleotides and ATP (adenosine triphosphate, which is used in energy-releasing processes), and by use of special techniques even the very small protein, insulin, have been synthesized under laboratory conditions.

When we consider them in relation to living things, they are as a few spare parts compared to a giant machine. It is important to point out that because of the complexity of life, production of a self-reproducing organism from simple precursors does not seem to be within the grasp of scientists at the present time. This fact not only should challenge, but also should encourage a cautious optimism among those engaged in basic biological research.

Faith in God, DNA Considered

Some scientists have tried to use recent knowledge of DNA, RNA, and various phenomena of life to discredit faith in a supernatural being. Often the "god" of these individuals becomes the quest for truth. For some, science has become a religion which teaches men to worship products of the human mind.

On the other hand, there are those scientists including myself, who have realized that there is a spiritual dimension beyond the millimicrons and cubic centimeters used in DNA and protein research. One day, some years ago while I was in graduate school, one of my associates told me in unforgettable terms what had happened to him at midnight the day before. He said he felt all of a sudden as though he had reached the top of his materialistic ladder. "There must be something beyond this" his very nature told him.

It seems to me that faith in God is logical. There are many philosophical arguments, which –true enough– do not prove His existence; yet, when taken together, they do show that faith in a supernatural God is *logical.* Also such faith is *satisfying.*

Speaking of God, St. Augustine said, "Thou hast made us for Thyself, and our hearts are restless till they find their rest in Thee." Some have said that God created man with an empty place that only He could fill. It is significant to me that belief in a supernatural being is found universally in all cultures, and as far as we know this always has been the case.

Relevance of Bible Indicated

The Bible indicates that the invisible things, from the time of creation, are seen and understood by the things that are made, and these point to God's power and deity (Romans 1:20). The story is told, that during the French revolution when some men were determined to remove churches, priests, Bibles, and everything that reminded people of God, that a farmer laughed when told this. When asked why he laughed, the farmer pointed upward to the stars and said, "I was just wondering how you will get them down." Psalm 19:1 tells us that the heavens declare the glory of God.

The Bible indicates that Jesus Christ actually is the creator of Genesis 1:1 (see John 1:1, 14; Hebrews 1:2; Colossians 1:16), and it says in Colossians 1:17 that in Christ all things hold together. I think this can refer to the stuff of life. Modern physico-chemical studies have given us considerable knowledge of the attractive forces existing within molecules and of the bonds holding molecules together. I thank God for this knowledge, because with it we can understand better His creation.

There is something about looking at starry "diamonds" glistening in the night sky, which causes us to meditate on the greatness of the God who created them; but there is something about DNA and its operation which gives me a similar thrill. Here is evidence of the handiwork of a God concerned with intricacy.

The greatest complexity of which we presently are aware exists in living "protoplasm." It seems to me that the human brain (probably the most complex organ in existence), if anything, would lead one at least to suspect the existence of a Higher Power.

Those of us who have experienced this Higher Power, in the person of Jesus Christ in our lives, have the most for which to be thankful. We can and do participate in various phases of scientific research, and we are thankful to God for all the new knowledge He allows us *to* obtain. We are analyzing components of living things and we are synthesizing some; thus we are learning more of His creative activity.

Will it ever be possible–starting with simple chemicals—to put together something alive, and have it maintain itself and reproduce in a test tube (or outside of a test tube)? This question remains unanswered. At any rate, as long as God ordains, we will go on researching and learning more about DNA and other aspects of His creation.

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