The history of dna replication biology essay

Science, Biology

College of Earth & Environmental Sciences

DNA Replication

SubmittedtoMa’m MariyamGeneral BiologyGroup Members: Muhammad Bilal, Maliha Shehr Bano, Gulanaz Shoaib Butt and FaheemBS Environmental SciencesSession 2012-2016January 2013

DNA Replication

TABLE OF CONTENTS

HISTORY OF DNA

Early scientists thought that protein was the cell’s hereditary material because it was more complex than DNA. Basically proteins were composed of 20 different amino acids in long polypeptide chains that’s why scientists take them as the most complex structure and considered them the

hereditary material. After that Fred Griffith (1928) worked with virulent S and non-virulent R strain. He studied Streptococcus pneumonia, the bacterium that causes pneumonia. The smooth outer core of the bacterium is known as strain S and the rough outer core of the bacterium is known as strain R. Griffith injected both strains into mice and found that only strain S bacteria were deadly. Then he injected live strain R bacteria and strain S bacteria that had been killed by heat but the mice died and he found a bug that living strain S bugs suddenly appeared. Then he injected only heated killed strain S bacteria and mice didn’t die. He concluded that something from the dead strain S bacteria and converted strain R bacteria into strain S bugs. He called it the " transforming principle." Thus Bacteria are capable of transferring of genetic material through a process known as transformation. After that in 1943 Oswald T. Avery put killed strain S in a test tube. When he added enzymes that destroy proteins the strain S bacteria were still able to transform strain R bacteria but when he added DNA-destroying enzymes the strain R bacteria couldn’t be transformed. Thus he concluded that DNA was the transforming agent. After that in 1950 Edwin Chargaff an Austrian biochemist studied the chemical composition of DNA molecule and had revealed that it contained a type of sugar called deoxyribose, plus a phosphate group and four different molecules or bases called adenine, thymine, guanine and cytosine. In a body or somatic cellA = 30. 3%T = 30. 3%G = 19. 5%C = 19. 9%

DNA

DNA stands for Deoxyribonucleic acid. It is the hereditary material in humans and almost all other organisms. Almost every cell in a person’s body has the

same DNA. Most DNA is located in the nucleus of the cell which is known as nuclear DNA, but a small amount of DNA can also be found in the mitochondria, known as mitochondrial DNA or mtDNA. All the information stored in DNA is stored as a code made up of four chemical basesadenine (A)guanine (G)cytosine (C)thymine (T)Human DNA consists of about 3 billion bases, and about 99 percent of those bases are same in all people. The order or sequence of these bases determines the information available for building and maintaining of an organism. These Four DNA chemical bases pair up with each other in specific wayA must pair with T (forming double bond between them)G must pair with C (forming triple bond between them)Each pair form a unit thus called base pairs. Basically DNA is made up of subunits called nucleotides. Each base is also attached to a sugar molecule and a phosphate molecule. This sugar molecule, phosphate molecule and a base pair joined together which is called a nucleotide. So a nucleotide is made up of following things: Phosphate group5 – Carbon sugarNitrogenous baseThese nucleotides are arranged into two long strands forming a spiral called a double helix. The structure of the double helix is like a ladder, with the base pairs forming the ladder’s rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

DNA Structure

In 1952 Rosalind Franklin took many diffraction x-ray photographs of DNA crystals but in 1953 James Watson & Francis Crick built the first model of DNA using Franklin’s x-rays. DNA structure is made up of two coiled strands called double helix in which sides are made up of pentose sugar (deoxyribose) bonded to phosphate groups by phosphodiester bonds and

their center is made up of nitrogen bases bonded together by weak hydrogen bonds.

Structure of DNA

HELIX

Most DNA has a right-hand twist with 10 base pairs in a complete turn. Left twisted DNA is called Z-DNA or southpaw DNA. Hot spots occur where right and left twisted DNA meat producing mountains.

ANTIPARALLEL STRANDS

One strand of DNA goes from 5’ to 3’ (sugars) while the other strand is opposite in direction going 3’ to 5’ (sugars)

DNA Replication

REPLICATION RULES

Some of the basic rules of replication areSemi-conservativeStarts at the ‘ origin’Can be uni or bidirectionalSemi-discontinuousSynthesis always in the 5-3’ directionRNA primers required

REPLICATION FACTS

DNA has to be copied before a cells dividesDNA is copied during the S phase or synthesis phase of interphaseNew cells will need identical DNA strands

SYNTHESIS PHASE (S PHASE)

Synthesis phase or " S phase" occurs during the interphase of a cell cycle between the G1 and G2 stages. During synthesis phase, DNA replication takes place. DNA molecules " unzip" and each old strand attracts free

nucleotides forming complementary new strands, leaving two strands of DNA identical to the original strand of DNA.

THREE PROPOSED MODELSS FOR DNA REPLICATION

Replication is the process by which a cell duplicates its DNA before division. In humans, for example, each parent cell must copy its entire six billion base pairs of DNA before undergoing mitosis. After the discovery of DNA structure the main objectives of scientists were to know the process of replication and how do the DAN replicate. For this purpose the scientists proposed three models of DNA. They are: Conservative replicationDispersive replicationSemi-conservative replication

CONSERVATIVE REPLICATION

According to the conservative replication model, the entire original DNA double helix serves as a template for a new double helix, so that each round of cell division produces one daughter cell with a completely new DNA double helix and another daughter cell with a completely intact old (original) DNA double helix. DISPERSIVE REPLICATIONIn the dispersive replication model, the original DNA double helix breaks apart into fragments and each fragment then serves as a template for a new DNA fragment. As a result, every cell division produces two cells with varying amounts of old and new DNA.

SEMI-CONSERVATIVE REPLICATION

According to the semi-conservative replication model, the two original DNA strands (i. e., the two complementary halves of the double helix) separate

during replication; each strand then serves as a template for a new DNA strand, which means that each newly synthesized double helix is a combination of one old (original) and one new DNA strand.

PREDICTIONS BASED ON THE MODELS

When these three models were first proposed, scientists had few clues about the process occurring at the molecular level during DNA replication. Fortunately, the models yielded different predictions about the distribution of old versus new DNA in newly divided cells. These predictions were as follows: According to the semi-conservative model, after one round of replication, every new DNA double helix would be a hybrid that consisted of one strand of old DNA bound to one strand of newly synthesized DNA. Then, during the second round of replication, the hybrids would separate, and each strand would pair with a newly synthesized strand. Afterward, only half of the new DNA double helices would be hybrids; the other half would be completely new. Every successive round of replication therefore would result in fewer hybrids and more completely new double helices. According to the conservative model, after one round of replication, half of the new DNA double helices would be composed of completely old, (original) DNA, and the other half would be completely new. Then, during the second round of replication, each double helix would be copied in its entirety. Afterward, one-quarter of the double helices would be completely old, and three-quarters would be completely new. Thus, each successive round of replication would result in a greater proportion of completely new DNA double helices, while the number of completely original DNA double helices would remain constant. According to the dispersive model, every round of replication

would result in hybrids, or DNA double helices that are part original DNA and part new DNA. Each subsequent round of replication would then produce double helices with greater amounts of new DNA.

MESELSON AND STAHL's ELEGANT EXPERIMENT (1958)

Matthew Meselson and Franklin Stahl were well familiar with these three predictions, and they reasoned that if there were a way to distinguish old versus new DNA, it should be possible to test each prediction. As they were aware of previous studies that had relied on isotope labels as a mode to differentiate between parental and progeny molecules, they decided to see whether the same technique could be used to differentiate between parental and progeny DNA. Thus they began their experiment by choosing two isotopes of nitrogen as their labels: The common and lighter 14NThe rare and heavier 15N (" heavy" nitrogen)As their sedimentation method they used a technique known as cesium chloride (CsCl) equilibrium density gradient centrifugation.

REASON FOR CHOOSING " N" FOR THE EXPERIMENT

Meselson and Stahl opted for nitrogen because it is an essential component of DNA; therefore, whenever a cell divides and its DNA replicates, it incorporates new N atoms into the DNA of its daughter cells, depending on which model was correct. If several different density species of DNA are present," they predicted, " each will form a band at the position where the density of the CsCl solution is equal to the density of that species. In this way, DNA labeled with heavy nitrogen (15N) may be resolved from unlabeled

DNA" They then continued their experiment by growing a culture of E. coli bacteria in a medium that had the heavier 15N (in the form of 15N-labeled ammonium chloride) as its only source of nitrogen. The bacteria divide will contain 15N. Then they changed the medium to one containing only 14N-labeled ammonium salts as the sole nitrogen source. So, from that point onward, every new strand of DNA would be built with 14N rather than 15N. Just before the addition of 14N and periodically thereafter, as the bacterial cells grew and replicated Meselson and Stahl sampled DNA for use in equilibrium density gradient centrifugation to determine how much 15N (from the original or old DNA) versus 14N (from the new DNA) was present. For the centrifugation procedure, they mixed the DNA samples with a solution of cesium chloride and then centrifuged the samples for enough time to allow the heavier 15N and lighter 14N DNA to move to different positions in the centrifuge tube. By centrifugation, the scientists found that DNA composed entirely of 15N-labeled DNA (i. e., DNA collected just prior to changing the culture from one containing only 15N to one containing only 14N) formed a single distinct band, because both of its strands were made entirely in the " heavy" nitrogen medium. Following a single round of replication, the DNA again formed a single distinct band, but the band was located in a different position along the centrifugation gradient. Specifically, it was found midway between where all the 15N and the entire 14N DNA would have migrated, in other words, halfway between " heavy" and " light" Based on these findings, the scientists were immediately able to exclude the conservative model of replication as a possibility. After all, if DNA replicated conservatively, there should have been two distinct bands after a single

round of replication; half of the new DNA would have migrated to the same position as it did before the culture was transferred to the 14N-containing medium (i. e., to the " heavy" position), and only the other half would have migrated to the new position (i. e., to the " light" position). That left the scientists with only two options: either DNA replicated semi-conservatively, as Watson and Crick had predicted, or it replicated dispersively. To differentiate between the two, Meselson and Stahl had to let the cells divide again and then sample the DNA after a second round of replication. After that second round of replication, the scientists found that the DNA separated into two distinct bands: one in a position where DNA containing only 14N would be expected to migrate, and the other in a position where hybrid DNA (containing half 14N and half 15N) would be expected to migrate. The scientists continued to observe the same two bands after several successive rounds of replication. These results were consistent with the semi-conservative model of replication and the reality that, when DNA replicated, each new double helix was built with one old strand and one new strand. If the dispersive model were the correct model, the scientists would have continued to observe only a single band after every round of replication.

Natural evidence for semi-conservative replication

Semi-conservative replication made sense in light of the double helix structural model of DNA, in particular its complementary nature and the fact that adenine always pairs with thymine and cytosine always pairs with guanine. Looking at this model, it is easy to imagine that during replication, each strand serves as a template for the synthesis of a new strand, with complementary bases being added in the order indicated.

DNA REPLICATION ENZYMES

DNA replication is possible because of the action of specific enzymes on DNA strands. Following are the enzymes which participate in DNA replication. GyraseDNA HelicasesSingle- Strand DNA binding proteinsDNA PrimaseDNA Polymerase IIIDNA Polymerase IDNA LigaseTelomeraseDNA ClampTopoisomerase

FUNCTION OF ENZYMES

DNA Ligase

Its function is to glue down the pieces of DNA created by DNA polymerase I and III. It also joins the Okazaki fragments of the lagging strand of DNA.

DNA PRIMASE

Its job is to lay down the initial RNA primers so that so Polymerase III can get to work.

DNA POLYMERASE III

It is the main enzyme that is responsible for the DNA replication. This enzyme sticks to the primer on the open DNA strand and build a second strand in 5’ to 3’ direction.

DNA POLYMERASE I

This enzyme helps out the DNA Polymerase III by replacing the RNA in the primer with the DNA. DNA HELICASESIt is a six protein complex arranged in a ring form. Its function is to unwind the double stranded DNA Helix and prying apart the double strands so primase, polymerase and ligase could act upon single strands of DNA.

SINGLE STRAND DNA BINDING PROTIENS

These proteins work together to bind the individual strands in the DNA double stranded helix and aid the helicases in opening it up into single strands. They are particularly useful in stabilizing the unwounded single-stranded conformation.

DNA GYRASE

It relieves the strain of unwinding by DNA helicase. DNA CLAMPIt helps the DNA Polymerase III from getting dissociated from the DNA parent strand. TOPOISOMERASEIt relieves the DNA from its super coiled nature. TELOMERASEIt causes the length of a telomeric DNA to increase by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes.

GENERAL MECHANISM OF DNA REPLICATION

DNA REPLICATION STAGES:

There are 3 stages of DNA replicationInitiationElongationTermination

INITIATION

It is the first stage and starting point of DNA replication. It has the following aspects and enzymes involved in it.

REPLICATION ORIGINS

Initiation always starts with replication origin. In order to begin the DNA replication the double stranded DNA helix must be opened. The site where first replication process occurs is called as replication origins.

UNWINDING OF THE DOUBLE HELIX OF DNA

DNA helicases are used to unwind the DNA into two single strands. The hydrogen bonds between the complementary nucleotide bases break down. As a result the coiled double helix structure changes to two single DNA strands.

REMOVAL OF TORSIONAL STRAIN

Topoisomerase is a protein complex that removes the torsional stain from the uncoiled DNA after it’s unwinding.

STABALIZATION OF DNA SINGLE STRANDS

Single- strand DNA binding proteins along with helicase acts on DNA strands and keep them separated and stabilized as the newly unwound DNA strands might have ability to twist again forming hydrogen bonds with complementary nucleotide bases.

REPLICATION BUBBLE

After such enzymes action replication bubble is created. It forms at multiple sites along the length of DNA. It catalyzes the replication process.

REPLICATION FORK

The open structure of DNA is often referred as replication fork. It is asymmetrical as two strands run anti-parallel directions. It forms as a result of helicase action as it breaks the hydrogen bonds between complementary nucleotide base pairs.

SYNTHESIS OF PRIMER

RNA polymerase enzyme or Primase is responsible for the formation of Primer. Primer is a short nucleotide structure containing 10 to 12 base pairs. Primer is significant as the DNA Polymerase Enzyme which is responsible for the elongation a new DNA strand can only add nucleotides to the complementary stand but is unable to synthesize. The primer serves as a new DNA strand containing ribose instead of deoxyribose. After elongation the Primer is removed and is replaced with DNA nucleotides by DNA Polymerase I.

ELONGATION

It is the process in which the DNA strand is synthesized from the template DNA by the action of DNA polymerase enzymes.

SYNTHESIS OF GROWING STRANDS

It involves the addition of nucleotides one by one in a sequence as specified by the original template strand. DNA is always synthesized in the 5' to 3' direction this means that nucleotides are added only to the 3' end of the growing strand. The 5’ - phosphate group of the new nucleotide binds to the 3' - OH group of the last nucleotide of the growing strand. The elongation differs for 5’-3’ and 3’-5’ template strands of DNA.

DNA POLYMERASE III ACTION

Since Polymerase III can only synthesize new strands in 5’ to 3’ direction the two strands replicate under slightly different mechanisms. The polymerase III add nucleotides to the new DNA strand and elongates it in single direction that is from 5’-3’ direction.

LEADING STRAND SYNTHESIS

The leading strands require fewer steps and therefore it synthesizes quickly. The daughter strand complementary to the template strand 5’-3’ proceeds in 3’-5’ direction and is known as leading strand. This daughter strand is known as leading strand as the DNA Polymerase III, identifies the template and add continuously the complementary nucleotides.

LAGGING STRAND SYNTHESIS

The daughter strand complementary to template strand 3’-5’ is known as lagging strand. This strand grows in discontinuous manner away from the replication fork. The DNA Polymerase delta is unable to read the template strand therefore DNA polymerase epsilon reads the template.

STEPS IN THE FORMATION OF LAGGING STRAND

Formation of a section RNA primer by the action of RNA Primase. Replacing the RNA primer with DNA with the help of DNA Polymerase I as it reads the fragments of RNA nd removes the PrimerFor the more formation of double helix the helix must continue to unwind.

OKAZAKI FRAGMENT

The lagging strand grows discontinuously in the form of series of short segments that become connected later. These fragments are called as Okazaki fragments. Each Okazaki fragment is created by DNA Polymerase III in 5’ to 3’ direction. The Okazaki fragment synthesis begins from the replication fork and moves away from it. Okazaki fragments are about 100-200 nucleotides long in eukaryotes. Length of Okazaki fragment in prokaryotes is about 1000-2000 nucleotides. The DNA ligase attaches the

Okazaki fragments in the lagging strand in this way the daughter strand completely synthesize.

TERMINATION

It is the last stage of DNA replication. It is the process in which the DNA replication ceases at Replication Fork . The two new DNA double helix structures are created. As it is Semi-conservative Replication the DNA double helix formed as a result of it consist of one parent DNA Strand and one daughter DNA strand. The daughter strands both leading and lagging twist around their respective DNA parental template strands and separates. Termination occurs as two replication forks meet each other at the opposite end of parental chromosomes. Following steps occur during termination:

TELOMERES FORMATION

In the last section of lagging strand when the RNA primer is removed, it is not possible for the DNA Polymerase to seal the gap as there is no primer available. So the end of the parental strand where the last primer binds isn't replicated. Because these ends of linear chromosomal DNA consists of non-coding DNA that contains repeat sequences and are called telomeres. As a result, a part of the telomere is removed in every cycle of DNA Replication. REPAIRINGThe DNA Replication is not completed before a mechanism of repair fixes possible errors caused during the replication. Enzymes like nucleases remove the wrong nucleotides and the DNA Polymerase fills the gaps.