

REVERSE TRANSCRIPTION, cDNA, AND AMPLIFICATION

Reverse Transcription

The process of making a segment of DNA from a segment of RNA is called **reverse transcription** and is mediated by the enzyme reverse transcriptase. In fact, this is the mechanism by which retroviruses convert single stranded

RNA inserted into a host to double stranded DNA. Since the process reverses the normal flow of genetic information, it is called reverse transcription.

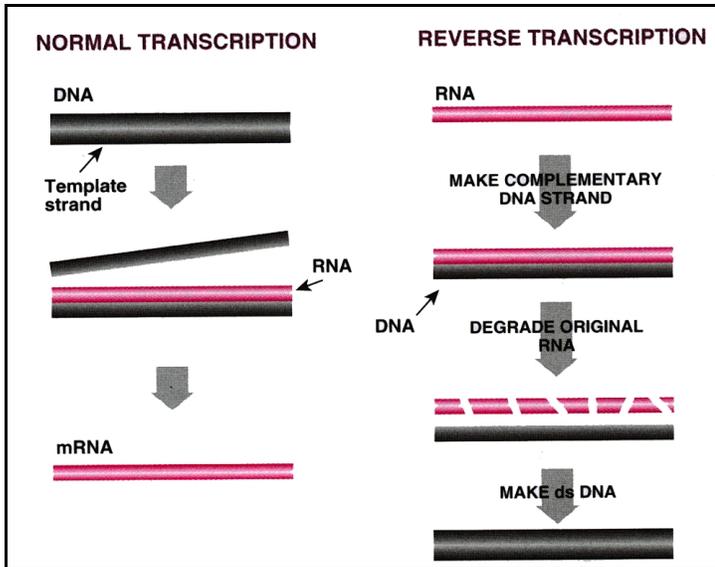


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cDNA

If we isolate a sample of mRNA from a cell or tissue, we can take advantage of the process of reverse transcription in vitro to make a copy of DNA from

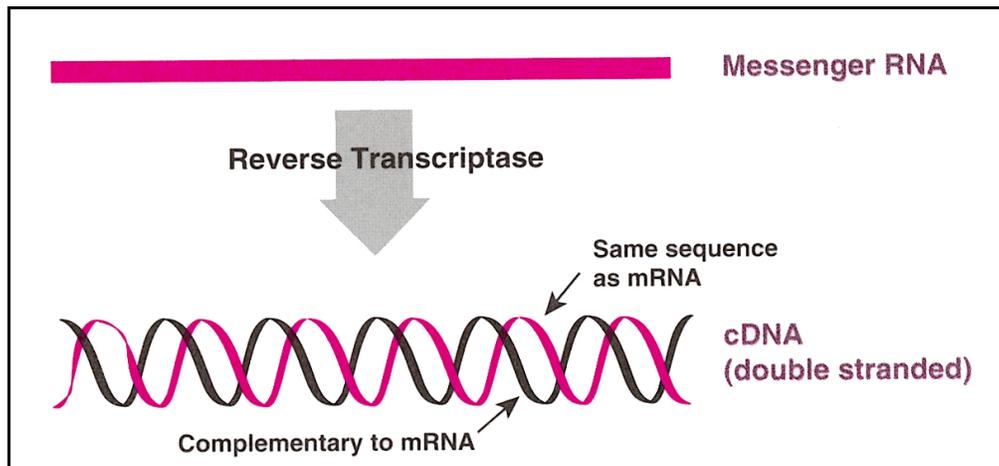


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the mRNA sample. The newly formed single stranded DNA is complementary, by virtue of the rules of base pairing, to the originally isolated mRNA. This is called complementary DNA (**cDNA**). Double stranded DNA can be derived from the cDNA by base pairing. Since the single stranded cDNA is complementary to the original mRNA, it can be tagged with an appropriate marker (radioactive or fluorescent) and used as a probe to identify the complementary mRNA in cells of another tissue.

Since the cDNA is derived from mRNA originally isolated from the cytoplasm, that mRNA has already been spliced to remove the introns. Consequently, the derived cDNA represents only the exons or coding region of the gene. The cDNA can be isolated and put into an appropriate plasmid or viral vector for cloning and, thus, can be propagated for a variety of uses. In order to obtain enough cDNA for use in

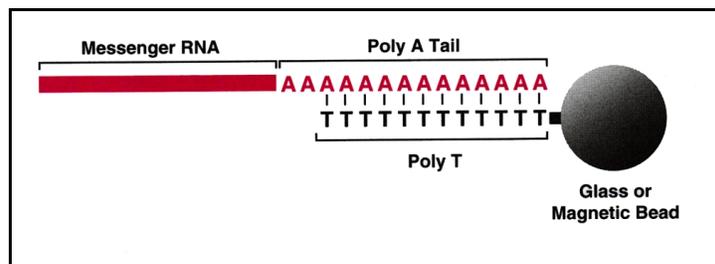


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the molecular biology laboratory, it can be amplified by a polymerase chain reaction (**PCR**). A **cDNA library** can thus be created to represent the mRNA present in a given tissue. The Cancer Gene Anatomy Project (CGAP) is based upon this technology of isolating mRNA from specific cancers, producing amplified quantities of cDNA from the isolated mRNA by reverse transcription polymerase chain reaction (**RT-PCR**), and establishing a library of expressed genes in specific cancers.

Amplification

One of the most powerful in vitro techniques in molecular biology involves amplification of DNA sequences by the **polymerase chain reaction (PCR)**. A series of discoveries are important in the application of PCR:

1. Isolation of bacterial **DNA polymerase** and elucidation of its mechanism of action wherein a **primer** is annealed to the template DNA and the DNA polymerase extends the primer by synthesizing new complementary DNA.
2. Isolation of thermostable DNA polymerases.

3. Development of a **thermal cycler**.

The reaction requires a template DNA and a primer to function as the anchor for the DNA polymerase. The primer will anneal to the template DNA where it is complementary to the nucleotide string. DNA synthesis then occurs by primer extension. PCR starts with as little as one molecule of double stranded DNA and through successive thermal cycling results in rapid production of millions of copies. After about 20 cycles, about one million copies are produced from one original molecule. There are actually two primers - one that works at the 5' end and one at the 3' end of the double stranded DNA of interest. Usually the primers are designed to recognize complementary sequences just upstream and downstream of the DNA of interest so that the entire sequence of interest is amplified.

As a practical consideration, it is currently difficult to amplify DNA fragments greater than 4 kb in length. However, the good news is that relatively crude preparations of DNA can be used in the PCR reaction.

The PCR cycles, which as successively repeated produce more and more copies of the DNA segment of interest, consist of three steps: denaturation, annealing of the primer, and primer extension.

Polymerase Chain Reaction

