The Role of Genetics
in the Treatment of Cancer

by Valerie Relias, PharmD, and Gregory T. Brennan, BS

Understanding how genetics and molecular techniques relate to the care of oncology patients has become increasingly important over the past decade. A basic understanding of these techniques is now necessary to help care appropriately for these patients. This article will review some basic concepts in molecular biology focusing on deoxyribonucleic acid (DNA) replication and production of proteins. It will also discuss how changes in the genetic sequence, whether through mutations or recombination, may affect patients. Finally we will discuss specific examples with direct correlation to patient care.

Bad Things Happen

A problem involving DNA will affect the cell's replication and production of proteins. Over time, DNA sequences can change due to mutations, while changes in translation can also lead to mutations. (See "Molecular Biology 101" on page 42 for more.)

Single nucleotide polymorphisms (SNPs) occur when one nucleotide is substituted for another, which may ultimately cause a change to protein sequences. These polymorphisms can be responsible for varying responses to drugs between individuals. There are many other types of mutations, such as single base substitutions, deletions, and insertions of nucleotides. Frameshift mutations disrupt translation, commonly resulting in proteins that have fewer amino acids than normal. Tandem repeat sequences occur when small identical sequences occur over and over again. These mutations are commonly associated with cancers and other diseases.[1]

Recombination of non-homologous chromosomes can result in novel gene products that sometimes lead to cancer. This type of mutation occurs when a piece of one chromosome breaks off and combines with another chromosome. This translocation can be reciprocal, when pieces of chromosomes break off and "switch" places on their respective chromosomes. In some instances this switching causes genes that are not ordinarily in close proximity to each other to now become "neighbors" (see Figures 1 and 2, page 41). This recombination can result in the formation of new protein, turn on genes, and ultimately cause malignant transformation of the cell.

DNA mutations can also give rise to cancer by producing gain-of-function mutations resulting in:

- Overactive gene products
- Loss of function mutations resulting in non-functional products
- Change in binding affinities
- Constitutively produced enzymes
- Proliferation.

Work in the Lab

Many laboratory tests have been developed that exploit the knowledge we have of DNA replication and translation. These tests are used in the clinic to help us to diagnose a patient's cancer, determine the most appropriate therapy for a patient's disease, or follow the progress of a patient's therapy. Here is a look at four of these tests:
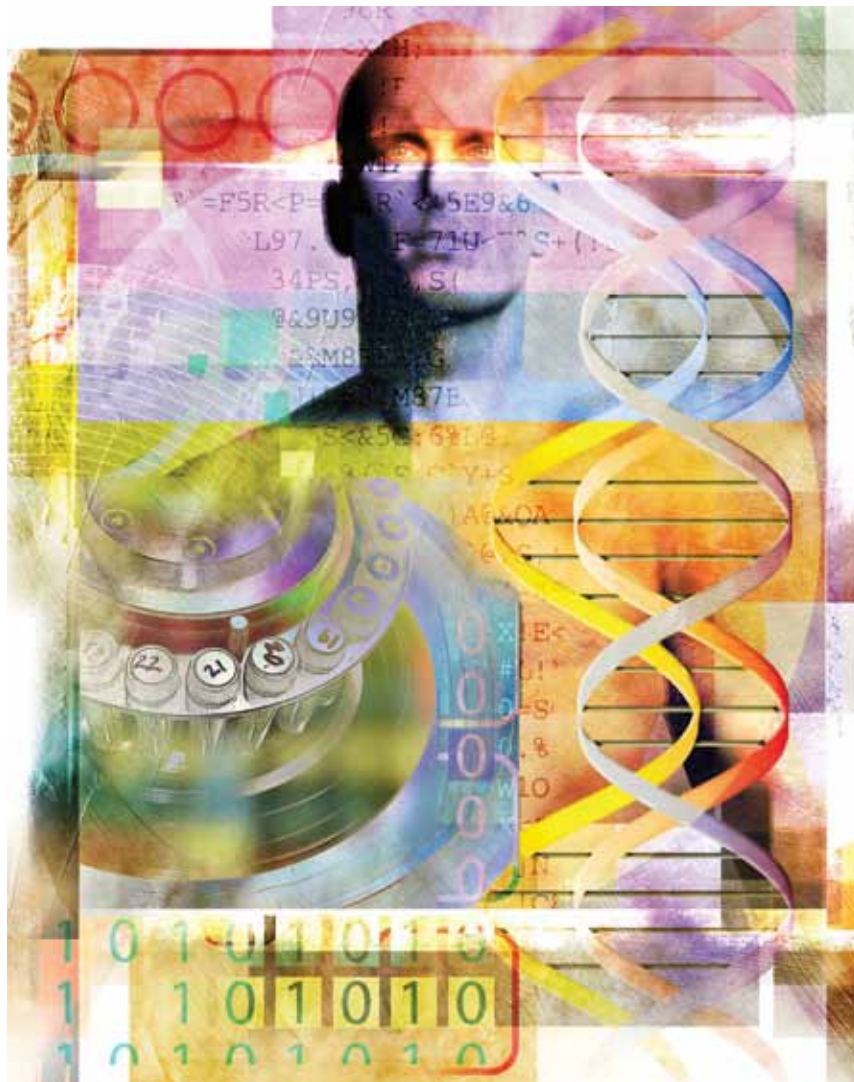
FISH. The fluorescent *in situ* hybridization (FISH) method uses DNA probes that are labeled with a fluorescent dye. These probes are complementary pieces of DNA. They attach to the desired gene or sequence of DNA with which they show a high degree of sequence similarity. Once bound to the gene, the cell is exposed to fluorescence microscopy to aid with visualization. FISH is helpful in determining the number of copies of a gene within the cell. For example, if you are "fishing" for HER2/neu overexpression in a breast cancer, this test may allow the pathologist to determine the number of copies of that gene within the tumor tissue. It is important with this test to know what you are "fishing" for since the outcome of the test is determined by the probes used. FISH is used in different tumor types to look for a variety of changes within the cell, such as overexpression of genes or the presence of certain sequences of genes.

Karyotype analysis. In contrast to FISH, a karyotype looks at the whole set of chromosomes within a cell. It is a snapshot that identifies the number, size, and structure of the chromosomes within the cell. In order to perform this technique, a viable, dividing cell is required. The cells are halted in metaphase using a drug such as colchicine. The cells are then crushed to form a single layer so that individual chromosomes are seen. A photomicrograph of the chromosomes is then taken. Individual chromosomes are then cut out of the photo and paired together until the chromosome pairs in a single cell are seen. Looking at the karyotype enables clinicians to determine chromosomes that have undergone translocations or deletions. It can also determine if there are extra chromosomes or if deletion of chromosomes has occurred. In clinical practice, this test may be used to help diagnose leukemias, such as chronic myelogenous leukemia (CML). The specific translocation between chromosomes 9 and 22 can be visualized on a karyotype, thus aiding in the diagnosis.

PCR. Another commonly used laboratory test that builds on the knowledge of molecular biology is the polymerase chain reaction (PCR) test. This test provides clinicians a means for amplifying the amount of DNA found in a specimen. It is performed in a temperature block that is able to cycle through many different temperatures. The steps involved in completing the PCR test include adding the DNA source (i.e., blood) into a test tube with DNA primers and DNA polymerase. The DNA primers are small pieces of DNA that attach and start the process of DNA replication, much like hitting a start button. The DNA polymerase is the enzyme that allows replication to take place.

All these processes must take place under ideal conditions, which is why the temperature block is needed. This block first cycles to a temperature of 96°C to allow the DNA to unwind. Then the temperature drops to 50°C to allow the primers to bind to the DNA template. Finally, the temperature rises to 72°C to allow extension of the DNA strand through the action of DNA polymerase. The temperature block cycles through these various temperatures many times before the process is complete.

Imagine beginning with one piece of double stranded DNA—after one cycle, the amount of DNA will double (two old strands plus two new strands). After five cycles through the temperatures, the process will produce 32 strands of DNA.

A variation of this test starts with RNA. The RNA is reverse transcribed (RT-PCR) back into DNA and then the process proceeds as described. This test is useful when only small amounts of DNA are available but larger amounts are needed.

RFLP. The test for restriction fragment length polymorphisms, or RFLP, can help identify if DNA from two different cells have a common origin. The test is done by isolating DNA from the two samples in question. Once isolated, each DNA sample is exposed to restriction endonucleases (enzymes that cut DNA at very specific points). The pieces of DNA are then run on a gel. If the DNA is from the same source, the gel pattern of the DNA fragments will be the same; however, if the DNA is from two separate DNA sources, the patterns will not line up.

Imagine two tumors in different lobes of a lung. Clinicians will want to know if these are two synchronous primary tumors or different primary tumors. RFLP may help distinguish between these two possibilities. This test is becoming the foundation for the development of newer technologies that may be useful in identifying tumor origins.

DNA Polymorphisms and SNPs—Predicting Toxicity

With a basic understanding of these techniques and processes, we can now begin to see their potential for use in clinical practice.

One example of how DNA polymorphisms are impacting clinical practice is in the area of colorectal cancer, specifically with the drug 5-fluorouracil (5-FU). This drug has been the mainstay of treatment for colorectal cancer for years and is generally well tolerated. However, we know that in a subset of patients this drug can become quite toxic. Studies have shown that over 30 percent of patients treated with 5-FU experience severe toxicity.[2] The question now becomes is there a marker that can be used to predict which patients will experience severe 5-FU induced toxicity?

The initial and rate limiting step in the catabolism of 5-FU is the enzyme dihydropyrimidine dehydrogenase (DPD). This enzyme accounts for 80 to 95 percent of the breakdown of the drug.[3] As such, this enzyme has been

looked at as a marker to identify patients at risk for 5-FU toxicity. Studies have shown that more than 30 SNPs and deletions have been associated with the DPD enzyme.[2] But do these mutations lead to toxicity?

A group in Amsterdam attempted to answer this question.[4] They took peripheral blood mononuclear cell samples from patients who had experienced grade 3 or greater toxicity to 5-FU and looked at DPD activity. They found that in 59 percent of cases where 5-FU toxicity was present there was also decreased DPD activity. Using PCR-based techniques to amplify the gene coding for DPD, these researchers were then able to identify mutations in 11 of 14 patients having reduced DPD activity. Other similar studies have shown that mutations in the DPD gene can be associated with severe 5-FU toxicity.[2,5,6,7]

So why do clinicians not routinely screen patients about to receive 5-FU for DPD deficiency? Currently there is no one test available that can easily be used in the clinic. Enzyme-based assays that look at DPD activity in peripheral blood mononuclear cells are time-consuming and not feasible. PCR-based testing to identify mutations in the DPD gene is currently used; however, no one specific mutation has been found that accurately predicts patients at risk. Usually, this test is performed after the patient has already experienced toxicity to document decreased DPD.

A newer breath test is being developed that can be used in real time in the clinic. The search for the most useful predictor of 5-FU toxicity will continue until a reliable marker for sensitivity to 5-FU becomes available as standard of care.

Mutational Analysis—Predicting Efficacy

While the role of genetics and molecular biology in helping clinicians predict who will encounter toxicity to a drug is an area under active research, the ability to determine who will respond to therapy has been proven to be a clinically viable tool. We now have good evidence that response to therapy can be determined by looking at the specific mutational analysis of tumor tissue.

Colorectal cancer is one of the top five cancers diagnosed in the United States, with more than 300,000 new cases per year.[8] Improvements in its treatment have occurred over the past several years with the addition of new treatment combinations of chemotherapy and the addition of biologic therapy. Targeted therapy with the monoclonal antibodies cetuximab (Erbitux®) and panitumumab (Vectibix®) have revolutionized the way clinicians treat these patients. These monoclonals have also ushered in an era in which genetic testing of tumor tissue has become standard. This genetic testing determines the likelihood of tumor response to the monoclonal antibody.

Both cetuximab and panitumumab are antibodies directed against the epidermal growth factor receptor (EGFR). The EGF receptor is part of a family of transmembrane receptors found on many cells. When activated, they send signals through downstream pathways, ultimately signaling cell proliferation. In colorectal cancer the actions of the EGFR are mediated by the downstream protein K-ras.

K-ras is a G protein which functions as an "on and off" switch for cell proliferation in colorectal cancer. If K-ras is in its normal or wild-type state, it requires a signal from EGFR to be activated. However if K-ras is mutated, it will "turn on" cell proliferation without the requisite signal from EGFR. This close association of EGFR with K-ras has major implications for the use of EGFR inhibitors, such as cetuximab and panitumumab.

This association between EGFR and K-ras was demonstrated in the study by Karapetis and colleagues.[9] They looked at more than 500 patients with metastatic colorectal cancer and randomized them to either best supportive care (BSC) or BSC plus the EGFR inhibitor cetuximab. Archived tumor tissue from about 400 of these patients was sent for mutational analysis using PCR techniques. Gene sequencing was able to identify mutations in the K-ras genes in approximately 40 percent of patients in both arms of the study. Interestingly, only those patients with unmutated or wild-type K-ras responded to treatment with cetuximab; none of the patients with mutated K-ras responded to therapy. The authors concluded that only patients with wild-type K-ras benefited with treatment of cetuximab. This and other studies have substantiated the link between mutational status of K-ras and response to the drug therapy with cetuximab.[8]

Not surprisingly, the same story has emerged with the use of panitumumab and K-ras.

Panitumumab is a fully humanized monoclonal antibody that also binds the EGF receptor. Amado and colleagues conducted a study very similar to the one previously described. They randomized more than 400 patients with metastatic colorectal cancer to BSC or BSC plus panitumumab. Mutational status of K-ras was determined via PCR-based techniques on the tumor cell blocks from these patients. Again, as in the previous study, 40 percent of the patients expressed a mutation in the K-ras protein. Those patients having the mutation did not respond to therapy with panitumumab. The authors concluded that K-ras mutational status should be determined prior to initiating treatment with panitumumab.[10]

Taken in combination, these studies have shown the effectiveness of doing mutational analysis on tumor tissue to determine efficacy of therapy. In fact, many payers now require that K-ras mutational analysis be done prior to use of the EGFR antagonists to ensure reimbursement. While this testing has become the standard for colorectal cancer, it is currently not required when using these drugs to treat other cancers, such as head and neck cancer. This is most likely due to the low mutational rate of K-ras in this type of cancer.

Chromosomal Translocations—Diagnosing Cancer

Genetics is also being used to help diagnose cancer. No where is this more evident than in the case of the leukemias, most especially acute myelogenous leukemia (AML).

AML has a specific chromosomal translocation (15;17 translocation) that identifies a subset of AML. This type of leukemia was first described as a distinct clinical entity in the late 1950s in patients who were found to have a rapid, fatal course with a majority of promyelocytes seen in the blood and severe coagulopathies. It is important to identify this subtype of AML because its treatment differs from that of the all the other subtypes of AML.[11]

Since these early reports, clinicians have identified the distinct molecular mechanism for this disease. The specific cytogenetic abnormality associated with this type of leukemia results from a reciprocal translocation between chromosomes 15 and 17. The promyelocytic leukemia (PML)

Figure 1. Reciprocal Translocation in CML

[Before Translocation]   [After Translocation]

t(9;22)

chromosome 22

bcr

ABL

chromosome 9
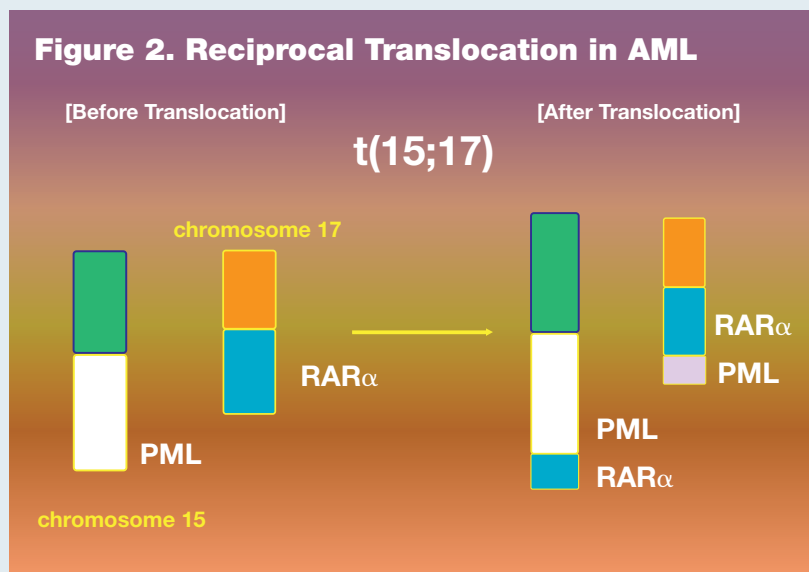
chromosome 22

bcr

9

ABL

22
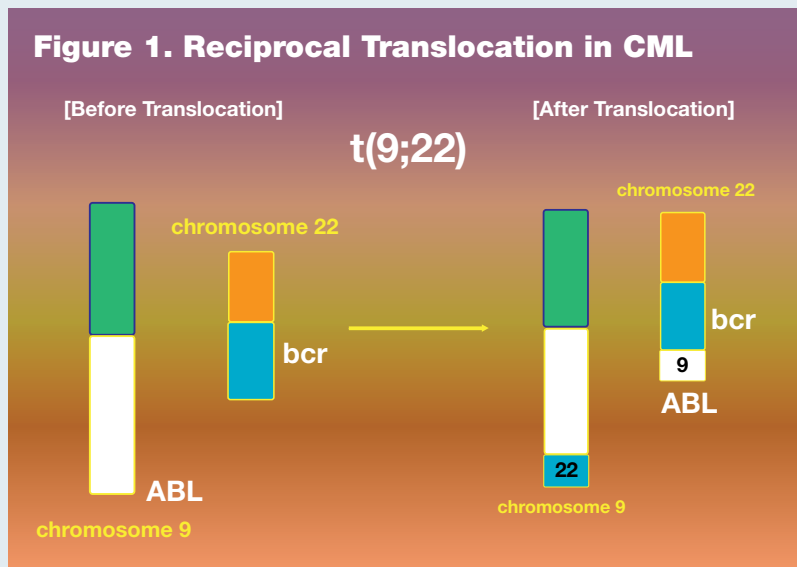
chromosome 9

Reciprocal translocation in CML. A piece of chromosome 9 containing the ABL gene is transferred to chromosome 22 bringing it adjacent to the bcr gene. Similarly, a piece of chromosome 22 is transferred to chromosome 9. The new bcr/ABL gene product confers the malignant phenotype to the cell.

Source: Relias V. PowerPoint Presentation; 2010.

Figure 2. Reciprocal Translocation in AML

[Before Translocation]   [After Translocation]

t(15;17)

chromosome 17

RARα

PML

chromosome 15

RARα

PML

PML

RARα

The reciprocal translocation between chromosomes 15 and 17 results in the production of a new fusion protein consisting of the PML and RARα genes. This new fusion protein blocks differentiation of promyelocytes causing malignancy.

Source: Relias V. PowerPoint Presentation; 2010.

gene resides on chromosome 15 and the retinoic acid receptor (RARα) gene resides on chromosome 17. When the translocation occurs, the PML gene is brought into close proximity to the RARα gene, creating a new fusion protein[12] (see Figure 2 above). This protein blocks the differentiation of the cell and suppresses transcription of factors needed to help the cells differentiate. This translocation confers the malignant phenotype to the cell, therefore it is imperative to identify the translocation.[13]

The most commonly used laboratory tests to help diagnose AML with 15;17 translocation are karyotyping, FISH, and RT-PCR. As described earlier, the karyotype can be done on bone marrow samples and will demonstrate the 15;17 translocation. Karyotyping is a somewhat more time-consuming process than the other tests available. FISH, which can be done on the peripheral blood or bone marrow of the patients, will identify the presence of the fusion protein. RT-PCR can be done on bone marrow samples, but the test can also be done on peripheral blood samples from patients with relatively low blood counts. The test is very sensitive and highly specific, and can usually be completed within 24 to 48 hours.[14] As you can see, these molecular tests are invaluable for the treatment of AML with the 15;17 translocation.
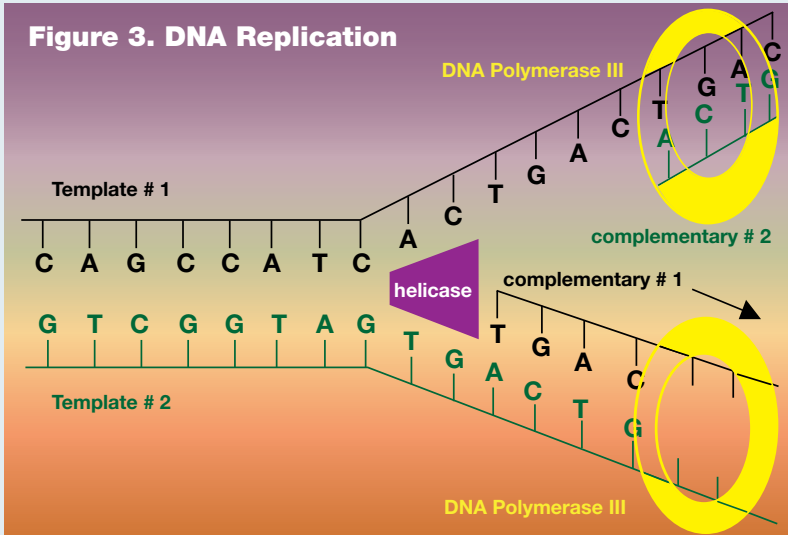
Other types of leukemia are also defined by specific translocations, including:

- Chronic myelogenous leukemia (CML) with its characteristic 9;22 translocation (see Figure 1 above)
- Acute lymphocytic leukemia (ALL) with the presence or absence of the Philadelphia chromosome (t9;22).

Genetics and molecular biology continue to play an important role in helping to care for cancer patients. While this article briefly discussed a few examples of how genetics and molecular biology have come to the forefront in the treatment of oncology patients, many more applications are being explored as clinicians gain more and more insight

Figure 3. DNA Replication

Template # 1

C A G C C A T C

helicase

complementary # 1

G T C G G T A G

Template # 2

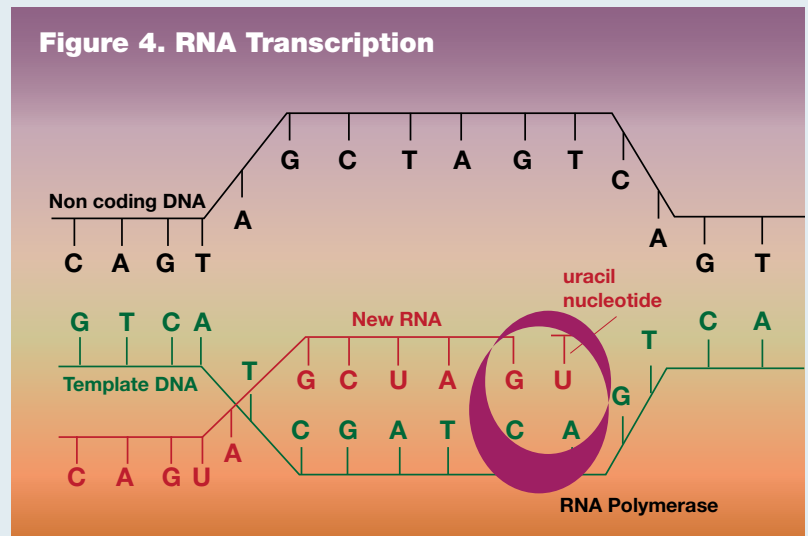DNA Polymerase III

complementary # 2

DNA Polymerase III

DNA strands serve as a template for replication. Double stranded DNA unwinds and a complementary strand is produced with the help of DNA polymerase.

Source: Ophardt CE. *Virtual Chembook*. 2003. Available online at: *http://www.elmhurst. edu/~chm/vchembook/582dnarep.html*.

A complementary strand of messenger RNA is produced from the DNA template. The synthesis is aided by RNA polymerase.

Source: Ophardt CE. *Virtual Chembook*. 2003. Available online at: *http://www.elmhurst. edu/~chm/vchembook/583rnatrans.html*.

Figure 4. RNA Transcription

Non coding DNA

Template DNA

New RNA

uracil nucleotide

RNA Polymerase

Molecular Biology 101

To understand the tests used commonly in the care of the oncology patient it is important to first review the role of DNA within a cell. DNA stores all the genetic information for the cell. It is housed within the nucleus and made up of a sugar, phosphate, and a base. The sugar and phosphate portion of the molecule form the backbone while the bases pair in specific and unique ways. DNA uses four bases: adenine (A), guanine (G), thymine (T), and cytosine (C). A DNA strand's sequence is determined by its collection of bases. When taken together as a unit, the complex of the sugar-phosphate-base is called the nucleotide.

In human cells, DNA exists as two anti-parallel strands wrapped around each other forming a double helix. The two stands are held together through extensive hydrogen bonding between the nitrogenous bases; adenine (A) always pairs with thymine (T) and cytosine (C) always pairs with guanine (G). DNA is packaged in cells in the form of chromosomes, which are very dense and associated with proteins. The human genome is approximately 30,000 genes found on 23 pairs of chromosomes. Every offspring possesses a maternal and paternal copy of a gene and, therefore, two alleles or versions of the same gene.

Replication. The ability to self-replicate is one of the important properties of DNA. Each DNA strand serves as a template for producing a complementary daughter strand. Replication occurs during S phase of the cell cycle and allows cells to rapidly divide. DNA itself forms the template on which it copies itself and thus is a semi-conservative process. For DNA replication to occur, the double-stranded DNA molecule must first unwind. After it has unwound, replication takes place along each strand of DNA. The process is initiated by the enzyme DNA polymerase. This polymerase synthesizes new DNA by adding nucleotides that are complementary to the DNA template strand. The final result of DNA replication is

Figure 5. Production of Proteins
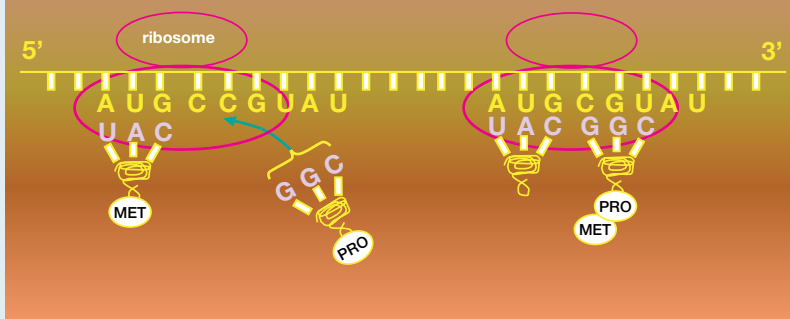
Transfer RNA carries amino acids (MET = methionine; PRO = proline) to the corresponding codon on the messenger RNA. Ribosomal RNA proceeds along the mRNA, allowing the amino acid chain to elongate until a stop codon is reached.

Source: Adapted from Ophardt CE. *Virtual Chembook*. 2003. Available online at: *http://www.elmhurst.edu/~chm/vchembook/584proteinsyn.html*.

the formation of two "new" strands of DNA that are exactly complementary to the two "old" strands (see Figure 3 at left). This method of replication allows new cells to have the exact same copy of the DNA that was in the old cell.

Protein production. DNA is also the molecule that directs the production of proteins within the cell. For this process, DNA acts through an intermediary—ribonucleic acid (RNA). RNA, like DNA, is composed of a sugar, phosphate and base; however, unlike DNA the bases in RNA are: adenine (A), uracil (U), guanine (G), and cytosine (C). These bases combine in specific ways, A with U and G with C.

The process of protein production begins with the transcription of messenger RNA (mRNA). The double helix of the DNA is unwound so that one strand of the DNA can serve as a template for the RNA synthesis. Synthesis is begun by the enzyme RNA polymerase binding to specific promoter regions of the DNA. The enzyme then rolls along the DNA, adding complementary bases until the "stop" signal is reached (see Figure 4 at left). This

process all takes place in the nucleus of the cell. Once production of the mRNA is complete, the next steps in protein synthesis occur: translation of mRNA into proteins.

Translation. Translation is mediated by two other types of RNA: ribosomal RNA (rRNA) and transfer RNA (tRNA). In transcription, RNA polymerase reads one gene and produces a single stranded mRNA. The mRNA is processed, exits the nucleus, and associates with a ribosome in the cytoplasm. Translation occurs outside the nucleus. With the help of ribosomes (rRNA) and tRNA carrying amino acids, the mRNA is translated into a polypeptide. The mRNA is made of nucleotides arranged in units of three bases (a codon). A codon represents a single amino acid. tRNA matches the codon of the mRNA to the appropriate amino acid it represents. Amino acids linked together form a polypeptide (see Figure 5 above).

Proteins are simply polypeptides or multiple polypeptides joined together. Proteins are the major players in carrying out processes in the cell.

at the molecular levels of what happens within the cancer cell. Bottom line: the future of oncology care will require practitioners at all levels to understand genetics and molecular biology in order to care for their patients. ◾

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