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A PRIMER OF GENETIC ENGINEERING I: BASIC STRUCTURAL COMPONENTS OF THE CELL.

*Cheryl Y. Bardales, Ph.D.**

Good morning. I would like to thank the sponsors and organizers for allowing me the opportunity to participate in today's symposium.

I'd like to start out by defining us, biologically, as being very complex organisms which are made up of many different subunit parts. For example, our bodies are made up of many different types of tissues and organs, such as the liver. These tissues and organs are, in turn, made up of smaller subunits which are called cells.

Cells can be thought of as being tiny factories which contain the machinery for carrying out the functions of life. The cells in our body carry out functions that are not only common to all the cells in our bodies, such as cell growth, and cell division, but, in addition, carry out specialized functions that for example, make liver cells liver cells and nerve cells nerve cells.

All living matter is made up of cells, and there is surprising similarities among different types of cells. For example, prokaryotic cells include bacteria such as *E. coli. E. coli* is a bacteria that's used quite commonly in scientific laboratories for studying cellular properties. Eukaryotic cells include all the cells in our bodies, such as liver cells or nerve cells.

We can grow both of these types of cells in the laboratory. In the case of bacterial cells, we can grow them either on a solid substrate in a petri dish, or we can also grow them in a liquid medium in a flask.

In the case of multi-cellular organisms, such as ourselves, the cells must first be disassociated away from the organ of origin, such as the liver. Cultured cell lines are then derived from a single cell. Therefore, each cell line constitutes a homogeneous cell mass.

These cells and cultures do not carry out the specific properties or functions of specialized cells. Rather, they're used to study the properties of cells that are common to all the cells in our body. Cell lines that were derived from a single cell, that was taken directly from the organism of origin, in this case liver, are what's known as a primary cell culture.

Primary cell cultures have a definite life span in culture. They will grow and divide only a certain number of times until they undergo a process known as senescence, followed possibly, by cell death. Occasionally these cells in culture acquire abnormal phenotypes, for example, the ability to grow indefinitely in cell culture. This property is known as immortalization and is thought to be one of the early steps that occurs in the development of a cancer cell. Immortalized cell lines constitute what's known as a secondary cell line.

The eukaryotic cells in our body contain two major subunit compartments: the nucleus and the cytoplasm. Both the nucleus and the cytoplasm are contained within the cell by a membrane, known as the plasma membrane. The cytoplasm contains many individual subunit compartments that carry out the cell's functions, such as the production of cellular

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energy. The nucleus can be thought of as being the brain of cellular operation.

The nucleus contains what is often referred to as being an instruction manual, which contains all the information for carrying out a cell's function. This instruction manual is what's known as the cell's genome. Each cell in our body contains the same genome inside, or instructional manual, the nucleus, but different cells in the body use different parts of this instruction manual to specify that cell's specific functions. This same instruction manual located in the nucleus allows a one-cell embryo, the fertilized egg, to become a fetus and a newborn child.

The cell's genome, or instruction manual, is made up of a molecule called DNA. DNA is in the shape of a double helix, which is made up of two strands wound around one another. It is often called a DNA ladder.

DNA is written in an alphabetic code, using four letters known as bases, which are shown by their initials A, C, G and T, as indicated on the interior of that double helix. A always pairs with T, as demonstrated in the top of the molecule, and C always pairs with G. A and T, and C and G, are what's known as base pairs. It is the precise sequence of these four letters in the DNA ladder that conveys biological information to the cell, much the same way that the letters in a word convey meaning.

The instructions that are located in the genome of our cells are organized into smaller units called genes. Genes are the functional units inside our cells and there are over 100,000 genes in every cell genome in our bodies.

I'd like to turn now to how the cell uses the instructions in this instruction manual to carry out cellular functions. DNA passes on its instructions by unwinding and using each of its two strands as a template for the synthesis of a new biological partner known as RNA.

RNA can be thought of as being the biological messenger in the cell. RNA conveys the information from the nucleus to the cytoplasm, where the information that is now carried in RNA's four letters, A, U, C and G, are copied into what's known as protein molecules. Proteins carry out the active role in a cell's functions.

Because the biological effects of DNA depend on the linear sequence of the four letters, DNA research relies heavily on techniques that reveal and compare these sequences. We can determine the sequence of the four letters in a piece of DNA, by a technique known as DNA sequencing.

The DNA must first be cut into smaller fragments and then the letters in each of these small fragments can be determined chemically. To do this, the pieces of DNA must be separated on a gel matrix. This gel matrix is similar to a slab of gelatin. The position of these fragments on the gel tells us the sequence of the piece of DNA.

DNA hybridization techniques make possible the direct comparison of sequences in different pieces of DNA and the detection of specific DNA sequences in a mixture of many different sequences. The DNA must first be broken into smaller fragments and separated on a gel matrix. The techniques used are very similar to those described before for the DNA sequencing technique.

The DNA fragments must then be transferred to a filter paper. This filter paper, now containing the DNA fragments bound to it, are then incubated with a smaller piece of DNA that is radioactively labeled. This radioactively labeled piece of DNA is what's known

as a probe.

If the radioactive probe finds a DNA sequence on the filter paper that is the mirror image of it, they will bind to one another. We can then determine the frequency of this occurrence by the amount of radioactivity that's bound to the filter paper.

Analysis of DNA and DNA sequences has allowed us to identify mutations or alterations in the base sequence of an organism's DNA. These mutations can take several different forms. For example, the top red DNA ladder indicates a normal DNA sequence, while the second DNA ladder indicates that a single base change has occurred. In this case, the base C has been changed to the base T. Additional alterations that can occur include the addition of DNA bases as indicated by the addition of the ATCGT fragment, and the deletion of bases.

These mutations serve as markers that allow us to specifically detect genetic diseases. For example, the disease cystic fibrosis may result from the deletion of three adjacent base pairs of DNA, as indicated in the bottom DNA ladder. As in the case of this particular disease, this very simple base change can have profound effects for the individual. Both these normal and altered DNA fragments can be cloned and examined in the laboratory and these techniques will be discussed in the next talk by Dr. Michael Roberts.

I will stop here and entertain any questions from the audience. One question that's often asked is that if every cell in our body contains the same instruction manual, and contains the same genome, how do different cells perform different functions?

Well, the answer is, even though every cell in our body contains the same 100,000 genes, not all of those genes are copied into protein molecules. Remember that the protein molecules inside our cells carry out the active functions. So, in certain cells, only certain protein molecules are expressed and functional.

Member of the Audience: And how does that cell know which one to express and function?

Dr. Bardales: That's a very complicated question and we could spend probably several hours just discussing that. The cell has many different types of control mechanisms, control sequences, even control proteins, which are responsible for turning on and turning off different genes. So the cell has built into its nucleotide sequence these mechanisms which allow it to determine what genes should be on and what genes should be off. Everything is detailed in that four-letter base code of the DNA sequence, the information for expressing proteins, and the information for expressing what proteins.

Member of the Audience: What are the mechanics involved in isolating a particular gene on a particular chromosome? I frequently read about how they are sure a gene is in a certain area of the DNA sequence, but how do they go from that to finding out which gene it is?

Dr. Bardales: That's an excellent question and that's something everyone should have a basic familiarity with, but I'm going to defer your question to Dr. Michael Roberts. He's going to talk about some of those techniques in the following talk.

Member of the Audience: You said there were 100,000 genes. How big is the average gene?

Dr. Bardales: The average gene really varies from several thousand base pairs to several hundred thousand base pairs. The entire genome contains over three billion base pairs. So it's rather large, and genes vary in their size.

Member of the Audience: You spoke of immortalization of the eukaryotic cells. That's only a phenomenon in eukaryotic cells. Aren't prokaryotic cells immortal to begin with?

Dr. Bardales: To my knowledge, all cells contain mechanisms that specifically define their life span. All cells control the amount of time in which they divide and grow, and all cells eventually do die and are replaced. It's only in the abnormal property of a cancer cell that those cells acquire the ability to grow indefinitely in culture in the laboratory.

Member of the Audience: That's true, because bacteria do not seem to have any mortality, in terms of the number of generations.

Dr. Bardales: Bacteria are very prolific. We can grow billions of them in the laboratory.