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From Gene to Oncogene

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The topic of this lecture -- from Gene to Oncogene -- is both historical and personal: historical because I wish to recall the story of a concept -- the gene concept -- with fifty years since the beginning of molecular biology; and personal, because fifty years ago the gene took me in its wake and molded my life, making a naive young physician into a molecular biologist and causing me fifty years later to end my career as a cancer research administrator.

The cancer of the gene itself was stupendous. The rise of molecular biology has been one of those exhilarating periods in the history of science in which a whole branch of science seems to fall into place. For molecular biology the process has been even faster than

for other sciences: what are fifty years compared with the course of mechanics to Galileo to Newton and _____ or that of chemistry from John Dalton to _____ _____ -- that is, from the first formulation of models to their establishment as all embracing theories?

Concepts are inventions that scientists produce in order to give body to their theories. Galileo's acceleration, Newton's mass and force and _____ waves were concepts of accidents (in the scholastic sum of the word), that is, of properties of the nation of matter. Dalton's alone was a concept of matter itself, included to explain gas pressure and the proportions of elements participating in chemical reactions.

The gene concept emerged form Mendel's algebra (and his rediscoverers) a concept of matter, the substance of heredity, a concept as naked and as perfect as Botticelli's Venus, and equally enigmatic. What kind of matter? Heredity from one generation to the next was atomized into the statistical assortment of _____ of discrete units, two copies of each in freely _____ pairs. Two copies of a unit could be sufficient enough to change shape or color or size of an organism but not to alter the plan, that is, the intrinsic program of the organizer. Organisms were born, developed, died, disintegrated; but through the process of reproduction genes escaped the fate of decay -- stable not as atoms are stable but because of _____ copied identically from one gener-

ation to the next and from one cell to its daughter cells -- stable while all other substances of living cells and organisms were in a state of chemical and structural flux.

Stable yet unstable only because of its ability to change could the existence of any gene be recognized (until molecular biology made other approaches possible). It had to exist in at least two alternative forms when assortment followed Mendel's algebra. From the work of Morgan, Sturbevant, Bridges, and Müller it became known that genes were linked together in _____ sequencing -- the chromosomes -- the sequence being itself almost perfectly stable. Heredity was determined by thousands of almost perfect pearls strung in a few almost perfect necklaces -- fit to adorn the body of

Botticelli's _____.

Yet, not pearls nor any other known substance. Until the late 1930's no one could do more than guess what genes really are. Then physics developed into quantum mechanics, and Heither and London developed the theory of the covalent bonds between atoms in molecules. And in 1937, the physicist Max Delbrück proposed that the stability of a gene was similar to the stability of molecules in general. Genes were molecules, large molecules, and mutations including those induced by radiation, were changes in the gene molecules, that is, chemical changes.

I do not know how many scientists were electrified by Delbrück's model of the gene. It influenced the great physicist Schraedinger

to write years later a famous book, What is Life?. At a humbler level, Delbrück's model influenced a young physician, myself, who was naively dreaming of joining physics to biology (knowing little of either) into jumping into the fray. I decided that I would try to test Delbrück's model of gene mutation by studying the action of radiation on organisms or agents as akin to a gene as possible. Thus I encountered bacteriophages, the viruses that pray on bacteria. And soon, with excitement and some trepidation, I discovered that Delbrück, who had migrated to the United States, had also by a strange chance started to work on bacteriophage. Only at the end of 1940 did Delbrück and I get together and joined forces.

The 1940's may be considered as the

"heroic" period of molecular biology by analogy with the heroic periods in history that precede the full flowering of each civilization. In 1941 Beadle and Tatum announced their first successes in the pursuit of the one-gene-one-enzyme theory, correlating individual genes with individual steps of metabolic pathways. Delbrück working together on bacteria and bacteriophage provided in 1943 the first genetic analysis of bacterial mutations, opening up the genetics of bacterial cells. At about the same time, Avery, MacLeod, and McCarty announced the identity of a Transforming Principle that changed the heredity of the pneumonia bacillus as DNA. The dream of convergence between genetics and biochemistry began to materialize.

Convergence, but not yet fusion -- not yet a _____ molecular biology, lack of a molecular model for the gene. We may compare the situation to that of planetary theory after 1620, when Kepler published his laws of planetary motion. The planets move in planetary ____ around the sun -- but what drove them to it? God? chance? or their own will? Fifty years later, Newton provided the explanation by inventing the concept of universal gravitation. In 1953 Watson and Crick discovered, or actually "invented" the structure of DNA -- the double helix. The convergence of genetics and biochemistry into molecular biology was an accomplished fact. The gene, the mysterious lump of genetic matter, the atom of Mendelian heredity, became a sketch of DNA fiber, more

akin to segment of computer tape than to a pearl in Venus' necklace.

Delbrück's hypothesis of mutations as chemical changes of gene was soon confirmed. Reshuffling of genes in chromosomes and even of parts of genes of recombination could be interpreted in chemical _____. And earlier interpretations of the gene as either a unit of function, or a unit of mutability, or of recombination were immediately reconciled.

In gaining chemical identity, genes lost their splendid isolation. Being part of a _____ -- DNA files in which all genetic information was stored in the four-letter alphabet of nucleotides -- a gene had to be "read" in order to be translated into the alphabet of proteins -- 20 amino acids. And

the reading of gene had to be responsible to the needs of the cell, that is, to the environment which the cell had to cope with, whether the external milieu or an internal one for cells of complex organisms. Thus, new lives of discovery grew, which together marked the triumph of Escherichia coli, an humble bacterium, which I take some irrational pride in having pushed into a career in molecular biology Escherichia coli, and its bacteriophages, were the protagonists of the major advances: the transcription and translation of the information of genes from DNA into RNA into proteins; and the regulation of the processes by classes of DNA-combining proteins -- repressors or activators -- controlled by enogenous substances.

By the middle 1965 the molecular biology of Escherichia coli, however, still incomplete in detail, had reached a satisfying state. Soon, however, it became clear that the dictum "What holds for Escherichia coli held, also for elephants" was somewhat optimistic. Elephants, or fruitflies, or humans have all that Escherichia coli does, plus something more. This something was what fifty years earlier geneticists and molecular biologists had deliberately put on a shelf for further attention -- embryology. Complex organisms have more than just many cells. They have a program, that is, a plan that makes it possible for cells with identical genetic information to become functionally and structurally different in different parts of an organism. Molecular

biologists tackling such complex organisms had to go back to the shelf and reexamine what they had ignored. They had to reverse the so-called Morgan derivation, that is, the choice by Morgan and his followers after 1910 to concentrate of genetics at the expenses of embryology and differentiation.

The molecular biology of the cells of complex organisms has provided a number of surprises. Some of these have been relatively easy to fit as extensions of the molecular biology of Escherichia coli, adding to it new complications. Thus the discovery of reverse transcription -- from RNA to DNA -- and that of enhancers of glue transcription did not change our view of the gene. Another line of discovery -- the presence within genes of

_____ whose information was not used but "spliced out" _____ before translating RNA _____ into protein sequences -- cut deeper into the gene concept itself. The gene that makes a protein is an expurgated region of the gene in the chromosome, freed of nonsense "junk" whose evolutionary significance remains a matter of speculation.

These complexities of molecular biology turn out to be of little relevance to the problem of development and differentiation, however. Contrary to what might have been hoped, gene splicing turned out not to be organ specific: it does not account for the differential expression of a given gene in different sets of cells. It throws no light on the secret of embryology: the preparing for differ-

ential gene expression is different cells of a plant or an animal.

The central problem of embryology may be posed as follows. At some points in the development of an organisms each line of cells becomes programmed (by evens intrinsic in the heredity of the organism) so that its cells will finally express certain genes and not to express others once the organism grows into its final form. For example, the nuclei of cells that will give rise to muscle fibers become programmed so that once in a muscle they will express genes that make contractible proteins and to lose expression of genes that function in cell division. Cells of the mammalian liver become programmed so that in the normal liver they express genes for liver proteins. They

still can make proteins needed for cell division but are prevented to do so by a signal that is made by the normal liver itself and that gears the total amount of liver cells to the normal requirement of the adult organism.

More generally, in a complex organism each cell is exposed to a more or less precisely regulated flow of chemical signals coming from other cells. Some of these signals are _____, many of which have already been identified. Other are "factors" that is, proteins or peptides whose presence is revealed by their effects on specific cells; many factors may turn out to be short-lived chemical substances appropriate for communication between adjacent cells; _____, some signals may be transmitted by physical contact between cells

without intervention of soluble substances. Any given cell will respond to certain signals if it has been programmed to express appropriate "receptors." The receptor protein convey signals to certain sets of genes turning them on a oft or modularity the level of their activity.

In this schematic view, the _____ of cellular differentiation becomes less mysterious: it consists of "marking" different clones of cells with the capacity to express specific sets of receptors that will determine specific sets of responses. The prescribed responses will consist of the activation of specific cell functions through regulation of gene activity. In some instances, the response can be a growth response. That is, cells are

stimulated to enter the "growth" cycle, duplicating their DNA and undergoing cell division. Alternatively, they may be restrained from growing and dividing.

Of these controls over cell responses, especially over cell growth and division, little was known until recently. The molecular biology of cancer may throw light in this area. Cancer cells differ from their normal counterparts both because of differences in function and because of altered growth capacity. Cancer cells have lost the discipline of the organism; they fail to respond to the signals that keep normal cell functionally and reproductively in their places within the plan of the organisms. This failure turns out to be due to the presence in cancer cells of certain altered genes

called oncogenes.

Evidence for the existence of oncogenes came first from the study of tumor-producing viruses, each of the viruses that causes leukemia or lymphoma turned out to be one gene whose presence was sufficient to induce the transformation of normal bone marrow cells into cancer cells. Loss of the oncogene made a tumor virus non-oncogenic. Other tumor viruses were found to have not one but two oncogenes which contributed to transformation into cancer cells. The presence of each of the viral oncogenes in tumor cells could be recognized by the presence of corresponding proteins detectable by serological tests.

It soon was clear that the transforming activity of viral oncogenes had no necessary

relation to their being part of viruses. Oncogenes were close relatives of normal cell genes (or proto-oncogene) whose presence could be discovered by the homology of their DNA sequence to that of oncogenes. From proto-oncogenes, the oncogenes had originated by mutation and had then been picked up by viruses, which transferred them from cell to cell or between organisms.

Next the activities of oncogene proteins were explored. Some turned out to be enzymes with altered specifications, for example, the ability to add phosphate residing to certain sites of cellular proteins. Others were enzyme proteins that had lost their catalytic activity. How did these altered functions explain the transformation of normal cells into cancer

cells?

More clues emerged when oncogenes were found not only in viruses but also in the cells of spontaneous cancers, especially human cancers, simply by extracting their DNA and transferring parts of it to normal cells in culture. Some of the recipient cells became cancer cells and if introduced into appropriate animals gave rise to cancers. Thus many different oncogenes were discovered, a few of them related to the viral ones. Among them are oncogenes that bring us closer to the central problem, how cell growth and cell division are regulated, and how oncogene alter that regulation.

Among the signals to which cells respond is a set of substances called growth factors. These are produced in different body organs and

their growth stimulating activity is specifically exerted on one or another class of cells. Their activity is to stimulate the specific target cells -- for example, epidermal cells -- to grow, enter the growth cycle, duplicate their DNA, and divide. Growth factors act on cells by combining on their surface with specific receptors for that factor. A receptor thus activated conveys to the cell nucleus a signal that triggers directly or indirectly the activity of genes that regulate cell growth. Such an activation can be recognized by the appearance of increased RNA or protein from the strain labeled genes. If a cell is a normal one, it's response to a growth factor remains within the limits of the needs of the organism. For example, epidermal growth factor may cause

regeneration of wounded skin epidermis until the wound is repaired. In cancer cells, however, growth responses are disorderly.

Certain oncogenes produce abnormal proteins related either to growth factors or growth factor receptors. If secreted, an abnormal growth factor can stimulate the receptors on the cell surface. Other oncogenes are altered forms of genes for receptors and make membrane proteins that behave as if they were permanently activated receptors, pushing the cell to grow and divide without responding to external controls: the cell becomes a tumor cell.

The role of oncogenes is upsetting the pattern of cell response to growth factors is one part of the story. There remains a second

part, the identification of the genes that in the cell nucleus respond to the signals and triggered cell growth and division. How is the expression of these genes regulated in normal cells and not regulated in cancer cells?

Here it is appropriate to recall that the problem of control of the cell cycle is still a biochemical mystery. Even for Escherichia coli we only know that initiation of a cycle of DNA replication is regulated by the nature and amount of nutrients; the set of genes that respond to this regulation is poorly known. Only for a few DNA elements such as plasmids we probably know all the component of the replicative response.

Oncogenes have begun to give us clues about growth control mechanisms of the mam-

malian cell. Among the oncogenes isolated from human cancer or from tumor viruses, there are some whose protein products are found within the nucleus itself. That is, after being made like all cell proteins in the cytoplasm, these oncogene proteins return to the nucleus. This is what we would expect of regulatory proteins, that "talk to genes" by combining directly with specific DNA sequences.

A further development has been the discovery that oncogenes with nuclear proteins can act in collaboration may with oncogenes with cytoplasmic proteins. Most oncogenes isolated from cancer cells were first recognized by their ability to transfer cells from established cell lines, but failed to transform newly cultured embryonal cells. The

established cultured cell were already capable of indefinite reproduction, potentially immortal. Transformation made them insensitive to growth inhibition from medicine components or given cell-to-cell contacts. Embryonal cells could be transformed only if two oncogenes were introduced into them. A successful pair had to consist of one nuclear-protein oncogene and one cytoplasmic-protein oncogene. The nuclear acting protein provides one immortalizing function: it makes the cellular response to the other oncogene a permanent growth response. It weakens the capacity for uncontrolled multiplication characteristic of the cancer cell.

The two-step mechanism of transformation of cells to cancer cells has several implica-

tions. It puts a solid genetic basis under the large body of observations that suggest a dual-step mechanism in the origin of cancer. And it opens the possibility of detecting the activity of single oncogenes within body cells before the second step of cancerous transformation has occurred.

As the standpoint of cell biology the two-step mechanism promises new insights. The failure of embryonal cells to become transformed by a single oncogene suggest a possible step in cell differentiation in the course of embryonal development. Cells that become committee to differentiation may lose "immortality" which can be restarted by the action of an exogenous oncogene. Differentiation may then imply limitation in cell lineage: in

culture, limited number of generations; in the body, dependence on specific growth factors.

The two classes of oncogenes suggest a model of cell regulation consisting of two separate but interactive levels. One of cellular responses to external stimuli, the other level dealing with responses within the cell nucleus. We may envision a double network, a cytoplasmic one modularity and transmitting signals from the outside, and an nuclear one regulating the extent of gene response to the signals. An oncogene expressing a cytoplasmic or membrane protein will disturb the first network and may cause among other effects, an excessive stimulation of a subset of genes concerned with cell growth. But the stimulation may remain within normal limits and fall short of cancerous

transformation unless the nuclear regulatory network has itself been altered by another oncogene.

Transformation may in a sense be seen as the opposite of differentiation, a reversal of the original "marking" of cells for orderly response to external signals.

The above model, however, speculative, is interesting for one additional reason. It suggests that oncogenes may become a major source of information concerning all genetics, particularly human cell genetics. Oncogenes are not infinite in number: among the first score or so, several repeats have been found. Clearly, only a small subset of the thousands of human genes can generate oncogenes. This may be the subset of genes that ____ at relevant nodes of

the networks, either as conveyors of signals or as controllers of responses. Through the analysis of many oncogenes of the altered cell functions they encode t should be possible to identify the components of the networks. Then we may also discover new sets of signals, such as short-lived soluble factors, from the response they evoke (or fail to evoke) in oncogene carrying cells. We may find in cancer an unexpected source of insight into human genetics.