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1989 or 1990

Genetic Engineering
or
The Splice of Life

Although at first thought a title such as The Splice of Life would bring to mind a sexy bit, and I suppose this would be true in this discussion only at a one-cell level, really I would like you to think with me as we review some of the basic consepts of genetics, of unbelievable advances in the field and of its application to modern life. I'm not going to end up telling you how to clone a human and how scientifically impossible it would ever be to bring forth an exact replica of any one Dutchman. You know even if it could be done who would be willing to finance such a cloning ?

What has awakened interest in the field of genetics is the new area of genetic engineering with the hundreds of millions of dollars that are being invested in search of a corner on humanity's future. What has them excited is the new technology of recombining DNA or gene-splicing and the prospect that it might be useful in the next decade to develope new resistant vaccines, more bountiful food supplies, even a substitute for oil and in cancer research.

The molecule DNA (deoxyribonucleic acid) as the basic hereditary material was established in 1944. The genetic information always has been ~~was~~ transferred between strains of micro-organisms simply by transferrence of DNA from one to another. So, DNA carried the genetic information and not the proteins. The structure of DNA was hypothesized in 1953.

In the early 1970's research groups working with the bacterium Escherichia coli uncovered possibilities. They learned (1) that the E. coli genetic material was a long strand of DNA the ends of which were joined together to form a circle. The E. coli cells could also carry other smaller circles of DNA known as plasmids. Plasmids are infectuous in that they can be passed from 1 E.coli cell to another and to related species and sometimes to even more

(1) - Diagram

distantly related bacteria. The plasmid DNA can be isolated by separating it from other cell substances in a centrifuge and the plasmid DNA can be re-introduced into bacterial cells.

The second research thread was the isolation of a class of enzymes called restriction enzymes. These can recognize a particular tiny segment of DNA molecule and cut the molecule at that site. Other enzymes called ligase enzymes can fuse together the free ends of 2 DNA molecules. Both kinds of enzymes can be extracted from some bacteria.

The third thread is the ease with which DNA can be purified from many organisms, plants, animals bacteria and viruses.

Foreign genes can be introduced into bacterial cells and in 1974 a gene from a toad was transferred into E. coli. The circular plasmid DNA molecule was cut with a restriction enzyme, the opened circles mixed with fragments of purified DNA from the toad and then using ligase enzymes, chemically linked the ends of the DNA fragments with the open ends of the plasmid DNA. It was like a jewler enlarging a ring by snipping the ring open, putting material in the gap and fusing the ends together. The new DNA ring too was larger--by the length of the toad gene.

These discoveries prompted The Natural Academy of SCiences to appoint a committee to study the problems that might arise in the applications of genetic tampering. Many investigators were concerned with the potential hazards and felt that safeguards principles should be set up and even that a voluntary ban on certain experiments was manditory. These fears proved groundless after suitable investigations.

Now I would like to digress for awhile and try to explain more about the fundamental molecules, their arrangements and interactions that take place in gene transmission in all forms of life. The simplest organisms are simple, free-living cells, and the cell is the basic unit of organization of all multicellular organisms.

The basic processes involved in the assembly of any cell appear to be fundamentally the same--a cell is primarily concerned with the synthesis and assembly of proteins. If we can think back far enough, we may recall that a cell has a nucleus with cytoplasm about it. In the nucleus (just like the front office in a company) much of the direction of a cell's activities occur. Here is the chromatin material (called chromatin because of the dye it takes up on staining the cell) later identified as containing chromosomes, which scientists have worked at and mulled over for about a hundred years. It was a great day when it was realized that somehow the chromosome could transmit characteristics from one generation to the next. One of the enigmas stated in 1910 "Since the number of chromosomes is realtively small and the characters of the individual are very numerous, it follows on the theory that many characters must be contained in the same chromosome." This seemed impossible and no explanation was forthcoming for some time. The genetic material in the chromosome (genes) was first called nuclein, later nucleic acid and finally deoxyribonucleic acid (DNA). ~~So, the DNA carried the genetic information and not the protein.~~ This is really microbiology for The DNA molecule consists of a double twisted chain or helix which when stretched out is 1 mm in circumference and is stuffed into a cell where the diameter is 1/1000 mm.

diagram

Slide (5)

(6) Structure DNA

In cell division, when DNA duplicates itself, it must involve the untwining of the double helix, leaving the bases that would normally be hooked together exposed to the contents of the cell's nucleus. This chemical warehouse contains the ingredients to create bases complementary to those exposed and by a specific pairing mechanism, 2 identical DNA molecules are produced (Mitosis) and when the copying isn't accurate, mutations are ~~produced~~ possible. The genetic information in the DNA molecule ~~are~~ contains the working instructions for the cell in its manufacturing of protein.

Slide (6) Replication diagram

In the primeval phase of evolution it is probable that amino acids in the environmental organic medium underwent spontaneous grouping together or polymerization to form peptide and polypeptide chains, in a process of self-assembly unaided by more than an input of energy. Yet it has been calculated that even if the whole earth had been made of nothing but amino acids which had arranged themselves randomly and completely 10 times a second, in the whole period of the earth's history there would have been little chance of ever producing one single molecule of insulin. Nature had to evolve the exquisite machinery of the system now generally known as the "central dogma" in order to direct within the confines of a cell the special assemblies of amino acids to form long-chain proteins with specific and useful properties. This system whereby biological information flows from DNA to RNA and then to a specific protein synthesis but never in the reverse order or direction is the ideological core of present molecular biology and accordingly of applied genetics in industry. How such a complicated mutually dependent system could have evolved is hard to imagine.

Slide (1)

As has been mentioned, the DNA in the nucleus carries the code. This information is taken out of the cell nucleus into the cytoplasm by one kind of RNA. From this point another kind of RNA begins the selection and assembly of amino acids following the code and with a specific enzyme for each amino acid required to form a string of connected amino acids, a polypeptide and eventually the protein molecule.

(8) DNA to ~~protein~~ polypeptide

(9) DNA to protein

When we consider the phantastic arrangement of mutually dependent reactions to form the structures that everywhere are about us and in us, we wonder why something doesn't go wrong more often than it does. We are all familiar with the selective "breeding in" of characteristics favorable to such industries as cattle, grain poultry and flora. The preferential selection of a desired strain took long periods of time because of necessary experimentation

took long periods of time because of necessary experimentation, gestation periods and maturation of the specie. Intentional mutations were induced by means of X-rays as early as 1927 and the discovery after 1945 of a wide range of other potent mutagenic and chemical mutagens gave microbiologists the tools to change genetic composition. Spontaneous mutations occur in only 1/100 million replications and at that are a random occurrence. In the mid 1940's it became possible to reshuffle genetic information by recombining genes from 2 or more organisms. After World War II, microbiological methods were found wherein new fermentations by micro-organisms for the first time yielded pure chemicals such as amino acids and nucleotides. Now we are up to the tremendous breakthrough in 1973 with the success of recombinant DNA and molecular cloning. Genes could now be transferred from any source into any micro organism. The techniques involved are powerful laboratory tools for revealing the structure and function of genes and potentially for the breeding of industrial strains of micro organisms that can make new fermentation products such as human insulin, growth hormones or new strains for improving fermentation products.

(10) Protoplast Fusion

(11) Homologous Recombination

The ability to isolate plasmid DNA from a culture and induce another culture to take it up is the basis of most recombinant DNA manipulations. A gene taken from an unrelated organism or an artificially synthesized gene can be spliced into a plasmid, the plasmid then introduced into a new microbiological host. The plasmid is thus a carrier for genes that can be passed indefinetly through successive generations as the plasmid reproduces. The DNA of certain viruses can also do this if they don't kill the host. In principle it should be possible to clone about any desired gene. Actually researchers take bacteria, viruses, animal or plant cells, break them apart and extract the DNA. Then enzymes cut the DNA

(15) Gene Splicing diagram

diagram

at specific points along its length. They then pull out a DNA fragment having the particular array of bases they want to study. This gene is linked to the DNA of one type of E. coli in the following manner. E. coli contains rings of DNA called plasmids. A plasmid is removed, its ring is opened with a restriction enzyme and the new DNA fragment inserted. An annealing enzyme, ligase, closes the ring and the plasmid is put back into the bacterium. Now cell division passes the new gene along to the next generation and in hours, there will be thousands of bacteria containing the hybrid new DNA. The new genetic clone colony will produce the specific protein determined by the inserted gene. Originally in 1973, a gene was inserted into E. coli that makes an intestinal germ resistant to the antibiotic streptomycin. In this way bacteria could be made resistant to antibiotics.

(13) Resistant gene

One of the critical steps is the restriction or cutting enzyme The DNA has to be cut at a certain spot. This enzyme can be isolated from bacteria or algae so that numerous cultures of the microorganism s are essential. The next step is to extract the enzyme from the cells.

Another problem is the need for fast reliable synthesis of DNA sequences 10 to 20 nucleotides long. These synthetic genes would have a known content, they could be labeled with a radioactive segment coupled with a natural gene and in this manner the natural gene could be isolated. Assembling a 20 nucleotide chain may take only 10 hours on the fastest machine but purification and verification of purity may take 2 more weeks. Basic chemistry approaches using phosphite and phosphotriester methods speed up the process so that a nucleotide can be added to a chain every 30 minutes. As we shall see later various research companys in the field are very competetive in providing and searching for newer better ways to obtain these new tools.

The discovery of the new science of genetic engineering has created an uproar in microbiological research laboratories both in industry and at the university. As more genetic manipulations succeed, the number of possibilities for this industry increase and the end is nowhere in sight.

In medicine genetic manipulation will unlock many of the mysteries related to genes. Each of the 100,000 genes in a human cell will be identified. The exact sequence of bases in a piece of DNA and the precise location of genes within a chromosome will be determined even though there are hundreds of thousands of possible combinations of sequences within genes. Because researchers have the ability to produce genes in enormous quantities they can finally study enough of them to map the pattern of the bases.

Biologists can tell how the total of 100,000 human genes fit in the 46 chromosomes. To do this, they clone a gene and mix it with chromosomes whose DNA spirals have been split down the middle. The DNA bases of the test gene automatically find their natural partners in the appropriate split chromosome, A to T and C to G etc Thus researchers will learn both (1) which chromosomes the gene naturally fits into and (2) where on that chromosome the gene normally rests. So this kind of gene mapping might make possible the cure of inherited diseases e.g. Sickel Cell Disease, and Hemophilia each of which result from a defect in a single gene. If scientists can locate the proper chromosome they could repair the defective gene or insert a properly functioning new gene into the cell.

Diagram

Check

One of the most startling discoreries by 2 independent U.S.A. groups is an answer to the question "What is the difference between a cancer gene and its benign physiologically indispensible version to be found in the normal cell ?" There is a gene which differs from the naturally occuring gene in a simple manner, the replace-

ment of 1 specific nucleotide in the DNA by another. The protein assembly governed by the gene differs from the normal. In bladder cancer cells, it was found that a normal amino acid is replaced by ~~another~~ an abnormal amino acid, ~~not normal~~. ~~The protein involved appears to be an enzyme~~. This is not a universal explanation of cancer. But it is marvelous that the techniques of gene manipulation uncovered a difference in normal & malignant cells ~~can be used to such purpose~~.

Another application to cancer study concerns the drug interferon. A victim of 1 kind of viral-caused-disease practically never comes down with another viral disease at the same time. The affected cells seemed to have been stimulated to produce something that interfered with other viral insults, and this substance remained behind when cells and viruses were removed. The verdict is not yet in on whether interferon is an effective anti-cancer agent. But in early 1980 the cloning and isolation of the interferon gene in a biologically active form, linked the recombinant DNA technique with the possibility of manufacturing this promising anti-cancer drug.

Insulin is a protein consisting of 2 chains of amino acids. In 1978 some fragments of the gene for insulin were assembeled and the synthetic gene for each of the 2 insulin chains were inserted into E. coli plasmid. Again, once the plasmids were put into the E. coli, the insulin genes responded and the bacteria began to turn out insulin chains. Normally $3\frac{1}{2}$ tons of animal pancreatic glands yield 1 pound of insulin. But the number of diabetics is increasing, the animal supply of insulin is not increasing, so the cost is very high. In addition 5 % of diabetics are allergic to animal insulin. The Eli Lilly Co. is building a $40 million plant to make this synthetic insulin and just recently the FDA gave approval for Humulin, the name for the synthetic insulin. However right now the cost of this is twice the cost of animal insulin. In the future it should be cheaper, and will be purer, non-allergic and the supply unlimited.

Other possible medical compounds that are being studied include the pituitary growth hormone, vaccines and opiate-like analgesics. In 1981 the first successful application of recombinant DNA technology in the diagnosis of an infectious disease, hepatitis-B commonly called serum hepatitis was announced. Serum hepatitis is a world-wide health problem with about 200 million carriers. The method may be applicable to detect other viral diseases.

In the herpes virus that affects the eye there appears to be a gene within the virus that determines whether virus production will be turned on for dormancy or for active infection. A recombinant that orders the system to go dormant would provide a tool to study latency. This gene in the recombinant herpes virus would multiply in the conjunctiva , induce an immune response without causing a corneal lesion, colonize the ganglia of the nerve and remain there permanently latent. If the ganglia were occupied at an early age by this latent form the cornea would never be affected. These same principles should apply to Herpes Type 2 Virus which is the venereal epidemic virus so alarming unpupular in these times.

The successful cloning of all the biosynthetic DNA enzymes needed to make 1 of the essential amino acids occurred in 1980. Although there are normally very stringent control circuits within a cell which prohibit it from producing more amino acids that necessary, through recombinant DNA techniques, specific genes were cloned and mutated to release some of these controls in the cell and allow the over production of amino acids. The same processes can be applied to the synthetic manufacture of other amino acids. Amino acids are food supplements for poultry, cattle and humans and the market is in the hundreds of millions of dollars per year.

There are numerous bazarre applications for the recombinant DNA. It would be possible to recover uranium, copper, tin and iron from low grade ores. A microbe called Thiobacillous ferroxidans

contains a gene that produces an enzyme that combines copper with other chemicals to give a soluble compound. From this compound, the copper can be leached out as solid copper. This same Thiobacillous could be implanted into organisms already living in uranium, iron and gold to follow the same plan. In the booze industry, carbohydrates & cellulose for centuries has been converted to sugar and then to alcohol, both steps by bacteria. But the relevant gene from 1 bacteria could be put in the other to perform the above in 1 step. In the fuel industry, some microbes contain genes that produce methane. These gas producing microbic genes, if transplanted into plants could turn leaves into gas wells. Researchers at Stanford are working on this. Our present gas and oil did come from plant breakdown as you know. Cheaper and E.P.A. approved fertilizers should be available in the future because there are bacteria that have genes that enable them to use atmospheric nitrogen. If these genes are spliced into plant DNA, the crops would be internally fertilized. For the dieters: a bacterium produces an enzyme that turna 100% corn syrup into fructose. Fructose is 1.5 x sweeter than glucose. ~~So, the enzyme producing gene is being spliced into a sturdier microbe.~~ . Dieters will be able to eat 1/3 less sugar with no decrease in sweetness. Finally, there are bacteria with genes that enable microbes to make a substitute for present day plastics, called Pullulan. These are delicate bacteria so more durable ones are being researched. Every aspect of industry is being investigated.

A great deal of money is at stake in biotechnology. By low estimates (1982) at least $ 500 million has gone into publicly and privately held new corporations. Some financial analysts say that those investments actually could be several times higher--and that is just looking at efforts under way at the relatively new resear-oriented firms and not counting how much has been invested in new programs at established chemical, pharmaceutical, and allied companies. The boom is only 2 years old but already talk of a shakeout is discussed. Money will be squandered here even though the people involved are far from stupid. The big gamblers are the venture capitalists. Venture capital investors can lure money away from

other investors for their own use. They tend to enter a company's developement early and often take a stance of staying with the company for 5-7 years. But in the case of genetic engineering, the time frame for realizing profits could be longer than 7 years. A venture capitalist in this field must have patience!

A majority of the 150 or so outfits that rushed into the field need capital to pay for their next research or developement steps. There have been no reported failures yet. But changes in the industry are evident. The depression and the uncertain stock market have scotched plans of many companies to sell their stock on the open market. Collaborative Research Inc. theought they would get $ 17 per share but settled for $ 11 and were happy about it. Genentech Inc. and Cetus Corp., two of the leaders possess sizable capital holdings raised in public offerings in the late 1980 and early 1981. But a lot of second-tier companies are going to run aground, either selling out to corporate bargain-hunters or just going belly-up. Examples of some of the companies, their interests and monies involved follows:

Genex Corp. (maryland) 75 million Sickel Cell Disease
Genetech Inc. (San Francisco) 70+ million Vaccine for Hoof and Mouth Dis.
Cetus Corp. (Berkley) 300 million Alcohol Production
Biogen (Swiss) Interferon
Merck Antibiotics
Upjohn Insulin
Eli Lilly Insulin
Du Pont Plant breeding

Other companies such as Bethesda Research Lab. Inc. are mainly interested in providing enzymes essential in the various steps in the genetic manipulations. Or, Vega Bio-Chemicals of Tucson Ariz. is an example of 1 of many companies interested in machine designs for the industry.

Still another ramification of the industry, many of the interested corporations have promised at least $ 240 million to those universities whose biomedical investigators can perform genetic alchemy. This figure equals the previous annual industry expenditure for university research in all scientific disciplines combined. In exchange the industrialists hope to obtain a host of genetically engineered drugs and biomediaal commodities with a projected commercial value of $ 100 billion from these academic investments. Private enterprise is here replacing academic monies that the federal government had previously supplied. And now we have the dilemna of the college prof. who may be offered stock in one of the

we have the dilemna of the college prof. who many be offered stock in one of the companies interested in his and his student's specific research. If he follows the direction of the companie's interests he will be financially rewarded but he and his department will lose the free spirit that is needed in basic research. This conflict of interest is a real thing and many academic biomicro-departments have had much difficulty over it.

Finally, this paper has been an attempt to review some of the cell mechanisms constantly going on within and about us. The new understanding of these, the manner in which normal function is being redirected spontaneously within the cell and by external forces such as radiation, trauma, poor nutrition and toxic exposures and deliberately by gene manipulation has been discussed. The exciting applications of gene splicing appears almost endless in theory. It will be most interesting to watch for the new substances and processes resulting from the gene machines that get into mass production and at a reasonable cost. As more of the complicated life within each cell of a living organism is being uncovered and with such understanding we can solve more of the problems connected with our medical, agricultural, industrial and waste problems. Ofcourse, there will have to be controls established so that the chance or deliberate production of material adverse to our culture will be prevented.

I'll never stop marveling that all of this has evolved with close to 100% percentage of perfection in the natural transmission of genes. What a mechanism! And to think that lower forms of life have the same purine bases in their genes, only of a different sequence & different length. (a forceful argument in evolution)