MENDEL TO BIOTECHNOLOGY

Books used:
Horace Freeland Judson: The Eighth Day of Creation.
James Watson: The Double Helix.
Erwin Schrodinger: What is Life: the physical aspect of the living cell.
John Brockman: The Third Culture: Beyond the Scientific Revolution
Richard Dawkins: The Selfish Gene.

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PART 1
A short history of the discovery of gene structure

Martin Hewlett from the Department of Molecular and Cellular Biology in the university of Arizona noted that by the sixth decade of the 19th century, all the pieces were in place for the transposition of a purely observational discipline into one with a strong theoretical basis. Some 80 years later, the field of molecular biology and its attendant sub-disciplines were grounded philosophically in a mechanistic, deterministic and reductionist view. In 1859 Charles Darwin published his Origin of Species and at the same time, Gregor Mendel, an Augustinian monk in Brunn, Austria was in the middle of his experiments with the breeding patterns of the common garden pea. It led to his publication of his laws of genetics in 1866. He described his conceptualization of discrete "factors," now called genes, as the units of inheritance.

Mendel had been trained in physics and chemistry at the University of Vienna and the monastery at Brunn was a center for scientific study. He had been stimulated by earlier observations made by botanists who had observed the products of crossing plants with different traits. His work, published in the Proceedings of the Brunn Society of Natural History, was all but ignored by his contemporaries. In 1902 deVries and his two colleagues discovered copies of Mendel's paper unopened among the papers of some prominent biologists, thus launching the field of genetics. The nature of the genetic material remained to be discovered. Mendel stated three principles. The first is that the sex cell of a plant or animal may contain one factor for each of different traits but not both factors needed to express those traits. The second principle stated that characteristics are inherited independently from other characteristics and the third stated that each inherited characteristic is determined by two hereditary factors, one from each parent that determines whether the characteristic will be dominant or recessive. Mendel analyzed patterns of inheritance of seven pairs of contrasting traits and, without going into details, showed by color and shape that there were dominant or recessive traits represented genetically.

It is only relatively recently that Mendelian inheritance patterns have been modified. When I was taking care of a large number of children with inborn errors of metabolism such as phenylketonuria, (PKU) I would explain the hereditary pattern to the parents using Mendelian rules. I must note however that I was greatly puzzled one year

by a sudden incidence of 5 infants in just over one month who were brought to my attention with this condition. They had been detected through the Ohio state newborn screening program. The statistical incidence of PKU is one in 10,000 live births, so this was unusual. In fact, I remember that I was participating in a University based genetics discussion in Columbus and my 5 patients made a huge peak in the annual statistics chart. I said then that there might be factors other than the pure genetics and this was, of course, not even discussed since genetically determined disease was considered to be by mutation of the gene. Later on in this presentation, I will show that this might well be something to be reconsidered.

In 1868, Miescher, a 24-year old Swiss went to Tubingen to study the chemistry of the cell nucleus and in 1869 he found a new, unexpected compound that was acidic, rich in phosphorus and made up of molecules that were apparently very large. He named it nuclein. What he had discovered turned out to be a complex of DNA and the protein normally associated with it in higher organisms. In 1870, Miescher isolateed pure DNA from salmon sperm and a pupil of his, Richard Altmann, introduced the term nucleic acid. The emphasis on it being acid turned out to be very important, as we shall see later. There was no basis for Miescher to guess at the function of this substance. The requirements of a science of heredity were still obscure, but he suggested in 1892 that some of the large molecules encountered in biology with a repetition of small chemical pieces could express the hereditary message "just like the words in a language construed from an alphabet." By 1900 it was known that nucleic acid contained 3 constituents, a pentose sugar called ribose, a phosphorus atom surrounded by 4 oxygen atoms called a phosphate, and base constituents built from nitrogen and carbon atoms. The bases were the central mystery of nucleic acid and were known as guanine, adenine, cytosine, thymine and uracil, often referred to by researchers as "GACTU."

By the 1920s, it was realized that there are two kinds of nucleic acid, now called ribonucleic acid or RNA, with the bases adenine, guanine, cytosine and uracil. The other was deoxyribose nucleic acid or DNA in which the uracil is replaced by thymine. In the early 30s, it became known that chromosomes are in large part DNA. The prevailing belief at that time was that DNA could only be some sort of structural stiffening like the laundry cardboard in a shirt, since the genetic material would have to be protein. Proof that the gene is DNA and not protein appeared in 1944 in a paper by Avery and his colleagues at the Rockefeller Institute and this paper is today universally cited as fundamental. At this time, Crick was working in the British Admiralty as a physicist designing naval mines and Watson a precocious college boy consumed by ornithology. When they met 7 years later, both knew of Avery's paper though for several years more it was generally believed that genes are protein since Avery's work was more or less ignored. The presence of protein in association with DNA confounded everyone for many years and its presence has only recently been detected for its importance, as we shall see.

Griffith, before Avery, had discovered that bacteria could transform from one type to another, but the mechanism was unknown. Using pneumococci, Avery and his coworkers had extracted something that they called "the transformation factor" and for the next decade he was increasingly preoccupied with step-by-step purification of the transforming agent from these bacteria. In defiance of the universal conviction that genes were protein, he proposed that the "transforming agent" was nucleic acid. Avery was a small man, a bachelor all his life and wore a pince-nez. He rolled his own cigarettes, was

fastidious with words, reserved with conclusions and was a gentle, versatile overwhelming monologuist for whom the pneumococcus was the microcosm of biology. Perhaps this might be seen in contrast to the bravado and aggressiveness of Watson and Crick, who when they started to work together, still believed that the gene was protein in spite of their knowledge of Avery's work.

An important figure in the history of discovery of DNA structure was Linus Pauling. His method of studying complex molecules was to build models, sometimes with pencil and paper at first, but eventually with precisely scaled physical representations of the atoms and one of Pauling's most remarkable contributions to molecular biology. His first Nobel prize was for his work on the structure of proteins. Although some of his rules turned out to be wrong, his insistence on reasoning rigorously from principles turned out to be decisive in the eventual solution of the structure of DNA.

In April 1948, Pauling was in bed with a cold and became bored. He called for paper, pencil and straightedge and drew the way a polypeptide chain would look spread out on a plane. He saw that if he folded the strip like a chain of paper dolls, the model represented a stable helix, somewhat like a spiral staircase. He was later able to use this paper model to work out the polypeptide molecule and called it the alpha helix. Watson, at the time, was in Europe doing experiments that, although not conclusive, heightened Watson's suspicion that DNA, not protein, was what genes were made of. Later, he attended a conference in Naples and was stimulated by seeing a slide in a lecture by Maurice Wilkins, a crystallographer from King's College, London. This was a new X-ray diffraction pattern from what Wilkins said was a crystalline form of DNA. Within a few months he went to Cambridge to study crystallography.

Wilkins had joined with Rosalind Franklin who, though an expert crystallographer, insisted that her photographs did not indicate a helical structure, thus misleading Wilkins in attaining the ultimate prize. The two had a deeply ingrained personality clash that made their cooperation virtually impossible. Pauling and Corey published a proposed structure for DNA, based on the alpha helix, some weeks ahead of Watson and Crick and got it wrong. Pauling's son, Peter, who was then working in Cambridge with Watson, received a copy of the manuscript from his father and showed it to Watson, whose report on this in his book "The Double Helix" is fascinating. The big error was that the structure was not an acid and Watson, in his rough style, pointed out that the greatest chemist in the world temporarily forgot basic college chemistry. Pauling knew of the X-ray photographs, taken by Rosalind Franklin, that were in the possession of Wilkins and wrote to him asking for prints. Wilkins replied that he was not ready to show them and an English historian of science, Robert Olby, later remarked that if Pauling had seen those photographs, he would have had the data for a correct structure of DNA.

It was these photographs that crystallized the concepts of Watson and Crick that enabled them finally to solve the structure of DNA. Borrowing from Pauling's work, they painstakingly built the model of the double helix, a structure of great elegance and beauty. Their paper entitled "Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid" appeared in Nature, (Volume 171, pages 737-738) on April 25th, 1953. It was a masterpiece of clearness and brevity, covering just over 1 page. The significance of this discovery was, as everyone now knows, the Pandora's Box that has led to extraordinary advances in our knowledge of hereditary phenomena. Watson and

Crick published a subsequent paper, again in Nature, entitled "Genetical implications of the structure of deoxyribonucleic acid" only a month later, in which they said--" The first feature of our structure which is of biological interest is that it consists not of one chain but of two. These two chains are both coiled around a common fibre axis etc." -- and " the important point is that only certain pairs of bases will fit into the structure. One member of a pair must be a purine and the other a pyrimidine in order to bridge between the two chains." After further discussion, "it follows that in a long molecule many different permutations are possible, and it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information." Later in the paper "our model of deoxyribonucleic acid is, in effect, a pair of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain, so that eventually we shall have two pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly." It is now known that the DNA of chromosomes is dispersed in the nucleus, but when a cell divides, the chromosomes condense and go through mitosis during cell division. Each half-chromosome replicates itself to create a new set in the daughter cell. When they are in this condensed state, known as metaphase, they are then visible in properly prepared cells and can be examined under a microscope. For about 30 years after chromosomes were discovered, it was thought that the number of chromosomes in the human cell was 48, but a researcher by the name of Tjio finally succeeded in preparing some cells when they were in metaphase and he found that all the unbroken cells in the preparation had 46 chromosomes.

When I was doing my residency at Cleveland Clinic in 1960, chromosome investigation was just beginning and it was my research project. The chromosome spreads were photographed and the prints enlarged. They were then all painstakingly cut out with scissors and paired to make up what is called a karyotype. Down's Syndrome, long a mystery, was the first human syndrome to be discovered with an abnormal karyotype. The specific feature is that all the cells of an affected person have 3 number 21 chromosomes, now known as trisomy 21. Many more conditions were later found with abnormalities in their chromosome complement.

In 1949, Barr and Bertram had shown that it was possible to determine the genetic sex of an individual according to whether there is a chromatin mass present on the inner surface of the nuclear membrane of cells with resting nuclei. This came to be known as the Barr body and was visible only in cells from females. It turned out to be one of the X-chromosomes and you will remember that females have two whereas males have one that is paired to a much smaller one known as the Y chromosome. It gave rise to the Lyon hypothesis. This stated that one of the two X-chromosomes in each somatic cell in a female is genetically inactivated. The Barr body represents the inactive one and this is now known to occur around the 16^{th} day of embryonic development. This inactivation, or silencing of a gene has now become of extreme importance in the new field of epigenetics to which this is all leading in my presentation.

Let us now look at the function of desoxyribonucleic acid or DNA and ribonucleic acid or RNA. Just as Watson and Crick forecast, DNA can replicate itself or it can transfer its message to messenger RNA that is then used to create a protein such as an enzyme. In replication of itself, during mitosis the double helix unwinds and the

strands separate, each one taking on a replication of itself to provide an identical copy for the daughter cell. Messenger RNA is synthesized in the cell nucleus by transcription of DNA. As in replication, a small section of DNA double helix unwinds and the bases on the two strands are exposed. Ribonucleotides line up in the proper order by hydrogen bonding to their complementary bases on DNA. The nucleotides are joined together by a DNA dependent polymerase enzyme. Unlike DNA replication where both strands are copied only one of the two DNA strands is transcribed into messenger RNA, a single stranded molecule. The DNA strand is known as the template strand while its complement is the informational strand. The action of transcription of RNA is a little like opening a zip fastener. An enzyme travels along one of the DNA strands and contrives to attach a series of ribonucleotides that make up a molecule of messenger RNA. When this traveling enzyme, known as RNA polymerase encounters a termination signal consisting of a specific sequence of nucleotides, it and its transcript are released from the DNA. The newly formed RNA is used to create a protein by linking a series of amino acids together. Transcription, therefore, is the process of converting information contained in a DNA segment, or gene, into a protein. Messenger RNA molecules contain anywhere from several hundreds to several thousand ribonucleotides depending on the size of the protein to be made. Each of the 100,000 or so proteins in the body is synthesized from a different messenger RNA that has been transcribed from a specific gene on DNA.

PART 2
Epigenetics

When a student has mastered the science briefly touched on here, he has to make yet another step and this may well be the most important one of all since it has fundamental clinical significance. This is the new science of epigenetics. We were all taught in biology that a genetic mutation is caused by a change in the DNA molecule that occurs but rarely. Epigenetics is defined as the study of heritable changes in gene function that occur without a change in the DNA sequence. An experiment was reported in the August 2003 issue of Molecular and Cellular Biology. These investigators used an unusual strain of mouse that carries a kind of trigger, known as a gene promoter, near the gene that determines not only the dirty yellow color of its coat but also its predisposition to obesity, diabetes and cancer. These obese mice breed true under normal conditions, their offspring having the same traits. When pregnant mice of this strain were fed extra vitamins and supplements they interacted with the trigger factor in the fetal mice and shut down the gene. As a result, obese yellow mothers gave birth to standard brown mice pups that grew up lean and healthy. We have known for a long time that what pregnant mothers eat, whether they are mice, fruit flies or humans, can profoundly affect the susceptibility of their offspring to disease. Until now, however, the mechanism has not been understood. Such factors have been shown to play a role in cancer, stroke, diabetes, schizophrenia, manic depression and other diseases as well as in shaping behavioral traits in offspring. Epigenetics may indeed hold answers to many mysteries that classical genetic approaches have been unable to solve.

This has inevitably blurred the neat picture of genetics that has surrounded the discovery of DNA as the substance of the gene. As you know, the analysis of the genome has given rise to a tremendous output of scientific papers that report the discovery of a gene that performs a specific disease role in reductionist terms. Such discoveries continually take medical science “off the hook” since the gene is considered to be responsible and nothing can be done about it until our scientific geniuses have solved the problem of gene replacement. Epigenetics involves an understanding of very complex mechanisms known as DNA methylation and histone acetylation and their effects in gene activation and inactivation.

Here is the problem for Mother Nature. The genomic DNA in a cell is estimated to be 1 millimeter long. It has to be incorporated into a nucleus that is only a few microns in diameter. Histones are a distinct form of protein and there are five different histones that react with DNA. Remember that it was the presence of protein that confused early DNA research referred to earlier. Four of these are joined together to form a disc-shaped complex around which a section of DNA is wound, much like winding a string on a spool. This forms a segment of the strand known as a nucleosome. The fifth histone is associated with another shorter strand of DNA and used to join one nucleosome to another in the strand. The result has been likened to pearls on a string. This raises an obvious question. When the whole strand of DNA is packed into the nucleus in this manner, how does the double helix unwind to begin transcription, the business of creating the template? You might remember that a methyl group is one carbon atom with three attached atoms of hydrogen, or CH3. An acetyl group is made up of a methyl group

attached to a carbon/oxygen combination and is described as CH3-CO. Methylation or acetylation refers to the attachment of one of these groups to a protein or to DNA.

Histones are kept in contact with DNA because they are positively charged while DNA is negatively charged. By adding an acetyl group to a histone the positive charge is neutralized and the dense packing of nuceosomes is relaxed. The double helix can unwind, enabling replication to take place when transcription occurs. Now we come to the process of methylation, perhaps the most important discovery of all for future therapy, since diet comes into the picture. Many natural food substances have methyl groups attached to their molecules. By means of certain enzymes, these can be detached and transferred to metabolites in the body and this process is of enormous importance in general physiology, quite apart from DNA chemistry. The science of epigenetics is concerned with their action on DNA and proteins.

There are many proteins, called transcription factors, used to initiate the replication mechanism. They do this by interacting with proteins called gene promoters that turn genes on, and with others that are gene silencers that turn them off. There are also gene enhancers that enable the process of transcription to accelerate and gene insulators that prevent a gene from being influenced by activation or repression of its neighbors. Any of these proteins, consisting of chains of amino acids, are made to be inactive by attaching a methyl group to a particular amino acid in the chain.

The human cell contains approximately 35,000 genes. Some, known as "housekeeping genes" are expressed all the time and are responsible for routine metabolic functions common to all cells, such as respiration. Some are expressed as a cell enters a particular pathway of differentiation and some only in those cells that have differentiated in a particular way. Others are expressed only as conditions around and in the cell change. For example, a hormone may turn a gene on or off. Think of the possible combinations. For example, methylation of a gene promoter makes it impossible for a transcription factor to bind to it. Thus the gene is silenced. On the other hand, if a gene silencer is methylated, the gene can continue to be expressed when it should be silenced.

Let me give you an example of this in disease. A gene codes for a growth factor called IGF2. The allele from the father is expressed but that from the mother is silenced under normal healthy conditions. If, however, both are expressed, the child may develop Wilm's tumor, a malignancy that affects the kidney in young children and is one of the most lethal malignancies in children. The secret is again methylation that silences the appropriate allele. Perhaps now we can see why the fat yellow mice were able to produce healthy pups by silencing the gene promoter that I referred to earlier as a trigger.

PART 3
The philosophical side

A good deal of modern biology is concerned with the identification of genes as the prime explanation for the biology of the organism. From this derives the impetus behind the Human Genome Project and much of the molecular approach to medical treatment. The current paradigm is reductionist and its extreme application is represented by Richard Dawkins, an evolutional biologist. Dawkins wrote "The Selfish Gene" in 1989 and stated that computers are by far the best metaphor for living things since they resemble them in so many respects. The whole idea of programming the behavior of a mechanism in advance is vital to the understanding of living organisms. From the selfish gene point of view, we are robot survival machines and because genes themselves can't pick things up, catch things, eat things or run around, they have to do it by proxy. They have to build machines to do it for them. The best way to look at ourselves is as a robot survival machine carrying around its own building program.

Daniel Dennett is quoted by John Brockman in "The Third Culture." He stated that Darwin's idea pulls the rug out from under the best argument for the existence of God that any theologian or philosopher has ever devised. The familiar argument is, of course, that the fantastic and ingenious design found in nature must be the work of a supremely intelligent God. It was remarkably persuasive until Darwin proposed a modest answer to the rhetorical question: natural selection. At least in the eyes of many academics, science has won and religion has lost. Darwin's idea has banished the Book of Genesis to the limbo of quaint mythology.

We are, however, still stuck with the conundrum proposed by a famous philosophical idea known as "Schrodinger's Cat." When the experiment begins, the box is opened and a cat is found in State A, alive, or in State B, dead. The biological paradox is that the difference between the cat in state A and state B is more than just a functioning cytochrome oxidase. We cannot simply assemble a cat from all of the molecular components, including the functioning enzyme systems and genetic information. Modern biology cannot definitively answer the question "What is the difference between the state of the cat in the two possible outcomes of the experiment?" The biologist, in other words, cannot determine what "life" really is.

Epigenetics represents a giant discovery whose impact will be extremely important. We can perhaps begin to understand why my PKU patients referred to earlier might not be mere coincidence. Perhaps the appearance of 5 rare, genetically determined diseases in one month from the same locality and from unrelated families was not a coincidence at all, but due to some unknown epigenetic factor. It may explain why I am able to treat a genetically determined disease like cystic fibrosis or muscular dystrophy with selected nutrients and improve the life of otherwise crippled children. If we discover the basic rules that were made by Mother Nature and do not obey them, we have nobody but ourselves to blame. There is a world of difference between ignorance of these rules and the knowledge gained from their discovery. We are just beginning to be aware that our food is the fuel that gives us function and is decisive in deciding how our genes give us maximum healthy life span.

I want to end with a story that might excite your interest. Years ago, when I was working as a consultant for inborn errors of metabolism, I was notified by the Columbus screening lab that a newborn girl had a positive PKU test. She turned out to have a raised blood

level of the amino acid phenylalanine but none of the biochemical complications of the more serious PKU. The blood test became normal after giving her large doses of vitamin C and she grew up as a healthy young woman who eventually delivered her own baby. This infant also had the screening test and it was negative. At the age of 9 months he received an inoculation of hepatitis B vaccine, after which his blood phenylalanine rose to abnormal levels and he became autistic. He has responded to nutritional therapy very well and the blood level of phenylalanine became normal. He is now , I am told, a healthy normal 2-year old.

I have told you this story because it strongly suggests that the present medical model is catastrophically wrong. In particular, we have to deal with the new concept that genetically determined disease often responds to a preventive approach that involves life style and nutrition as basic components well within the affordable reach of anyone.