MODERN BIOTECHNOLOGY

Principles and Applications

About the author

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Note to the Students

There are no short cuts in life and it becomes even more relevant when we are giving time to understanding of a subject. There is no alternative to reading a complete textbook from cover to cover in order to develop comprehensive understanding of a field. Therefore, I am not even going to try to make these hand outs such that they can be used as a replacement for a text book, instead, these hand outs will take you either deeper or will provide you with more breadth on the subject discussed in the corresponding video lecture. There are parts of these hand-outs which are written by me and there are parts which are copied from the best suited text book usually with my opinion on the subject matter.

Biotechnology is a very interdisciplinary field and as it draws upon from it's grounding in biochemistry, molecular biology, microbiology, chemical engineering, materials chemistry and biophysics. I would attempt to point you in the right direction for further reading wherever necessary. However, the following two text books you have to read from cover to cover to pass this course:

1) Molecular Biotechnology (Principles & Applications of Recombinant DNA) by Glick, Pasternack and Patten, 4th Edition, 2010, ASM Press, USA.
2) Pharmaceutical Biotechnology (Concepts and Applications) by Gary Walsh, 2007, John Wiley & Sons.

Topic 1 to 11

Biotechnology

Bio (Life) + Technology (the application of scientific knowledge for practical purposes)

Simply put Biotechnology is application of biology to create products.
There are various ways and manners in which you can define Biotechnology (Table 1) however, a more traditional comprehensive definition could be:

"Biotechnology is the use of microbes, animal/plant cells and their products to synthesize, break down or transform materials. Primarily it includes the use of recombinant DNA technology and genetic engineering techniques to improve upon the quality of processes"

Table 1: Selected Definition of Biotechnology[1]

A collective noun for the application of biological organisms, systems or processes to manufacturing and service industries.
The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application capabilities of microorganisms, cultured tissue cells and parts thereof.
A technology using biological phenomena for copying and manufacturing various kinds of useful substances.
The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.
The science of the production processes based on the action of microorganisms and their active components and of production processes involving the use of cells and tissues from higher organisms. Medical technology, agriculture and traditional crop breeding are not generally regarded as biotechnology.
Really no more than a name given to a set of techniques and processes.
The use of living organisms and their components in agriculture, food and other industrial processes.
The deciphering and use of biological knowledge.
The application of our knowledge and understanding of biology to meet practical needs.

Traditional biotechnology refers to the conventional techniques that have been used for many centuries to produce beer, wine, cheese etc. These techniques are still being used and if they don't include the use of any genetically modified organisms, they are considered to be under traditional biotechnology. A significant proportion of food market can be taken as an example. Market is replete with yogurts that contain active bacteria. Exhibit 2 shows two such products where the label of one highlights as many as five strains. Most of these are the naturally occurring bacterial strains and are common inhabitants of your gastrointestinal tract. It will not be wrong to say that these bacteria are at the

[1] Smith, J. E. (2009). Biotechnology, Cambridge University Press.

forefront of 'probiotics revolution'. If you get food poisoning (i.e. ingestion of food contaminated with pathogenic bacteria), these bacteria can restore and regulate the intestinal microbial flora. Pakistan has a growing problem of antibiotic resistance and physicians should use probiotics as a first line of defense against stomach bugs or at least ask patients to use products like yogurts and Lassi to provide prophylaxis. European and American markets are brimming with such products e.g. one brand of yogurt drink (Actimel®) boasts on having 10 billion L. casei live cultures alongside vitamins B6 and D in single serving.

Figure 1 A) A popular brand of Biolive Yogurt and the label showing the diversity of the species contained in the pot; B) Actimel a yogurt drink beaming with L. Casei

Roots of traditional biotechnology goes all the way back to 7000 BC, where Chinese, Sumerians and Babylonians are considered to understand **anaerobic principles** which helped yeast to make alcohol. Egyptians on the other hand showed adeptness at using **aerobic principles** to leaven bread (Figure 2 A). Excavations in Egypt have revealed figurines showing bread making process dating back to 4000 BC (Figure 2C). To date yeast is routinely used to leaven bread and is now produced industrially (Figure 2B). Pakistan used to produce its own yeast at the industrial scale but those companies were unfortunately closed during Bhutto's industrial reforms of 1972 where they were nationalised.

Figure 2: A) Note the increase in the size of fermented dough B) A block of industrially produced yeast C) Figurines excavated in Egypt showing bread making process

It was not only food, Chinese identified insecticides as well e.g. they used the extracts of the Chrysanthemum to drive the mosquitoes away by 100 BC. In the very similar way we use mosquito repellents like Mospel® by Abbott (Figure 3A).

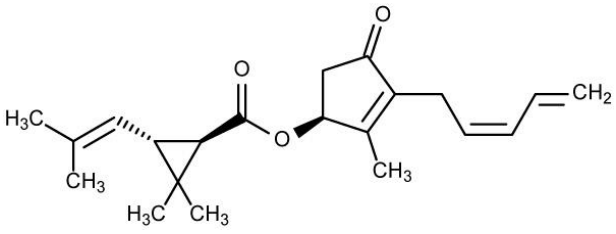
Food for thought: One of the goals I have with these notes and the course is for you to develop the ability to start asking the revealing and divulging questions. For example:

1) What compound was in the plant, which made mosquito run away?
2) How would you isolate and scale up that particular compound?
3) What would be more economical in this context: biotechnology or synthetic chemistry?
4) What makes mosquitoes run away from this compound? How does mosquito recognize this compound?
5) At the molecular level how does this compound work……does it bind to a receptor or an enzyme? What signalling pathway it initiates or blocks?
6) Do not hesitate to rely on your basic chemistry which surprisingly comes in very handy e.g. how volatile is this compound?
7) How would you formulate it to stay on your skin for longer periods?

The ability to ask the correct questions is all you need to be able to successful in the world. Then answers are almost a click away on your internet browsers. Information technology has changed the ground game for better. Two decades back, a good student was working twice as hard to be information laden and the employers wanted to tap into his ability to make use of that information when required. However, now information is readily available but you need to know what questions to ask and how to find answers! Therefore, it is up to you how to make better use of this long distance course.

Coming back to the example, chrysanthemum has chemicals known as pyrethrins (Figure 3A), however, modern mosquito repellents (Figure 3B) such as **diethyltoluamide (DEET)** are much less bulky and are relatively less heat labile and are more photostable.

A)



B)

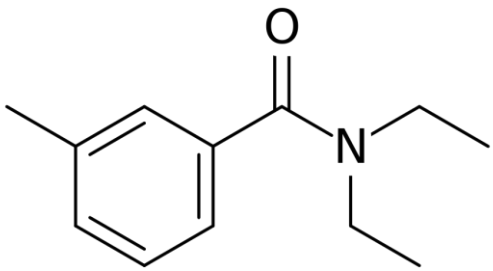


Figure 3: (A) Chrysanthemum Flower and structure of pyrethrins (B) A popular brand of mosquito repellents and structure the active ingredient DEET.

Table 2 outlines some of the historic processes which were unknowingly laying out the foundations of biotechnology.

Table 2: Discoveries before common era[2]

Era (BCE)	Discoveries
7000	Chinese discover fermentation through beer making
6000	Yogurt and cheese made with lactic acid-producing bacteria by various peoples
4000	Egyptians bake leavened bread using yeast
500	Moldy soybean curds used as an antibiotic
250	The Greeks started to practice crop rotation for maximum soil fertility
100	Chinese use chrysanthemum as a natural insecticide

The demarcation between **Modern Biotechnology** and traditional biotechnology is the advent of **recombinant DNA technology**. For practical purposes, Ullmann's Encyclopaedia of Industrial Chemistry categorises Biotechnology into 3 distinct areas:

- Red Biotechnology
- White Biotechnology
- Green Biotechnology

Red biotechnology deals with the applications in the healthcare, medicine or in the pharmaceutical industry. Today, 20 % of marketed medicines, 50 % of those in clinical trials, and 80 % in early development are biotech-based products. Forty percent of these candidate medicines are for the treatment of cancer. Almost 6 out of 10 most sold drugs are biologicals. Typical products of red biotechnology are recombinant vaccines, antibodies, blood clotting agents, and hormones. In addition, tissue engineering and stem cells is a part of this area of biotechnology. Tissue engineering aims at the functional regeneration of tissues through implantation of tissue cultured in vitro. After the successful discovery of modern genome editing tools such as CAS9-CRISPR, gene therapy also seems to be close to fruition.

White biotechnology deals with the industrial applications of biotechnology. The chemical industry has produced coal, gas, and petroleum-based goods and products for over 150 years. This era is likely to end within the next 100 years at the latest. The underlying reasons for a future era of new raw materials are the finite nature of fossil resources on the one hand, and the connection with many environmental problems on the other hand. New screening methods and tools such as metabolic engineering and global analysis methods such as genomics, proteomics and metabolomics are leading the way for white biotechnology. One

[2] Table adapted from the Wikipedia article on 'Timeline of Biotechnology' accessed on April 8, 2016

key area which has been and still continues to be an area of focus is the development of biocatalysts also known as enzymes. Modern day chemistry still struggles to mimic the substrate specificity of the enzymes. Industries like detergents, leather and textiles, chemicals and food have greatly benefitted from enzymes. Your modern day detergents include lipases, amylases, cellulases and proteases. Table 3 shows the difference between biological as well non biological detergents.

Table 3: Difference between Biological and Non-biological washing powder

The differences between biological and regular washing powders

	Biological washing powder	Regular washing powder
Detergents Mix greasy dirt with H_2O so it can be washed away	**(+)**	**(+)**
Enzymes **Braking** down stains and dirt in fabrics	**(+)** **Proteases** → proteins (blood, egg, gravy*...) **Amylases** → starches **Lipases** → fats and grease **Cellulase** → micro fibrils on cotton, brightening color of washed clothes They work efficiently at 40°C.	**(-)**
Remove **difficult stains** (blood, gravy, egg yolk, sweat, fats and grease)	**Easily** by decomposing the stains.	**Difficultly**. **Heat** alone makes stains coagulate and attach more firmly to the clothing.

**Gravy: juices that drip from cooking meat.*

We use proteases in tenderizing meat in day to day kitchen. Leather is softer and harder based upon the extent of treatment with enzymes. Traditional industrial processes have harsher environments like high pressures, temperature and extreme pH, while most enzymes like to function in moderate environments and like to work in physiological pH range around 7.4. Therefore, for last few decades most of the protein engineering efforts have been geared towards stabilizing these enzymes. To see the extent of enzymes used in the industry, I would advise you to look at the product range on the website of the world's largest enzyme producer known as Novozymes (Figure 4). Please have a look at Table 5[3] to see a detailed list of the enzymes used in the industry.

Figure 4:
Logo of the largest enzyme manufacturer

One of the added advantages of the biological methods are that they are environment friendly as well when compared to the chemical methods. To give you one example, fracking which is the process of drilling down into the earth before a high-pressure water and chemical mixture is directed at the rock to release the gas inside. This method uses copious amounts of water and the chemical mixture contains toxic chemicals such as lead, uranium, mercury, ethylene glycol, radium, methanol, hydrochloric acid and formaldehyde. The recent evidence suggests that these chemical leach out in underground water (deep aquifer) causing enormous discomfort to the communities drinking that water. Biotechnology may have solution to this problem in the form of biological detergents and microbial enhanced oil recovery (Figure 5)[4].
White biotechnology has also made an impact in the polymer market as well which is traditionally considered to be a very strong hold of chemistry. Following excerpt from Europa Bio report will give you an idea of how biotech is leaving its mark on the leading companies making polymers:

Mitsubishi Rayon has incorporated an enzymatic process step at the beginning of their polyacrylamide production process. They use an immobilised bacterial enzyme (nitrile hydratase) to produce acrylamide from an acrylonitrile feedstock. The acrylamide is then polymerised using a conventional chemical process. This is one of the first examples of biological processing in the bulk chemicals industry. Not only does it eliminate the use of sulphuric acid, but it also gives higher yield, less waste and lower energy costs. To add to these advantages, product quality is also better. More than 100,000 tonnes are produced annually by this route.

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[3] Chapter 7, Enzymes, Modern Biotechnology by Mosier & Ladisch (2009)

[4] By Mike Norton (accessed through https://commons.wikimedia.org/w/index.php?curid=25855892)

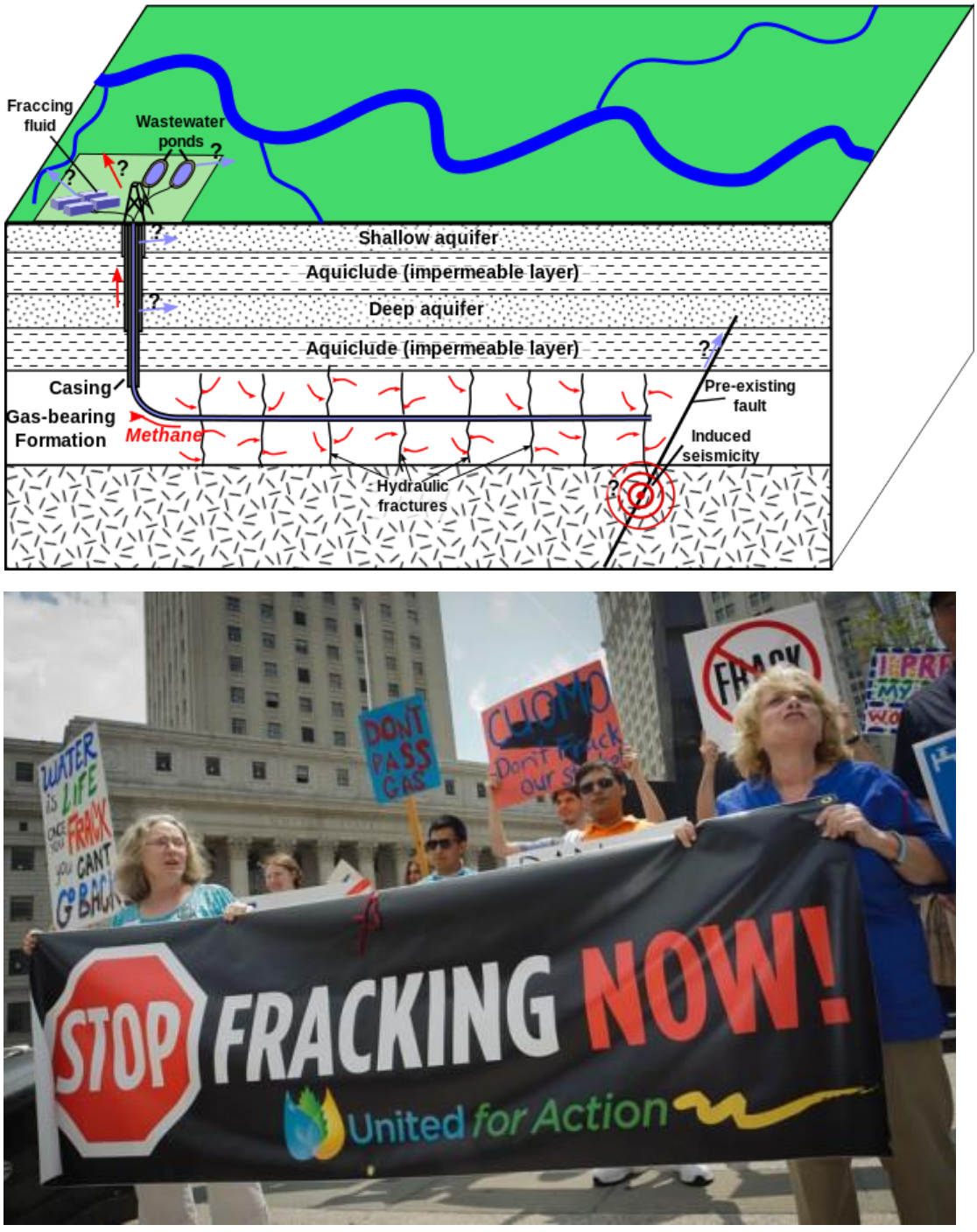

Figure 5: (A) Schematics of fracking (B) People protesting against fracking

Cargill started the production of poly-lactic acid (PLA) under the Natureworks™ name, by fermentation of corn-starch. Although having properties comparable to conventional polymers, which make it suitable for use in the packaging and textile industries, PLA is completely biodegradable. After use, cups and other articles can simply be composted with organic waste. Annual production has now reached 140,000 tonnes.

DuPont manufactures a copolymer of 1,3-propanediol (PDO) and terephthalic acid under the Sorona® name. Sorona® has been produced using propanediol synthesized from petrochemical feedstock. Recently, DuPont and Tate & Lyle formed a joint venture to commercialize the production of PDO from glucose using a modified bacterium. A large-scale fermentation facility has been set up in 2006, and it has a capacity of 90,000 tonnes of PDO annually. Bio-PDO is produced more economically than petrochemical PDO, it can be produced at higher purity and in turn yields a superior polymer, and it reduces dependence on non-renewable resources[5].

[5] Industrial or White Biotechnology, Report by EuropaBio

Table **Industrial Uses of Carbohydrate-Hydrolyzing Enzymes, Proteolytic Enzymes, Other Types of Hydrolytic Enzymes, Oxidoreductases, Isomerase, and Other Enzymes, and Selected Research, Medical, and Diagnostic Use of Enzymes[a]**

Common Name	Enzyme EC Number	IUB Name	Range of Optimal pH	Reaction Catalyzed	Applications
α-Amylase	3.2.1.1	α-1,4-Glycanhydrolase	4.8–6.3	Starch → glucose + maltose + oligosaccharides	Breadmaking Mashing in alcohol production Desizing of fabrics Wallpaper removal
β-Amylase	3.2.1.2	α-1,4-Glycanhydrolase	5.0–5.5	Starch → maltose + dextrin	Maltose production
Amyloglucosidase	3.2.1.3	α-1,4-Glycanhydrolase	3.8–5.5	Starch → glucose + oligosaccharides	Glucose from corn syrup Low-carbohydrate beers
Cellulase	3.2.1.4	System of enzymes β-1,4-Glucanglucanohydrolase β-1,4-Cellobiohydrolase β-Glucosidase	4.0–6.0	Cellulose → glucose + polysaccharides	Treatment of cotton fabrics Laundry detergents Glucose production from cellulose
Dextranase	3.2.1.11	Dextranase	4.0–6.5	Dextran → glucose + oligosaccharides	Reduce viscosity of sugar syrup
Diastase (mixture of α- and β-amylase)	NA	NA	Slightly acid pH[1,2] 6.0–7.0[3]	Starch → glucose	Glucose from corn syrup

Common Name	Enzyme EC Number	IUB Name	Range of Optimal pH	Reaction Catalyzed	Applications
Chymosin[b]	3.4.23.4	NA[c]	2.5–5.10[4–6]	κ-Casein → *p*-κ-casein	Cheese production
Hemicellulases	NA	NA	4.5 to 7.5	Xylans, Arabans, Glucans → Xylose, arabinose, glucose, oligosaccharides	Viscosity reduction, antispoiling agent
Invertase	3.2.1.26	β-Fructofuranosidase	3.0-8.0[7]	Sucrose → glucose + fructose	Confectionery products
Lactase	3.2.1.23	β-Galactosidase	2.4-9.0[8–10]	Lactose → glucose + galactose	Prevent are lactose crystallization in ice cream
Lysozyme	3.2.1.17	Muramidase *N*-acetylmuramide glycanhydrolase	3.5-8.0[11,12] PH: 6.0–9.0 (*Staphylococcal* and egg white lysozyme)	Bacterial cell walls → acetylglucouronides	Ophthalmic preparations
Naringinase	Naringinase is an enzyme complex consisting of α-rhamnosidase (EC 3.2.1.40) and flavonoid β-glucosidase (EC 3.2.1.21)	Hesperidinase	4.0–6.5	Naringin → Prunin + Rhamnose Prunin → naringenin + glucose	Removal of bitter taste from grapefruit juice and peel

Common Name	Enzyme EC Number	IUB Name	Range of Optimal pH	Reaction Catalyzed	Applications
Pectinase	NA	Three major types of pectinase[d]	3.0–10.5[13,14]	Pectin → galacturonic acid	Clarification of fruit juices Coffee bean fermentation Citrus oil recovery
Pullulanase	3.2.1.41	Amylopectin 6-glucanohydrolase[1,2]	5.0–8.5[15–17]	Starch → maltose + maltotriose	Treatment of wort (beer brewing)
Bacterial proteases	3.4.24.4	Mixture of enzymes	7.0–11.0	Protein → amino acids (broad specificity)	Laundry detergents
Fungal proteases	3.4.24.3	Mixture of enzymes	6.8–8.5	Protein → amino acids (broad specificity)	Food processing (dough softening, sake production)
Papain	3.4.22.2	Papain	6.0–9.5[18,19]	Hydrolyzes bonds adjacent to basic L-amino acids, of Leu or Gly	Meat tenderizer Shrinkproofing of wool
Rennin (*see* Chymosin above)	3.4.23.4	Rennet		κ-Casein → *p*-κ-casein	Cheese manufacture
Streptokinase	3.4.21.7	Fibrinolysin, plasminokinase	8.5 (*Homo sapiens*)[20,21]	Hydrolyzes proteins at Arg and Lys	Anticoagulant (medical use)
Lipase	3.1.1.3	Lipase	4.9–7.5	Fats → fatty acids + glycerol	Flavor improvements in refrigerated dairy products, chocolates

Common Name	Enzyme EC Number	IUB Name	Range of Optimal pH	Reaction Catalyzed	Applications
Histinase	4.3.1.3	L-Histidine ammonia lyase	4.0–6.5	Histidine *m*-urocanoate + NH_3	Production of urocanoic acid (used in sunscreen)
Lipoxygenase	1.13.11.12	Lidoxidase	4.5–9.5[28,29]	Caretenoids + linoleic acid + O_2 *m*-peroxidized linoleic acid	Bread whitening Peroxidized oils used in flavorings
Tryptophanase	4.1.99.1	Tryptophanase	7.0–9.0[30,31]	Indole + pyruvate + NH_3 *m*-L-tryptophan + H_2O	Amino acid production
Asparaginase	3.5.1.1	L-Asparagine amidohydrolase	5.0–9.5[32–34]	—	Treatment of acute lymphatic leukemia
Cholesterol oxidase	1.1.3.6	Cholesterol oxidase	4.0–8.0[35–37]	—	Assay of cholesterol
DNA polymerase	2.7.7.7	DNA nucleotidyl transferase	6.5–9.2[38–40]	$(DNA)_n$ + dNTP → $(DNA_{n+1}$ + $Pp_i)$	DNA synthesis
Glucose oxidase	1.1.3.4	Glucose oxidase	4.0–7.0[41–43]	See Table 4.3	Assay of glucose (used together with catalase)
Lactate dehydrogenase	1.1.1.27	L-Lactate: NAD oxidoreductase	4.5–9.8[44–46]	L-Lactate + NAD^+ → pyruvate + NADH	Diagnosis of myocardial infarction and leukemia

Common Name	Enzyme EC Number	IUB Name	Range of Optimal pH	Reaction Catalyzed	Applications
Acetylcholine esterase	3.1.1.7	Acetylcholine esterase	4.8–9.0[47–49]	Acetylcholine + H_2O → choline + acetic acid	Assay of neuroactive peptides
Uricase	1.7.3.3	Uric acid: oxygen oxidoreductase, urate oxidase	7.0–9.5[50–52]	Uric acid + H_2O + O_2 → allantoin + H_2O	Diagnosis of gout

[a]References cited in this table:

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54. Zhao et al. (2006)
55. BRENDA (2008)

[b]Other names for chymosin are prorennin, rennin (old name—the term rennin appears rarely today in some fungal milk-clotting aspartic proteinases such as *Mucor* rennin), and prochymosins A, B, and C (for bovine isozymogens).

[c]See I. Gritti, G. Banfi, and G. S. Roi, "Pepsinogens: Physiology, Pharmacology, Pathophysiology, and Exercise," *Pharmacol. Res.* **41**(3), 265–281 (2000). Per IUB's Enzyme Nomenclature rules, proteases are designated and classified by three code numbers, referring to class, subclass, and sub-subclass.

[d]Pectinases are classified under three headings according to the following criteria: whether pectin, pectic acid or oligo-D-galacturonate is the preferred substrate, whether pectinases act by *trans*-elimination or hydrolysis, and whether the cleavage is random (endo-, liquefying, or depolymerizing enzymes) or endwise (exo- or saccharifying enzymes). The three major types of pectinase are pectinesterases (PE), depolymerizing enzymes, and protopectinase.

Source: Adapted from Atkinson and Mavituna (1983).

Green Biotechnology or agricultural biotechnology deals with plants and crops which are genetically engineered.

Why do we need to genetically engineer crops?

It is a fair question and the reason is that population of the world is increasing very fast, owing gratitude to the progress in modern medicine specially development of antibiotics and aseptic surgical methods. Since 1960s when the world population was only 3 billion people now in 2016 we are standing at 7.2 billion. If you think that feeding them was easy then you are wrong. In our attempt to feed them we have lost 1/5th of the top soil to erosion, desertification and salinity. On top of it we have the problem of food distribution. On one hand, we have people who have food and resources in excess and are obese and on the other hand we have people dying of starvation (Figure 6). About 30,000 people dies of huger every day and nearly 1.2 billion people live on less than dollar a day. If we wouldn't increase our productivity on existing farms, we will need additional 1.6 billion hectares of arable land by 2050. This means an area eight times the size of Pakistan. This means to feed these people we need crops which have enhanced yield, nutrition and better shelf life for transportation.

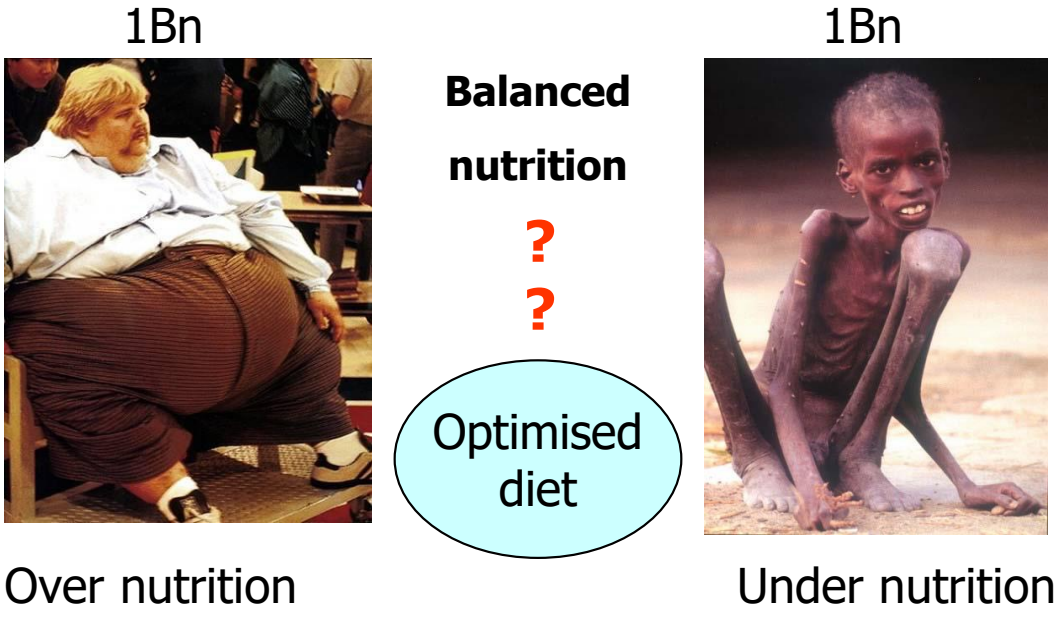
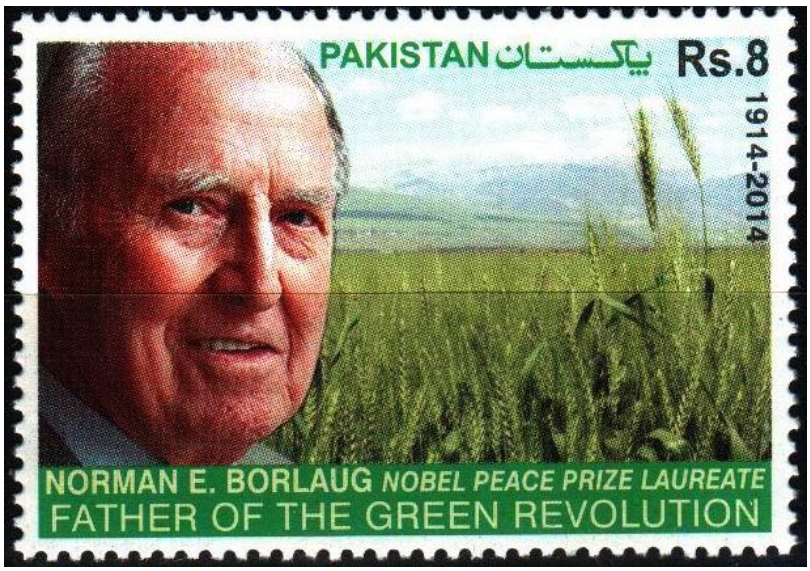


Figure 6: The disparity between nutritional status and the need of optimised diet

Some of you should take this chance to look into wheat varieties we eat in Pakistan. You will find yourself to be thankful to an American Nobel Prize winner, Prof. Norman Borlaug whose research on wheat kick started the green revolution. He developed where semi-dwarf, high-yield, disease-resistant wheat varieties. the variety known in Pakistan as Mexipak which had increased yield per hectare (Figure 7). Pakistani government has celebrated Borlaug's achievements by

issuing a postage stamp, however, they did make a mistake as he died in 2009 not 2014.

A)



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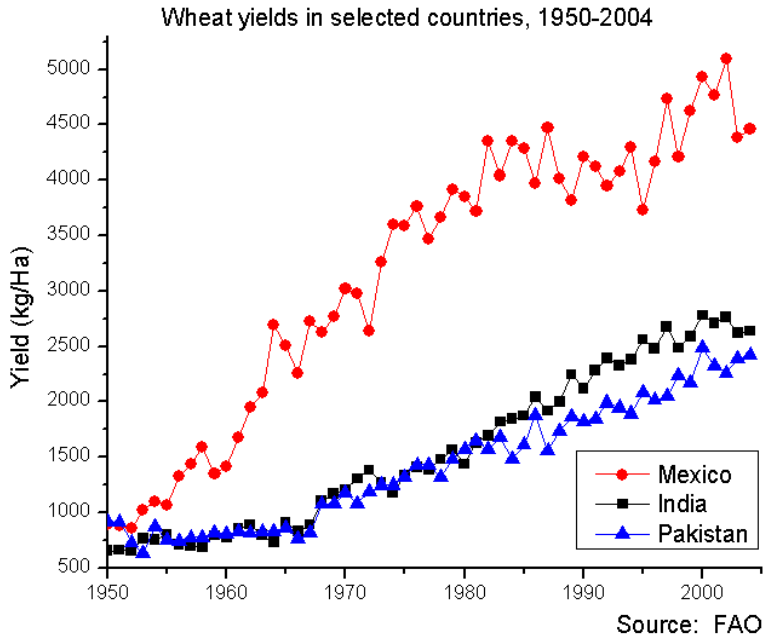


Figure 7: (A) Pakistan Post issued stamps in honour of Norman Borlaug (B) Increase in wheat yields per hectare from 1950 till 2004 in Mexico, India and Pakistan

It may be interesting for you that genetically engineered crops are banned in Europe. This unfortunate anomaly is due to two reasons:

a) There are a lot of people who genuinely believe that genetically engineered crops would harm them.
b) Europe is bread basket of the world and there is no shortage of food to worry them.

However ironical it may be, the world's second largest agri-biotech company is located in Europe in Basel, Switzerland. Moreover, agricultural biotechnology has gotten a lot of bad press due to Monsanto's (leading world agri-biotech company) aggressive policy against the farmers which has led to various lawsuits both in United States and outside.
I am not going to cover Plant Biotechnology in this course as Virtual University has a separate course planned in this area.

Some classifications of Biotechnology also consider Marine Biotechnology as **Blue Biotechnology** which is probably less relevant in case of Pakistan. However, with algal biofuels booming it is becoming an area of active research.

Roots of Biotechnology are founded in the development of microbiology and the cell theory. It is indeed a fascinating journey worth reading about.

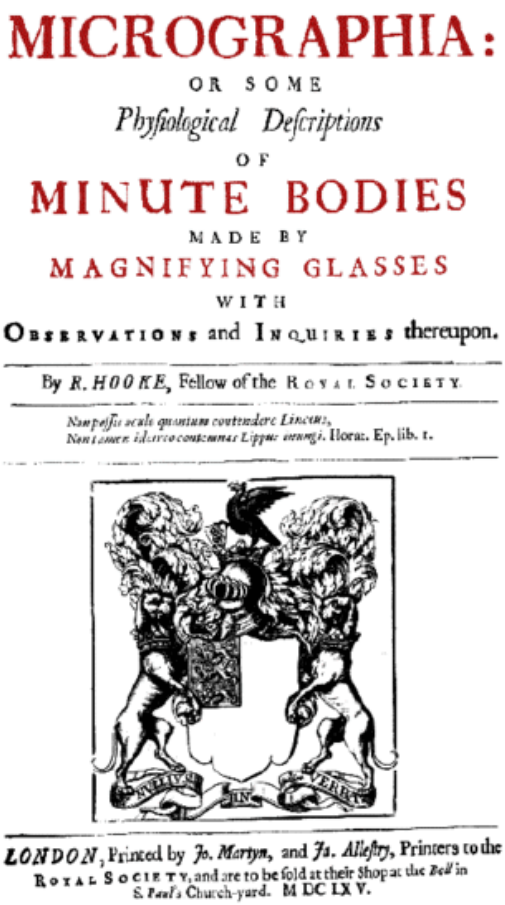
1628 William Harvey, a physician graduated from Cambridge, was the first one in in the English world to describe accurately how a heart functions in a book titled 'An Anatomical Study of the Motion of the Heart and of the Blood in Animals'. The key was his deciphering the function of the valves to understand circular nature of blood flow in human body. However, from biotech perspective it was his observation that humans and other mammals reproduced via the fertilization of an egg by the sperm.

1665 First recorded description of living cells by Robert Hooke. Figure 8 shows the title page of the probably first scientific best seller, where Hooke recorded his observations of microorganisms. Some of you may remember Hooke's law from your physics texts. Hooke was a polymath indeed, however, a lot of his work remained unnoticed due to his bitter arguments with his contemporaries such as Isaac Newton and Henry Oldenburg. His tirade with Newton on law of universal gravitation is worth reading for the students who are interested in history.

1676 Antonie Philips van Leeuwenhoek is usually credited to be the father of Microbiology. His observations were results of his ability to design lenses for the microscope. Anton possessed some microscopes which could magnify up to 500 times and some of the lenses were so finely made that it was not until 1957 that they were replicated.

One of his single lens microscope is shown in the Figure 9. Van Leeuwenhoek's single-lens microscopes were relatively small devices, the biggest being about 5 cm long. They worked by placing the lens very close in front of the eye, while looking in direction of the sun. The other side of the microscope had a pin, where the sample was attached in order to stay close to the lens. There were also three screws to move the pin and the sample, along three axes: one axis to change the focus, and the two other axes to navigate through the sample. Using this sort of microscope some of his discoveries were:

- the infusoria (protists in modern zoological classification), in 1674
- the bacteria, (e.g., large Selenomonads from the human mouth), in 1676
- the vacuole of the cell
- the spermatozoa in 1677
- the banded pattern of muscular fibers, in 1682



MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

MAGNIFYING GLASSES

WITH

OBSERVATIONS and INQUIRIES thereupon.

By *R. HOOKE*, Fellow of the ROYAL SOCIETY.

Nam posse oculo quantum contendere Lynceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the *Bell* in S. *Paul's* Church-yard. M DC LX V.

Figure 8
First Scientific best seller by Robert Hooke

Figure 9
Replica of microscope by Leeuwenhoek

1798 Edward Jenner uses first viral vaccine to inoculate a child from small pox. More details on him are in the vaccines section and in the corresponding lecture.

1802 Jean-Baptiste Lamarck publishes *Research on the Organization of Living Bodies,* in which he claims that species become more perfect or complex and pass on acquired characteristics to their offspring. This hypothesis was later to be overturned by evolution.

1824 Henri Dutrochet discovers described osmosis, respiration and effect of light on the plant.

1832 Joseph Jackson Lister builds a new type of microscope correcting chromatic aberration that removes distortion and greatly increases resolution. Using it he was able to visualize blood cells* and identify Hodgkin's disease while working with Dr. Thomas Hodgkin, they published their paper '*On Some Morbid Appearances of the Absorbent Glands and Spleen'.*

Note: Students need to note that this is before cell was established as a unit of life and scientists and physicians like Hodgkin were making notes of the changes they were seeing in blood

1833 Robert Brown**,** named the cell nucleus. The nucleus had been observed before, perhaps as early as 1682 by the Dutch microscopist Leeuwenhoek, and Franz Bauer had noted and drawn it as a regular feature of plant cells in 1802, but it was Brown who gave it the name it bears to this day (while giving credit to Bauer's drawings). Neither Bauer nor Brown thought the nucleus to be universal, and Brown thought it to be primarily confined to Monocotyledons.

1838 Matthias Schleiden discovers that plants are made of cells and laid foundations to the cell theory.

1840 Theodor Schwann discovers that all animal tissues are made of cells. To understand why this was game changing! Students need to read on vitalism and on its eventual refutation. Here is a Wikipedia excerpt for the students who are too lazy to google:

Cell theory

In 1837, Matthias Jakob Schleiden viewed and stated that new plant cells formed from the nuclei of old plant cells. While dining that year with Schwann, the conversation turned on the nuclei of plant and animal cells. Schwann remembered seeing similar structures in the cells of the notochord (as had been shown by Müller) and instantly realized the importance of connecting the two phenomena. The resemblance was confirmed without delay by both observers, and the results soon appeared in Schwann's famous *Microscopic Investigations on the Accordance in the*

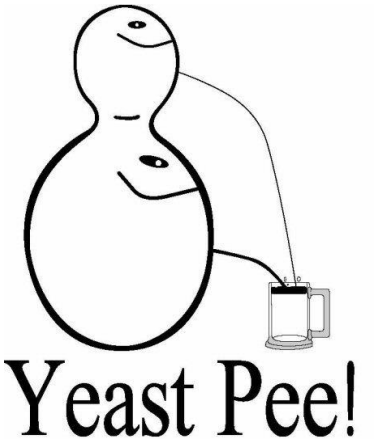
Structure and Growth of Plants and Animals, in which he declared that "All living things are composed of cells and cell products". This became ***cell theory*** or *cell doctrine*.
In the course of his verification of cell theory, Schwann proved the cellular origin and development of the most highly differentiated tissues including nails, feathers, and tooth enamel. Schwann also established a basic principle of embryology by observing that the ovum is a single cell that eventually develops into a complete organism.

Vitalism and germ theory

Schwann was amongst the first to break with **vitalism** (Concept that living organisms are fundamentally different from non-living entities because they contain some non-physical element or are governed by different principles than are inanimate things) and worked towards a physico-chemical explanation of life. Schwann also examined the question of spontaneous generation, which led to its eventual disconfirmation. In 1836, Schwann went beyond others who had noted simply the multiplication of **yeast** during alcoholic fermentation, as Schwann assigned yeast, the role of primary causal factor, and then went further and claimed it was alive. Embattled controversy ensued as two eminent chemists like Friedrich Wohler and Justus von Liebig ridiculed Schwann's views on fermentation, because Schwan's ideas were contradictory to his theory that chemical ferments due to their interaction with Oxygen.
After publishing anonymous mockery (Figure 10) in a journal of their own editorship they published a purely physicochemical if also hypothetical explanation of the interaction resulting in fermentation. As both the rival perspectives were hypothetical, and there was not even an empirical definition of 'life' to hold as a reference frame, the controversy—as well as interest itself—fell into obscurity unresolved until Pasteur resolved it in 1857 by conforming that yeast were alive. In retrospect, the germ theory of Pasteur, as well as its antiseptic applications by Lister, can be traced to Schwann's influence.

Figure 10

Wohler's and Leibig's unfortunate but successful mockery of Schwann's explanation of fermentation, which destroyed his career as an academic.



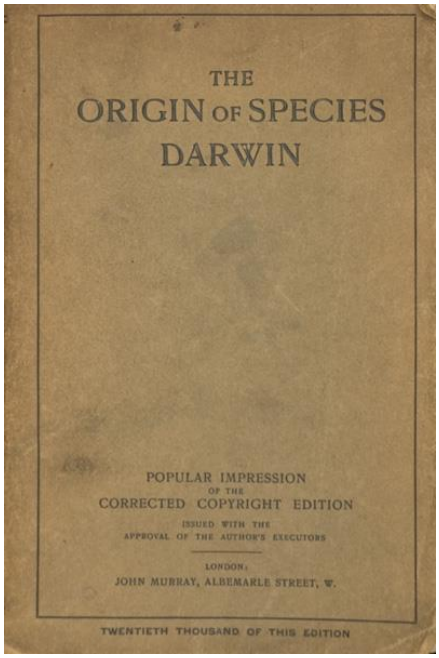
1857 In 1857, pathologist Rudolf Virchow posed the maxim *Omnis cellula e cellula*—that every cell arises from another cell. By the 1860s, cell doctrine became the conventional view of the elementary anatomical composition of plants and animals.

1857 Joseph von Gerlach discovers a new way of staining cells that revealed their internal structures.

1858 The theory of evolution is made public at a meeting of the Linnean Society in London with the reading of papers by Charles Darwin and Alfred Russel Wallace.

1859 Charles Darwin publishes *On the Origin of Species* (*Figure 11*). The complete first print sells out on the first day.

Figure 11
Cover page of the most significant book of the nineteenth century



1865 Gregor Mendel presented his paper "Experiments in Plant Hybridization" in meetings of the Society for the Study of Natural Sciences in Brnø, Moravia. The paper outlined the basic principles of the modern science of genetics. It was published the next year but received little attention.

1868 Fredrich Miescher isolated DNA from the nuclei of cells; he called it nuclein.

1871 Francis Galton carries out experiments in rabbits that disprove Darwin's hypothesis of how heredity functions. He is considered to be the pioneer of Eugenics and started the "nature vs. nurture" debate.

Food for thought

Consider some challenging questions:

Are we all born equal? I am not talking about advantages one get could from being born rich or disadvantage from being poor. All we all born genetically equal? Are there any genes which may make someone superior or inferior?

Students who are interested in this subject should read The New York Times Bestseller, **The Blank State** by famous Psychologist from MIT, Steven Pinker (Figure 12).

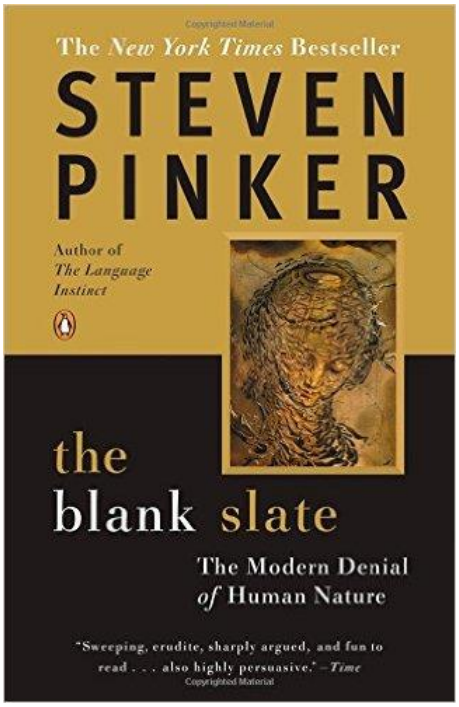


Figure 12
The Blank State by Steven Pinker, published in 2002

1876 Oscar Hertwig observed the fusion of sperm and egg nuclei during fertilization.

1878 Albrecht Kossel discovers five DNA bases Adenine, Guanine, Cytosine, Thymine and Uracil

1879 Walther Flemming observed the behavior of chromosomes during cell division and published them in his book in 1882 (Figure 13).

1885 August Weismann stated that organisms compartmentalise reproductive cells from the rest of their bodies, which helps explain why Lamarck's concept of evolution and inheritance is wrong. He tried and failed to observe Lamarckian inheritance in the laboratory by cutting off the tails of mice for many generations. Thus producing his **germ plasm theory** which at one point in time was also known as *Weismannism*. The theory presented that in a multicellular organism, inheritance only takes place by means of the germ cells—the gametes such as eggs and sperms. Other cells of the body—somatic cells—do not function as agents of heredity. The effect is one-way: germ cells produce somatic cells and are not

affected by anything the somatic cells learn or therefore any ability an individual acquires during its life. Genetic information cannot pass from soma to germ plasm and on to the next generation. This is referred to as the Weismann barrier. This idea, if true, rules out the inheritance of acquired characteristics as proposed by Jean-Baptiste Lamarck.

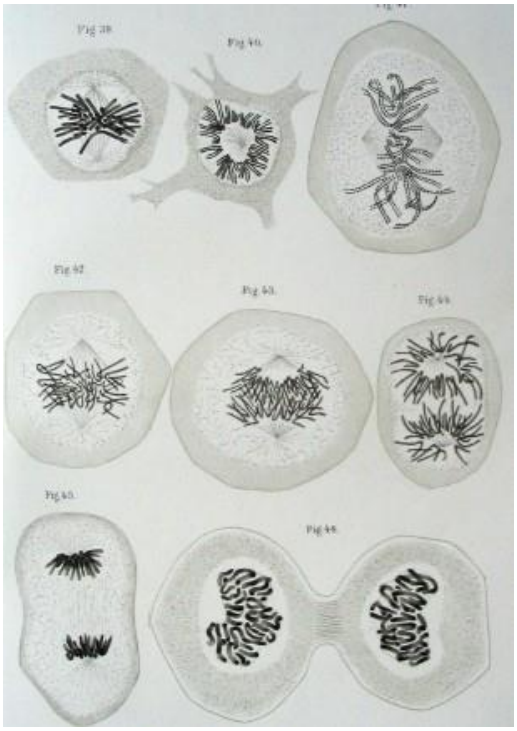


Figure 13
Illustrations of cells with chromosomes and mitosis, from the book *Zellsubstanz, Kern und Zelltheilung*, 1882

1900 Hugo de Vries, Carl Correns, and Erich von Tschermak-Seysenegg independently publish papers that confirm Mendel's principles of heredity in a wide range of plants. Archibald Garrod identifies the first disease (Alkaptonuria) that is inherited according to Mendelian laws, which means that it is caused by a defective gene. Theodor Boveri demonstrates that different chromosomes are responsible for different hereditary characteristics.

1901 Karl Landsteiner identifies the ABO blood groups. The excerpt from Wikipedia on his discovery is worth reading:

'In 1900 Karl Landsteiner found out that the blood of two people under contact agglutinates, and in 1901 he found that this effect was due to contact of blood with blood serum. As a result, he succeeded in identifying the three blood groups A, B and O, which he labelled C, of human blood. Landsteiner also found out that blood transfusion between persons with the same blood group did not lead to the destruction of blood cells, whereas this occurred between persons of different blood groups. Based on his findings, in 1907 the first successful blood transfusion was performed by Reuben Ottenberg at Mount Sinai Hospital in New York.

Today it is well known that persons with blood group AB can accept donations of the other blood groups, and that persons with blood group O can donate to all other groups. Individuals with blood group AB are referred to as *universal recipients* and those with blood group O are known as *universal donors*. These donor-recipient relationships arise due to the fact that persons with AB do not form antibodies against either blood group A or B. Further, because type O blood possesses neither characteristic A nor B, the immune systems of persons with blood group AB do not refuse the donation. In today's blood transfusions only concentrates of red blood cells without serum are transmitted, which is of great importance in surgical practice. In 1930 Landsteiner was awarded the Nobel Prize in Physiology or Medicine in recognition of these achievements. For his pioneering work, he is recognised as the father of transfusion medicine.

1902 William Bateson popularizes Mendel's work in a book called *Mendel's Principles of Heredity: A Defense.*

1903 Walter Sutton connects chromosome pairs to hereditary behavior, demonstrating that genes are located on chromosomes. Nikolai Koltsov proposed that the shape of cells was determined by a network of tubules which he termed the cytoskeleton.

1905 Nettie Stevens and Edmund Wilson independently discover the role of the X and Y chromosomes in determining the sex of animal species.

1906 William Bateson discovers that some characteristics of plants depend on the activity of two genes i.e. polygenic traits.

1908 Archibald Garrod shows that humans with an inherited disease are lacking an enzyme (a protein), demonstrating that there is a connection between genes and proteins. The disease was Alkaptonuria.

1910 William Bateson coins the term *genetics.*

1910 The Eugenics Record Office is opened at Cold Spring Harbor, New York. Thomas Hunt Morgan discovers the first mutations in fruit flies, *Drosophila melanogaster,* bred in the laboratory. This leads to the discovery of hundreds of new genes over the next decades.

1911 Morgan discovers some traits that are passed along in a sex-dependent manner and proposes that this happens because the genes are located on sex chromosomes. He proposes the general hypothesis that traits that are likely to be inherited together are located on the same chromosome.

1913 Alfred Sturtevant constructs the first genetic linkage map, allowing researchers to pinpoint the physical locations of genes on chromosomes.

1919 Károly Ereky, a Hungarian agricultural engineer, first uses the word biotechnology in a book he published in Berlin called *Biotechnologie der Fleisch-, Fett- und Milcherzeugung im landwirtschaftlichen Grossbetriebe* (Biotechnology of Meat, Fat and Milk Production in an Agricultural Large-Scale Farm) where he described a technology based on converting raw materials into a more useful product. For Ereky, the term "biotechnologie" indicated the process by which raw materials could be biologically upgraded into socially useful products.

1920 Hans Spemann and Hilde Proescholdt Mangold begin a series of experiments in which they transplant embryonic tissue from one species to another. The scientists show that particular groups of cells they called organizers send instructions to neighboring cells, changing their developmental fates.

1921 Erwin Baur, Eugen Fischer, and Fritz Lenz publish a book called *Menschliche Erblichkeits lehre und Rassenhygiene,* which attempts to link genetics to race and is used by eugenicists in the United States and Germany as a justification for declaring that there are inferior races and a motivation for sterilizing and killing social undesirables.

Note: A lot of people believe that fanatics only come from religion however above examples show that they can come from extreme interpretation of scientific theories as well.

1922 Ronald A. Fisher uses mathematics to show that Mendelian inheritance and evolution are compatible.

1927 Koltsov proposed that inherited traits would be inherited via a "giant hereditary molecule" which would be made up of "two mirror strands that would replicate in a semi-conservative fashion using each strand as a template".

1927 Hermann Muller shows that radiation causes mutations in genes that can be passed down through heredity.

1928 Fredrick Griffith discovers that genetic information can be transferred from one bacterium to another, hinting that hereditary information is contained in DNA.

The account of his famous experiment is fairly detailed on Wikipedia and is abridged here for your convenience:

Griffith's experiment was the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as transformation.
Pneumonia was a serious cause of death in the wake of the post-WWI Spanish influenza pandemic, and Griffith was studying the possibility of creating a vaccine. Griffith used two strains of pneumococcus

(*Streptococcus pneumoniae*) bacteria which infect mice – a type III-S (smooth) which was virulent, and a type II-R (rough) strain which was non-virulent. The III-S strain covered itself with a polysaccharide capsule that protected it from the host's immune system, resulting in the death of the host, while the II-R strain did not have that protective capsule and was defeated by the host's immune system. A German bacteriologist, Fred Neufeld, had discovered the three pneumococcal types (Types I, II, and III) and discovered the Quellung reaction to identify them *in vitro*. Until Griffith's experiment, bacteriologists believed that the types were fixed and unchangeable, from one generation to another.

In this experiment, bacteria from the III-S strain were killed by heat, and their remains were added to II-R strain bacteria. While neither alone harmed the mice, the combination was able to kill its host. Griffith was also able to isolate both live II-R and live III-S strains of pneumococcus from the blood of these dead mice. Griffith concluded that the type II-R had been "transformed" into the lethal III-S strain by a "transforming principle" that was somehow part of the dead III-S strain bacteria.

Today, we know that the "transforming principle" Griffith observed was the DNA of the III-s strain bacteria. While the bacteria had been killed, the DNA had survived the heating process and was taken up by the II-R strain bacteria. The III-S strain DNA contains the genes that form the protective polysaccharide capsule. Equipped with this gene, the former II-R strain bacteria were now protected from the host's immune system and could kill the host (Figure 14). The exact nature of the transforming principle (DNA) was verified in the experiments done by Avery, McLeod and McCarty and by Hershey and Chase.

1931 Barbara McClintock shows that as chromosome pairs line up beside each other during the copying of DNA, fragments can break off one chromosome and be inserted into the other in a process called recombination. Archibald Garrold proposes that diseases can be caused by a person's unique chemistry—in other words, genetic diseases may be linked to defects in enzymes.

1933 Theophilus Painter discovers that staining giant salivary chromosomes in fruit flies reveal regular striped bands.

1934 Calvin Bridges shows that chromosome bands can be used to pinpoint the exact locations of genes.

1935 Nikolai Timofeeff-Ressovsky, K. Zimmer, and Max Delbrück publish a groundbreaking work on the structure of genes that proposes that mutations alter the chemistry and structure of molecules.

1937 George Beadle and Boris Ephrussi show that genes work together in a specific order to produce some features of fruit flies.

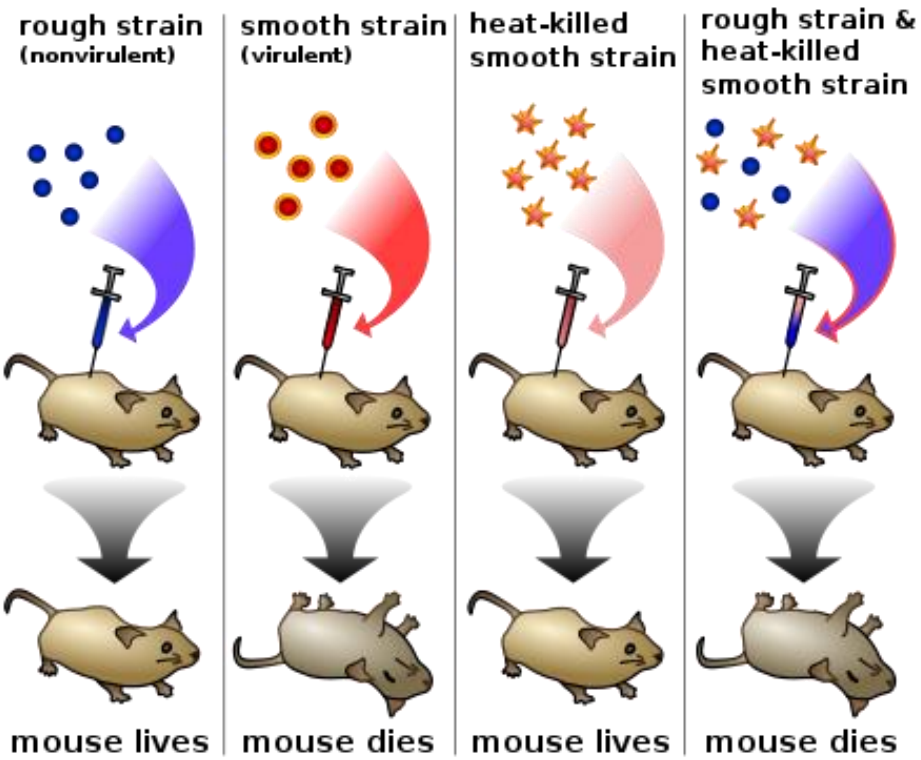


Figure 14
Frederick Griffith's transformation experiment

Figure 15
Avery, MacLeod & McCarty's Experiment

1940 George Beadle and Edward Tatum prove that a mutation in a mold destroys an enzyme and that this characteristic is inherited in a Mendelian way, leading to their hypothesis that one gene is related to one enzyme (protein), formally proposed in 1946.

1943 Max Delbruck and Salvador Luria demonstrate evolution in the laboratory by showing that bacteria evolve defenses to viruses through mutations that are acted on by natural selection.

1944 Oswald Avery, Colin MacLeod, and Maclyn McCarty show that genes are made of DNA by isolating DNA, white precipitate in the bottom of the jar (Figure 15). Erwin Schrödinger publishes *What Is Life? from Cambridge University Press.*

In the book, Schrödinger introduced the idea of an "aperiodic crystal" that contained genetic information in its configuration of covalent chemical bonds. In the 1950s, this idea stimulated enthusiasm for discovering the genetic molecule. Although the existence of DNA had been known since 1869, its role in reproduction and its helical shape were still unknown at the time of Schrödinger's lecture. In retrospect, Schrödinger's aperiodic crystal can be viewed as a well-reasoned theoretical prediction of what biologists should have been looking for during their search for genetic material. Both James D. Watson, and independently, Francis Crick, co-discoverers of the structure of DNA, credited Schrödinger's book with presenting an early theoretical description of how the storage of genetic information would work, and each respectively acknowledged the book as a source of inspiration for their initial researches.

1948 The American Society for Human Genetics is founded.

1950 Barbara McClintock publishes evidence that genes can move to different positions as chromosomes are copied. Erwin Chargaff discovers that the proportions of A and T bases in an organism’s DNA are identical, as are the proportion of Gs to Cs.

1951 Rosalind Franklin uses X-ray diffraction to obtain images of DNA; the patterns reveal important clues to the building plan of the molecule.

1952 The irrefutable evidence of DNA as a hereditary material came from Alfred Hershey and Martha Chase.

While DNA had been known to biologists since 1869, many scientists assumed at the time that proteins carried the information for inheritance because DNA appeared simpler than proteins. In their experiments, Hershey and Chase showed that when bacteriophages, which are composed of DNA and protein, infect bacteria, their DNA enters the host bacterial cell, but most of their protein does not. Although the results were not conclusive, and Hershey and Chase were cautious in their interpretation, previous, contemporaneous and subsequent discoveries all

served to prove that DNA is the hereditary material (Figure 16).

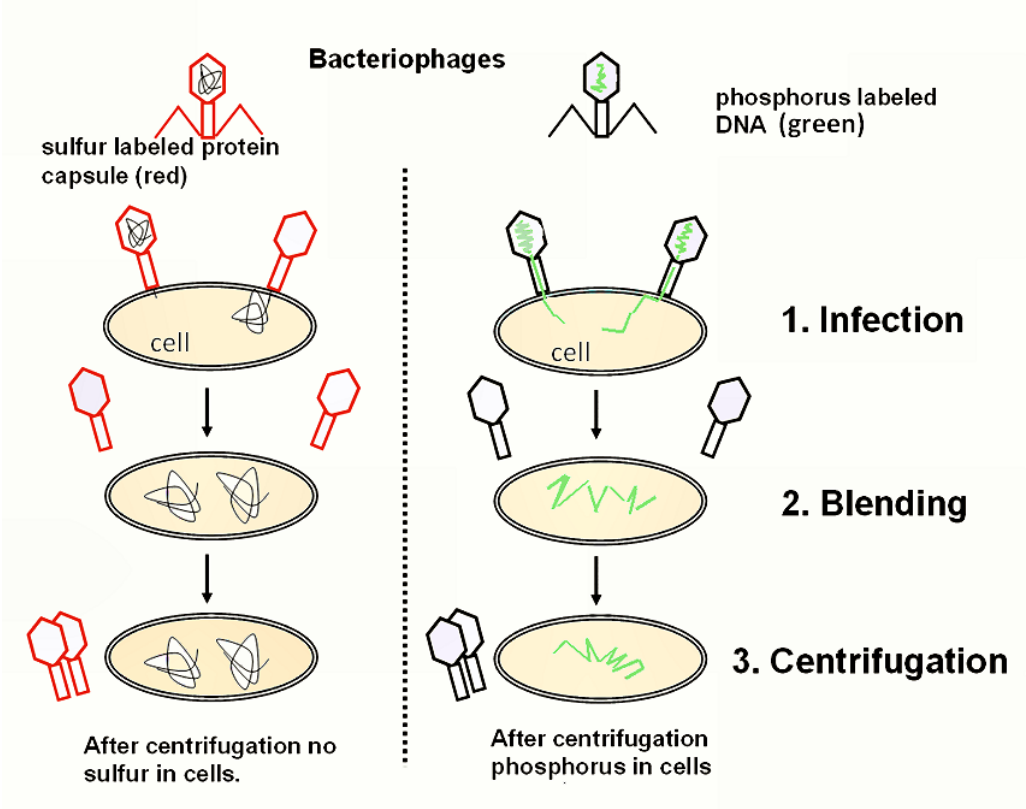


Figure 16
Hershey and Chase experiment

Hershey and Chase needed to be able to examine different parts of the phages they were studying separately, so they needed to isolate the phage subsections. Viruses were known to be composed of a protein shell and DNA, so they chose to uniquely label each with a different elemental isotope. This allowed each to be observed and analyzed separately. Since phosphorus is contained in DNA but not amino acids, radioactive phosphorus-32 was used to label the DNA contained in the T2 phage. Radioactive sulfur-35 was used to label the protein sections of the T2 phage, because sulfur is contained in amino acids but not DNA.

Hershey and Chase inserted the radioactive elements into the bacteriophages by adding the isotopes to separate media within which bacteria were allowed to grow for 4 hours before bacteriophage introduction. When the bacteriophages infected the bacteria, the progeny contained the radioactive isotopes in their structures. This procedure was performed once for the sulfur-labeled phages and once for phosphorus-labeled phages. The labeled progeny were then allowed to infect unlabeled bacteria. The phage coats remained on the outside of the bacteria, while genetic material entered. Centrifugation allowed for the separation of the phage coats from the bacteria. These bacteria were lysed

to release phage progeny. The progeny of the phages that were originally labeled with ^{32}P remained labeled, while the progeny of the phages originally labeled with ^{35}S were unlabeled. Thus, the Hershey–Chase experiment helped confirm that DNA, not protein, is the genetic material.
Hershey and Chase showed that the introduction of deoxyribonuclease (referred to as DNase), an enzyme that breaks down DNA, into a solution containing the labeled bacteriophages did not introduce any ^{32}P into the solution. This demonstrated that the phage is resistant to the enzyme while intact. Additionally, they were able to plasmolyze the bacteriophages so that they went into osmotic shock, which effectively created a solution containing most of the ^{32}P and a heavier solution containing structures called "ghosts" that contained the ^{35}S and the protein coat of the virus. It was found that these "ghosts" could adsorb to bacteria that were susceptible to T2, although they contained no DNA and were simply the remains of the original bacterial capsule. They concluded that the protein protected the RNA from RNAse, but that once the two were separated and the phage was inactivated, the DNAse could hydrolyze the phage DNA.

1953 James Watson and Francis Crick (Figure 17) publish the double helix model of DNA, which explains both how the molecule can be copied and how mutations might arise.

In the same issue of the journal *Nature,* Rosalind Franklin and Maurice Wilkins publish X-ray studies (Photo 51) that support the Watson-Crick model. This launches the field of molecular biology that shows, over the next 20 years, how the information in genes is used to build organisms.

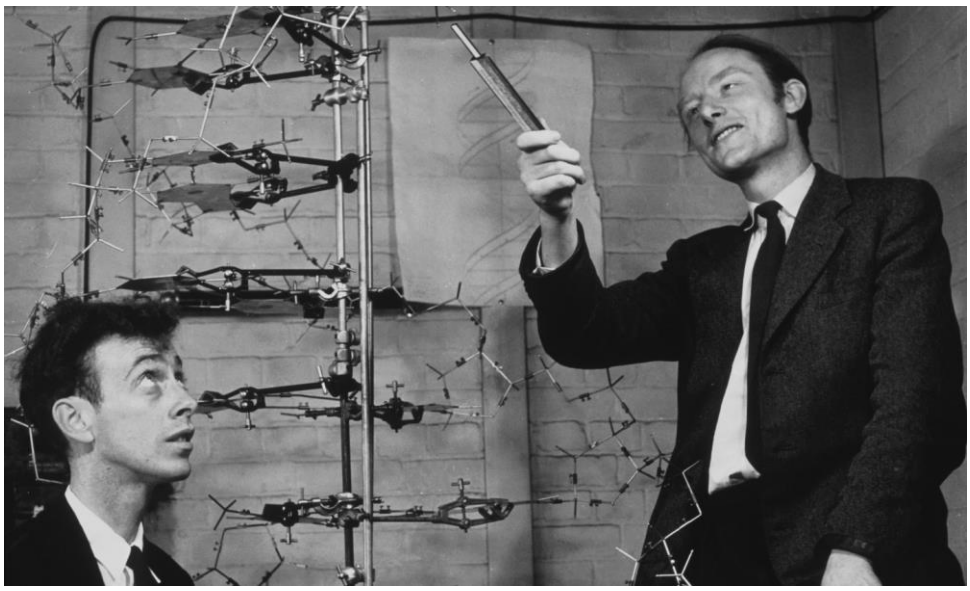


Figure 17
James D. Watson and Francis Crick

Photo 51

Photograph 51 (Figure 18) is the nickname given to an X-ray diffraction image of DNA taken by Raymond Gosling in May 1952, working as a PhD student under the supervision of Rosalind Franklin at King's College London in Sir John Randall's group. It was a critical piece of evidence in identifying the structure of DNA. James Watson was shown the photo by Maurice Wilkins without Rosalind Franklin's approval or knowledge (although by this time Gosling had returned to the supervision of Wilkins). Along with Francis Crick, Watson used characteristics and features of Photo 51 to develop the chemical model of the DNA molecule. In 1962, the Nobel Prize in Physiology or Medicine was awarded to Watson, Crick and Wilkins. The prize was not awarded to Franklin; she had died four years earlier, and the Nobel Prize's rules require that it be awarded only to living persons.

The photograph provided key information that was essential for developing a model of DNA. The diffraction pattern determined the helical nature of the double helix strands (antiparallel). The outside of the DNA chain has a backbone of alternating deoxyribose and phosphate molecules, and codes for inheritance are inside the helix. Watson and Crick's calculations from Franklin's photography gave crucial parameters for the size and structure of the helix.

Photo 51 became a crucial data source that led to the development of the DNA model and confirmed the prior postulated double helical structure of DNA, which were presented in the articles in the Nature journal by Raymond Gosling.

As historians of science have re-examined the period during which this image was obtained, considerable controversy has arisen over both the significance of the contribution of this image to the work of Watson and Crick, as well as the methods by which they obtained the image. Franklin was hired independently of Maurice Wilkins, who, nonetheless, showed Photo 51 to Watson and Crick, without her knowledge. Whether Franklin would have deduced the structure of DNA on her own, from her own data, had Watson and Crick not obtained Gosling's image, is a hotly debated topic, made more controversial by the negative caricature of Franklin presented in the early chapters of Watson's history of the research on DNA structure, The Double Helix. Watson admitted his distortion of Franklin in his book, noting in the epilogue: "Since my initial impressions about [Franklin], both scientific and personal (as recorded in the early pages of this book) were often wrong, I want to say something here about her achievements."

1954 First organ transplant (kidney) on identical twins.

1958 Francis Crick describes the central dogma of molecular biology: **DNA creates RNA creates proteins.** He challenges the scientific community to figure out the molecules and mechanisms by which this happens.

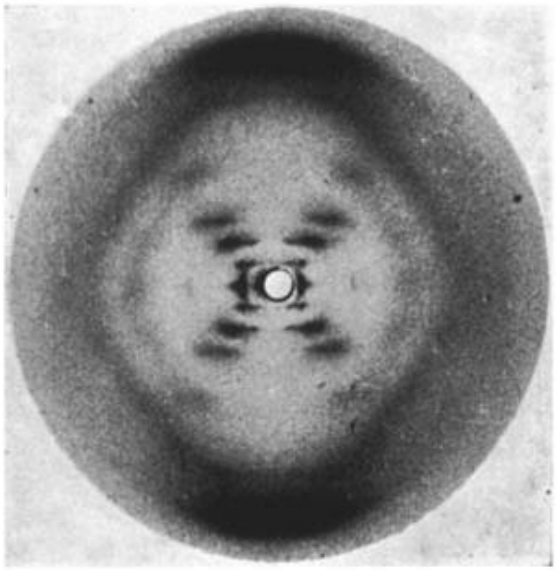


Figure 18
Photograph 51 showing X-ray diffraction pattern of the DNA

1959 Jerome Lejeune discovers the first disease due to defects in chromosomes: Down syndrome is caused by the inheritance of an extra chromosome.

1961 Sidney Brenner, François Jacob, and Matthew Meselson discover that messenger RNA is the template molecule that carries information from genes into the protein form. Crick and Brenner suggest that proteins are made by reading three-letter codons in RNA sequences, which represent three-letter codes in DNA.

The number of bases found in each code word, or codon, during transcription was one of the major mysteries in the 1960s. Scientists knew there was a total of four bases (guanine, cytosine, adenine, and thymine) and they also knew that there were 20 known amino acids.

George Gamow suggested that the genetic code was at least made of three nucleotides per amino acid, because there are 20 amino acids and only four bases, the coding units could not be single (4 combinations) or pairs (only 16 combinations). Rather, he thought triplets (64 possible combinations) were the coding unit of the genetic code. However, he proposed that the triplets were overlapping and non-degenerate.

Sidney Brenner is one of the 25 Nobel laureates, who won Nobel Prize while working at Laboratory of Molecular Biology at University of Cambridge, UK. Cambridge takes pride in having 92 Nobel laureates and it is an amazing feat that 25 of them are associated with this one institute. I was lucky to do experiment there while doing my PhD at Cambridge. I would urge all the students to apply for higher studies there, if they get the opportunity.

1965 Leonard Hayflick discovers that human cells raised in laboratory cultures have a limited life span, prompting a search for molecular mechanisms of aging. He coined the term **Hayflick limit** which is the number of times a

normal human cell population will divide until cell division stops. Empirical evidence shows that the telomeres associated with each cell's DNA will get slightly shorter with each new cell division until they shorten to a critical length (Figure 19)[6]. He found that normal fetal cells will divide between 40 to 60 times before entering a senescence phase.

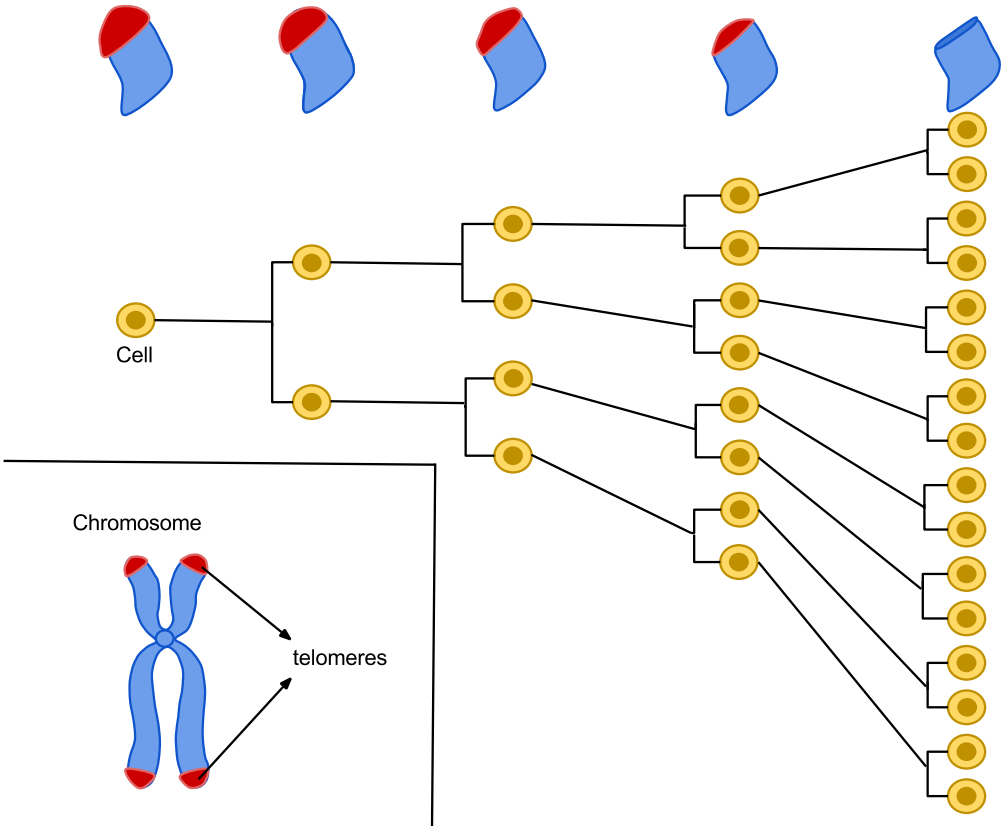


Figure 19
The average cell will divide between 50-70 times before cell death. As the cell divides the telomeres on the end of the chromosome get smaller. The Hayflick Limit is the theory that due to the telomeres shortening through each division, the telomeres will eventually no longer be present on the chromosome. This end stage is known as senescence and proves the concept that links the deterioration of telomeres and aging.

1966 Marshall Nirenberg and H. Gobind Khorana worked out the complete genetic code—the DNA recipe for every amino acid.

They needed a cell-free system that would build amino acids into proteins. They achieved this by rupturing *E. coli* and releasing the contents of the cytoplasm, allowing them to synthesize proteins in vitro. They could control translation by adding the synthetic RNA. The experiment used 20 test tubes, each filled with a different amino acid. For each individual

[6] Picture from Wikipedia designed by Azmistowski

experiment, 19 test tubes were "cold", and one was radioactively tagged with ^{14}C so they could detect the tagged amino acid later. They varied the "hot" amino acid in each round of the experiment, seeking to determine which amino acid would be incorporated into a protein following the addition of a particular type of synthetic RNA. In their experiments in late May 1961 they had narrowed down the amino acids encoded by Poly-U to Phenylalanine or Tyrosine. Matthaei used phenylalanine for the "hot" test tube. After an hour, the control tubes showed a background level of 70 counts, whereas the hot tube showed 38,000 counts per milligram of protein. Figure 20 shows a few pages from Nirenberg's laboratory notebook.

The experiment showed that a chain of the repeated uracil bases produced a protein chain made of one repeating amino acid, phenylalanine. Therefore, polyU coded for polyphenylalanine, consistent with UUU coding for phenylalanine. At the time the number of bases per codon could not be determined. The two kept their breakthrough a secret from the larger scientific community until they could complete further experiments with other strands of synthetic RNA (such as Poly-A) and prepare papers for publication. Using the three-letter poly-U experiment as a model, the research team discovered that AAA (three adenosines) was the code word or "codon" for the amino acid lysine, and CCC (three cytosines) was the code word for proline. They also discovered that by replacing one or two units of a triplet with other nucleotides, they could direct the production of other amino acids. They found, for example, that a synthetic RNA GUU codes for a valine be added to a developing amino acid chain.

1970 Hamilton Smith and Kent Wilcox isolate the first restriction enzyme, a molecule that cuts DNA at a specific sequence—which will become an essential tool in genetic engineering. Today restriction enzymes are used to cut DNA in almost all molecular laboratories. Several restriction enzymes have been found and created to target specific sites (Table 6).

There are various types of restriction endonucleases and students would learn more about them in the courses on Biochemistry and Molecular Biology.

1972 Janet Mertz and Ron Davis use restriction enzymes and DNA-mending molecules called ligases to carry out the first recombination: the creation of an artificial DNA molecule. Paul Berg creates a new gene in bacteria using genetic engineering.

1973 Stanley Cohen, Annie Chang, Robert Helling, and Herbert Boyer create the first transgenic organism by putting an artificial chromosome into bacteria.

1975 Edward Southern creates Southern blotting, a method to detect a specific DNA sequence in a person's DNA; the method will become crucial to genetic testing and biology in general.

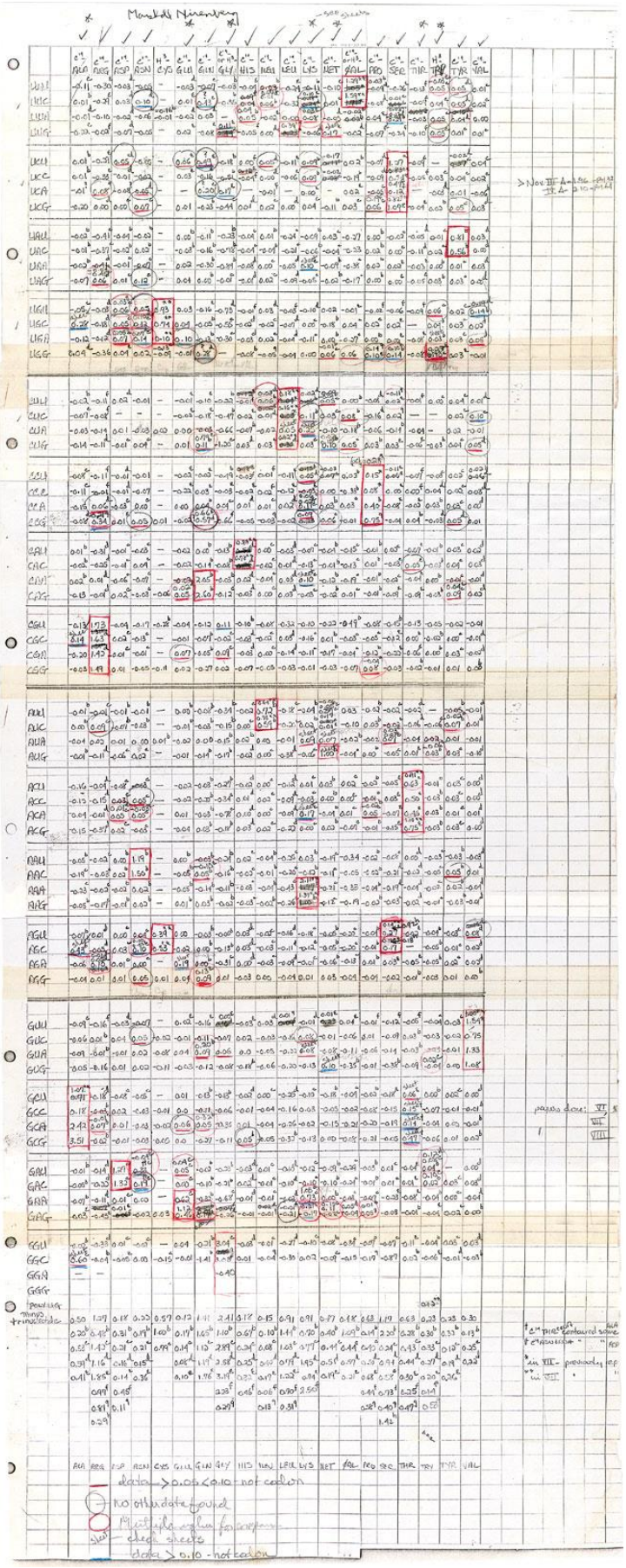


Figure 20 A few pages of Nirenberg's laboratory notebook

Table 6: Recognition sequences of some restriction endonucleases[7]

Enzyme	Recognition site	Type of cut end
EcoRI	G↓A—A—T—T—C C—T—T—A—A↑G	5′ phosphate extension
BamHI	G↓G—A—T—C—C C—C—T—A—G↑G	5′ phosphate extension
PstI	C—T—G—C—A↓G G↑A—C—G—T—C	3′ hydroxyl extension
Sau3AI	↓G—A—T—C C—T—A—G↑	5′ phosphate extension
PvuII	C—A—G↓C—T—G G—T—C↑G—A—C	Blunt end
HpaI	G—T—T↓A—A—C C—A—A↑T—T—G	Blunt end
HaeIII	G—G↓C—C C—C↑G—G	Blunt end
NotI	G↓C—G—G—C—C—G—C C—G—C—C—G—G—C↑G	5′ phosphate extension

Arrows denote cleavage sites.

1975 Cesar Milstein, Georges Kohler, and Niels Kai Jerne develop a method to make monoclonal antibodies.

1977 Walter Gilbert and Allan Maxam develop a method to determine the sequence of a DNA molecule; Fredrick Sanger and colleagues independently develop another very rapid method for doing so, launching the age of high-throughput DNA sequencing.

Frederick Sanger finishes the first genome, the complete nucleotide sequence of a bacteriophage.

Phillip Sharp and colleagues discover introns, information in the middle of genes which do not contain codes for proteins and must be removed before an RNA can be used to create a protein.

1977 Genentech, the first biotech firm, is founded based on plans to use genetic engineering to make drugs.

1978 Recombinant DNA technology is used to create the first human hormone.

1980 Christiane Nüsslein-Volhard & Eric Wieschaus discover the first patterning genes that influence the development of the fruit fly embryo, bringing together the fields of developmental biology and genetics.

1980 The U.S. patent for gene cloning is awarded to Cohen and Boyer.

1981 Three laboratories independently discover oncogenes: proteins that lead

[7] Copied from Molecular Biotechnology by Glick, Pasternak and Paten

to cancer if they undergo mutations.

1982 Insulin becomes the first genetically engineered drug.

1983 Walter Gehring's laboratory in Basel and Matthew Scott and Amy Weiner, working at the University of Indiana, independently discover HOX genes: master patterning molecules for the creation of the head-to- tail axis in animals as diverse as flies and humans.

1985 Kary B. Mullis publishes a paper describing the polymerase chain reaction, a method which rapidly and easily copies DNA molecules.

1986 First outbreak of BSE (mad cow disease) among cattle in the United Kingdom.

1987 First human genetic map was published

1988 The Human Genome Project is launched by the U.S. Department of Energy and the National Institutes of Health, with the aim of determining the complete sequence of human DNA.

1989 Alec Jeffreys discovers regions of DNA that undergo high numbers of mutations. He develops a method of DNA fingerprinting that can match DNA samples to the person they came from and can also be used in establishing paternity and other types of family relationships. The Human Genome Organization (HUGO) is founded.

1990 W. French Anderson carries out the first human gene replacement therapy to treat an immune system disease in four-year-old Ashanti DeSilva.

1993 The company Monsanto develops and begins to market a genetically engineered strain of tomatoes called Flavr Savr. The Huntington disease gene is found. Cynthia Kenyon discovers mutations in *C. elegans* that double the worm's life span.

1994 Mary-Claire King discovers BRCA1, a gene that contributes to susceptibility to breast cancer.

1995 The first confirmed death from Creutzfeldt-Jakob disease, the human form of BSE, is reported in the United Kingdom.

1996 Researchers complete the first genome of a eukaryote, baker's yeast. The completion of the genome of *Methanococcus jannaschii,* an archaeal cell, confirmed that archaea are a third branch of life, separate from bacteria and eukaryotes. Gene therapy trials to use the adenovirus as a vector for healthy genes are approved in the United States.

1997 Ian Wilmut's laboratory at the Roslin Institute produces Dolly the sheep, the first cloned mammal.

1998 Scientists obtain the first complete genome sequence of an animal, the worm *Caenorhabiditis elegans.*

1999 Jesse Gelsinger dies in a gene therapy trial, bringing a temporary halt to all viral gene therapy trials in the United States.

2000 The genome of the fruit fly *Drosophila melanogaster* is completed. Scientists complete a working draft of the human genome. The complete genome is published in 2003.

2001 Celera Genomics and the Human Genome Project create a draft of the human genome sequence. It is published by *Science and Nature* Magazine.

2002 The mouse and rice genome is completed.

2003 The Human Genome Project is completed, providing information on the locations and sequence of human genes on all 46 chromosomes.

2004 Scientists in Seoul announce the first successful cloning of a human being, a claim which is quickly proven to be false.

2008 Samuel Wood of the California company Stemagen successfully uses his own skin cells to produce clones, which survive five days.

2008 Japanese astronomers launch the first Medical Experiment Module called "Kibo", to be used on the **International Space Station**.

2009 Cedars-Sinai Heart Institute uses modified SAN heart genes to create the first *viral pacemaker* in guinea pigs, now known as iSANs.

2012 Thirty-one-year-old Vawter successfully uses a nervous system-controlled bionic leg to climb the Chicago Willis Tower.

2012 Jennifer Doudna and Emmanuelle Charpentier first showed CRISPR to work as a genome-engineering and editing tool in bacterial cell cultures.