

Recombinant DNA

Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) that bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

Recombinant DNA is the general name for a piece of DNA that has been created by combining two or more fragments from different sources. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, differing only in the nucleotide sequence. Recombinant DNA molecules are sometimes called **chimeric DNA** because they can be made of material from two different species like the mythical chimera. rDNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

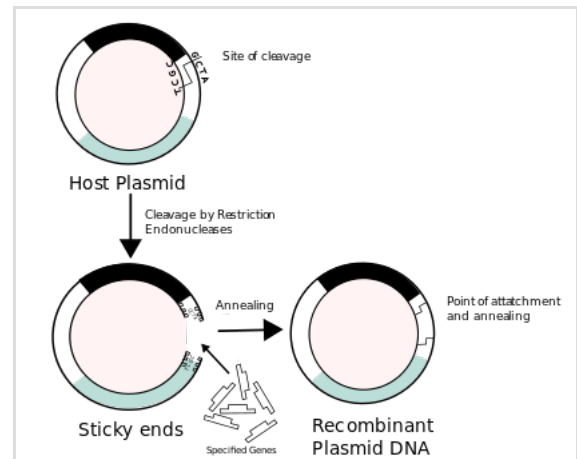
The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA can be joined to bacterial DNA, or human DNA can be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature can be created by the chemical synthesis of DNA and incorporated into recombinant DNA molecules. Using recombinant DNA technology and synthetic DNA, any DNA sequence can be created and introduced into living organisms.

Proteins that can result from the expression of recombinant DNA within living cells are termed *recombinant proteins*. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced.^[1] Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences.^[2]

Recombinant DNA differs from genetic recombination in that the former results from artificial methods while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

Production

Molecular cloning is the laboratory process used to produce recombinant DNA.^{[3][4][5][6]} It is one of two most widely used methods, along with polymerase chain reaction (PCR), used to direct the replication of any specific DNA sequence chosen by the experimentalist. There are two fundamental differences between the methods. One is that molecular cloning involves replication of the DNA within a living cell, while PCR replicates DNA in the test tube, free of living cells. The other difference is that cloning involves cutting and pasting DNA sequences, while PCR amplifies by copying an existing sequence.



Construction of recombinant DNA, in which a foreign DNA fragment is inserted into a plasmid vector. In this example, the gene indicated by the white color is inactivated upon insertion of the foreign DNA fragment.

Formation of recombinant DNA requires a cloning vector, a DNA molecule that replicates within a living cell. Vectors are generally derived from plasmids or viruses, and represent relatively small segments of DNA that contain necessary genetic signals for replication, as well as additional elements for convenience in inserting foreign DNA, identifying cells that contain recombinant DNA, and, where appropriate, expressing the foreign DNA. The choice of vector for molecular cloning depends on the choice of host organism, the size of the DNA to be cloned, and whether and how the foreign DNA is to be expressed.^[7] The DNA segments can be combined by using a variety of methods, such as restriction enzyme/ligase cloning or Gibson assembly.

In standard cloning protocols, the cloning of any DNA fragment essentially involves seven steps: (1) Choice of host organism and cloning vector, (2) Preparation of vector DNA, (3) Preparation of DNA to be cloned, (4) Creation of recombinant DNA, (5) Introduction of recombinant DNA into the host organism, (6) Selection of organisms containing recombinant DNA, and (7) Screening for clones with desired DNA inserts and biological properties.^[6] *These steps are described in some detail in a related article (molecular cloning).*

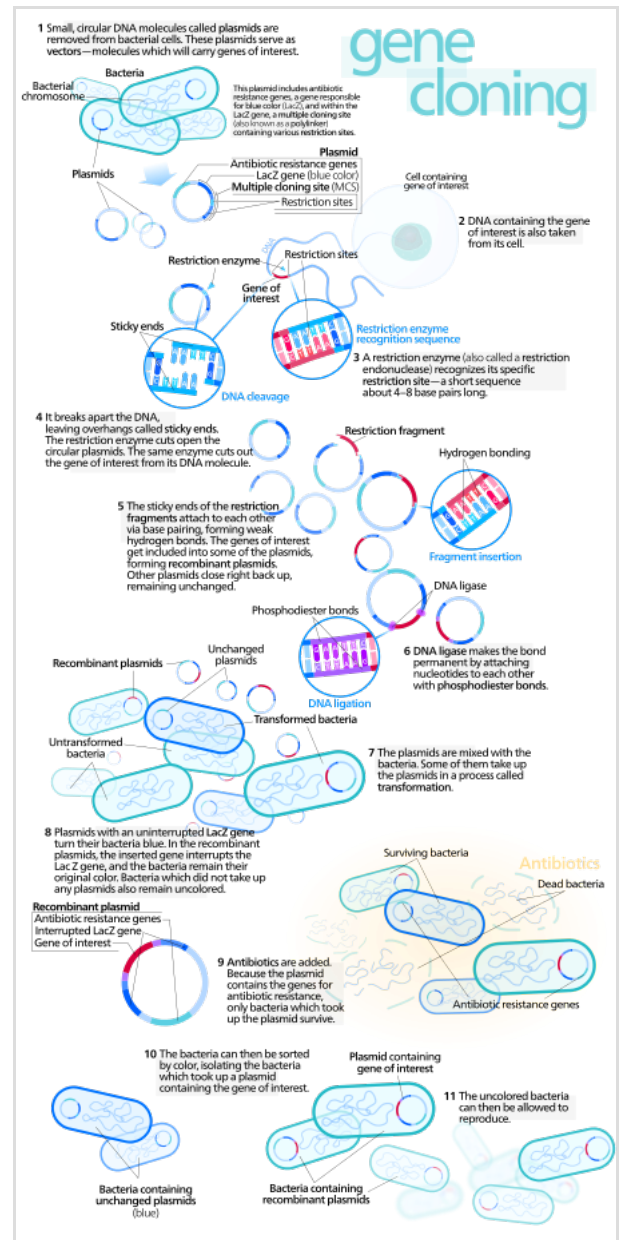
DNA expression

DNA expression requires the transfection of suitable host cells. Typically, either bacterial, yeast, insect, or mammalian cells (such as Human Embryonic Kidney cells or CHO cells) are used as host cells.^[8]

Following transplantation into the host organism, the foreign DNA contained within the recombinant DNA construct may or may not be expressed. That is, the DNA may simply be replicated without expression, or it may be transcribed and translated and a recombinant protein is produced. Generally speaking, expression of a foreign gene requires restructuring the gene to include sequences that are required for producing an mRNA molecule that can be used by the host's translational apparatus (e.g. promoter, translational initiation signal, and transcriptional terminator).^[9] Specific changes to the host organism may be made to improve expression of the ectopic gene. In addition, changes may be needed to the coding sequences as well, to optimize translation, make the protein soluble, direct the recombinant protein to the proper cellular or extracellular location, and stabilize the protein from degradation.^{[10][11][12]}

Properties of organisms containing recombinant DNA

In most cases, organisms containing recombinant DNA have apparently normal phenotypes. That is, their appearance, behavior and metabolism are usually unchanged, and the only way to demonstrate the presence of recombinant sequences is to examine the DNA itself, typically using a polymerase chain reaction (PCR) test.^[13] Significant exceptions exist, and are discussed below.



If the rDNA sequences encode a gene that is expressed, then the presence of RNA and/or protein products of the recombinant gene can be detected, typically using RT-PCR or western hybridization methods.^[13] Gross phenotypic changes are not the norm, unless the recombinant gene has been chosen and modified so as to generate biological activity in the host organism.^[14] Additional phenotypes that are encountered include toxicity to the host organism induced by the recombinant gene product, especially if it is over-expressed or expressed within inappropriate cells or tissues.

In some cases, recombinant DNA can have deleterious effects even if it is not expressed. One mechanism by which this happens is insertional inactivation, in which the rDNA becomes inserted into a host cell's gene. In some cases, researchers use this phenomenon to "knock out" genes to determine their biological function and importance.^[15] Another mechanism by which rDNA insertion into chromosomal DNA can affect gene expression is by inappropriate activation of previously unexpressed host cell genes. This can happen, for example, when a recombinant DNA fragment containing an active promoter becomes located next to a previously silent host cell gene, or when a host cell gene that functions to restrain gene expression undergoes insertional inactivation by recombinant DNA.

Applications of recombinant DNA

Recombinant DNA is widely used in biotechnology, medicine and research. Today, recombinant proteins and other products that result from the use of DNA technology are found in essentially every western pharmacy, physician or veterinarian office, medical testing laboratory, and biological research laboratory. In addition, organisms that have been manipulated using recombinant DNA technology, as well as products derived from those organisms, have found their way into many farms, supermarkets, home medicine cabinets, and even pet shops, such as those that sell GloFish and other genetically modified animals.

The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences.^[13] Recombinant DNA is used to identify, map and sequence genes, and to determine their function. rDNA probes are employed in analyzing gene expression within individual cells, and throughout the tissues of whole organisms. Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.^[4]

Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering.^[4] Some specific examples are identified below.

Recombinant chymosin

Found in rennet, chymosin is the enzyme responsible for hydrolysis of κ -casein to produce para- κ -casein and glycomacropeptide, which is the first step in formation of cheese, and subsequently curd, and whey.^[16] It was the first genetically engineered food additive used commercially. Traditionally, processors obtained chymosin from rennet, a preparation derived from the fourth stomach of milk-fed calves. Scientists engineered a non-pathogenic strain (K-12) of *E. coli* bacteria for large-scale laboratory production of the enzyme. This microbiologically produced recombinant enzyme, identical structurally to the calf derived enzyme, costs less and is produced in abundant quantities. Today about 60% of U.S. hard cheese is made with genetically engineered chymosin. In 1990, FDA granted chymosin "generally recognized as safe" (GRAS) status based on data showing that the enzyme was safe.^[17]

Recombinant human insulin

Recombinant human insulin has almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of type 1 diabetes. A variety of different recombinant insulin preparations are in widespread use.^[18] Recombinant insulin is synthesized by inserting the human insulin gene into *E. coli*, or yeast (*Saccharomyces cerevisiae*)^[19] which then produces insulin for human use.^[20] Insulin produced by *E. coli* requires further post translational modifications (e.g. glycosylation) whereas yeasts are able to perform these modifications themselves by virtue of being more complex host organisms. The advantage of recombinant human insulin is after chronic use patients don't develop an immune defence against it the way animal sourced insulin stimulates the human immune system.^[21]

Recombinant human growth hormone (HGH, somatotropin)

Administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers. This unsafe practice led to some patients developing Creutzfeldt–Jakob disease. Recombinant HGH eliminated this problem, and is now used therapeutically.^[22] It has also been misused as a performance-enhancing drug by athletes and others.^{[23][24]}

Recombinant blood clotting factor VIII

It is the recombinant form of factor VIII, a blood-clotting protein that is administered to patients with the bleeding disorder hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation.^[25] Before the development of recombinant factor VIII, the protein was obtained by processing large quantities of human blood from multiple donors, which carried a very high risk of transmission of blood borne infectious diseases, for example HIV and hepatitis B.

Recombinant hepatitis B vaccine

Hepatitis B infection can be successfully controlled through the use of a recombinant subunit hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells. The development of the recombinant subunit vaccine was an important and necessary development because hepatitis B virus, unlike other common viruses such as polio virus, cannot be grown in vitro.^[26]

Recombinant antibodies

Recombinant antibodies (rAbs) are produced in vitro by the means of expression systems based on mammalian cells. Their monospecific binding to a specific epitope makes rAbs eligible not only for research purposes, but also as therapy options against certain cancer types, infections and autoimmune diseases.^[27]

Diagnosis of HIV infection

Each of the three widely used methods for diagnosing HIV infection has been developed using recombinant DNA. The antibody test (ELISA or western blot) uses a recombinant HIV protein to test for the presence of antibodies that the body has produced in response to an HIV infection. The DNA test looks for the presence of HIV genetic material using reverse transcription polymerase chain reaction (RT-PCR). Development of the RT-PCR test was made possible by the molecular cloning and sequence analysis of HIV genomes. HIV testing page from US Centers for Disease Control (CDC) (<https://www.cdc.gov/hiv/topics/testing/index.htm>)

Golden rice

Golden rice is a recombinant variety of rice that has been engineered to express the enzymes responsible for β -carotene biosynthesis.^[14] This variety of rice holds substantial promise for reducing the incidence of vitamin A deficiency in the world's population.^[28] Golden rice is not currently in use, pending the resolution of regulatory and intellectual property issues.^[29]

Herbicide-resistant crops

Commercial varieties of important agricultural crops (including soy, maize/corn, sorghum, canola, alfalfa and cotton) have been developed that incorporate a recombinant gene that results in resistance to the herbicide glyphosate (trade name *Roundup*), and simplifies weed control by glyphosate application.^[30] These crops are in common commercial use in several countries.

Insect-resistant crops

Bacillus thuringiensis is a bacterium that naturally produces a protein (Bt toxin) with insecticidal properties.^[28] The bacterium has been applied to crops as an insect-control strategy for many years, and this practice has been widely adopted in agriculture and gardening. Recently, plants have been developed that express a recombinant form of the bacterial protein, which may effectively control some insect predators. Environmental issues associated with the use of these transgenic crops have not been fully resolved.^[31]

History

The idea of recombinant DNA was first proposed by Peter Lobban, a graduate student of Prof. Dale Kaiser in the Biochemistry Department at Stanford University Medical School.^[32] The first publications describing the successful production and intracellular replication of recombinant DNA appeared in 1972 and 1973, from Stanford and UCSF.^{[33][34][35][36]} In 1980 Paul Berg, a professor in the Biochemistry Department at Stanford and an author on one of the first papers^[33] was awarded the Nobel Prize in Chemistry for his work on nucleic acids "with particular regard to recombinant DNA". Werner Arber, Hamilton Smith, and Daniel Nathans shared the 1978 Nobel Prize in Physiology or Medicine for the discovery of restriction endonucleases which enhanced the techniques of rDNA technology.

Stanford University applied for a US patent on recombinant DNA in 1974, listing the inventors as Herbert W. Boyer (professor at the University of California, San Francisco) and Stanley N. Cohen (professor at Stanford University); this patent was awarded in 1980.^[37] The first licensed drug generated using recombinant DNA technology was human insulin, developed by Genentech and licensed by Eli Lilly and Company.^[38]

Controversy

Scientists associated with the initial development of recombinant DNA methods recognized that the potential existed for organisms containing recombinant DNA to have undesirable or dangerous properties. At the 1975 Asilomar Conference on Recombinant DNA, these concerns were discussed and a voluntary moratorium on recombinant DNA research was initiated for experiments that were considered particularly risky. This moratorium was widely observed until the National Institutes of Health (USA) developed and issued formal guidelines for rDNA work. Today, recombinant DNA molecules and recombinant proteins are usually not regarded as dangerous. However, concerns remain about some

organisms that express recombinant DNA, particularly when they leave the laboratory and are introduced into the environment or food chain. These concerns are discussed in the articles on genetically modified organisms and genetically modified food controversies. Furthermore, there are concerns about the by-products in biopharmaceutical production, where recombinant DNA result in specific protein products. The major by-product, termed host cell protein, comes from the host expression system and poses a threat to the patient's health and the overall environment.^{[39][40]}

See also



Biology portal

- Asilomar conference on recombinant DNA
- Genetic engineering
- Genetically modified organism
- Recombinant virus
- Vector DNA
- Biomolecular engineering
- Recombinant DNA technology
- Host cell protein
- T7 expression system

References

1. Rosano, Germán L.; Ceccarelli, Eduardo A. (2014-04-17). "Recombinant protein expression in *Escherichia coli*: advances and challenges" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4029002>). *Frontiers in Microbiology*. **5**: 172. doi:10.3389/fmicb.2014.00172 (<https://doi.org/10.3389%2Fmicb.2014.00172>). ISSN 1664-302X (<https://www.worldcat.org/issn/1664-302X>). PMC 4029002 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4029002>). PMID 24860555 (<https://pubmed.ncbi.nlm.nih.gov/24860555>).
2. "Promoters used to regulate gene expression" (<http://www.cambia.org/daisy/promoters/818.html>). *www.cambia.org*. Retrieved 16 February 2018.
3. Campbell, Neil A. & Reece, Jane B.. (2002). *Biology (6th ed.)*. San Francisco: Addison Wesley. pp. 375–401. ISBN 978-0-201-75054-6.
4. Peter Walter; Alberts, Bruce; Johnson, Alexander S.; Lewis, Julian; Raff, Martin C.; Roberts, Keith (2008). *Molecular Biology of the Cell (5th edition, Extended version)*. New York: Garland Science. ISBN 978-0-8153-4111-6.. Fourth edition is available online through the NCBI Bookshelf: [link \(https://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4\)](https://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4)
5. Berg, Jeremy Mark; Tymoczko, John L.; Stryer, Lubert (2010). *Biochemistry, 7th ed. (Biochemistry (Berg))*. W.H. Freeman & Company. ISBN 978-1-4292-2936-4. Fifth edition available online through the NCBI Bookshelf: [link \(https://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer\)](https://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer)
6. Watson, James D. (2007). *Recombinant DNA: Genes and Genomes: A Short Course*. San Francisco: W.H. Freeman. ISBN 978-0-7167-2866-5.
7. Russell, David W.; Sambrook, Joseph (2001). *Molecular cloning: a laboratory manual* (http://archive.org/details/molecularcloning0000samb_p7p5). Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory. ISBN 978-0-87969-576-7.
8. Eberle, Christian (December 2022). "Recombinant DNA technology – Steps, Methods & Examples" (<https://www.evitra.com/journal/recombinant-antibodies/recombinant-dna-technology/>). Retrieved July 18, 2023.

9. Hannig, G.; Makrides, S. (1998). "Strategies for optimizing heterologous protein expression in *Escherichia coli*". *Trends in Biotechnology*. **16** (2): 54–60. doi:10.1016/S0167-7799(97)01155-4 (https://doi.org/10.1016%2FS0167-7799%2897%2901155-4). PMID 9487731 (https://pubmed.ncbi.nlm.nih.gov/9487731).
10. Mahmoudi Gomari, Mohammad; Saraygord-Afshari, Neda; Farsimadan, Marziye; Rostami, Neda; Aghamiri, Shahin; Farajollahia, Mohammad M. (1 December 2020). "Opportunities and challenges of the tag-assisted protein purification techniques: Applications in the pharmaceutical industry" (https://www.sciencedirect.com/science/article/abs/pii/S0734975020301555). *Biotechnology Advances*. **45**: 107653. doi:10.1016/j.biotechadv.2020.107653 (https://doi.org/10.1016%2Fj.biotechadv.2020.107653). ISSN 0734-9750 (https://www.worldcat.org/issn/0734-9750). PMID 33157154 (https://pubmed.ncbi.nlm.nih.gov/33157154). S2CID 226276355 (https://api.semanticscholar.org/CorpusID:226276355).
11. Brondyk, W. H. (2009). "Chapter 11 Selecting an Appropriate Method for Expressing a Recombinant Protein". *Guide to Protein Purification, 2nd Edition*. Methods in Enzymology. Vol. 463. pp. 131–147. doi:10.1016/S0076-6879(09)63011-1 (https://doi.org/10.1016%2FS0076-6879%2809%2963011-1). ISBN 9780123745361. PMID 19892171 (https://pubmed.ncbi.nlm.nih.gov/19892171).
12. Ortega, Claudia; Prieto, Daniel; Abreu, Cecilia; Oppezzo, Pablo Javier; Correa, Agustin (2018). "Multi-compartment and multi-host vector suite for recombinant protein expression and purification" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6030378). *Frontiers in Microbiology*. **9**: 1384. doi:10.3389/fmicb.2018.01384 (https://doi.org/10.3389%2Fmicb.2018.01384). ISSN 1664-302X (https://www.worldcat.org/issn/1664-302X). PMC 6030378 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6030378). PMID 29997597 (https://pubmed.ncbi.nlm.nih.gov/29997597).
13. Brown, Terry (2006). *Gene Cloning and DNA Analysis: an Introduction*. Cambridge, MA: Blackwell Pub. ISBN 978-1-4051-1121-8.
14. Ye, X.; Al-Babili, S.; Klöti, A.; Zhang, J.; Lucca, P.; Beyer, P.; Potrykus, I. (2000). "Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm". *Science*. **287** (5451): 303–305. Bibcode:2000Sci...287..303Y (https://ui.adsabs.harvard.edu/abs/2000Sci...287..303Y). doi:10.1126/science.287.5451.303 (https://doi.org/10.1126%2Fscience.287.5451.303). PMID 10634784 (https://pubmed.ncbi.nlm.nih.gov/10634784). S2CID 40258379 (https://api.semanticscholar.org/CorpusID:40258379).
15. Koller, B. H.; Smithies, O. (1992). "Altering Genes in Animals by Gene Targeting". *Annual Review of Immunology*. **10**: 705–730. doi:10.1146/annurev.iy.10.040192.003421 (https://doi.org/10.1146%2Fannurev.iy.10.040192.003421). PMID 1591000 (https://pubmed.ncbi.nlm.nih.gov/1591000).
16. "Chymosin - an overview | ScienceDirect Topics" (https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chymosin#:~:text=The%20action%20of%20chymosin%20(rennet,formation%20of%20curds%20and%20whey.)). Archived (https://web.archive.org/web/20231210152147/https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chymosin#:~:text=The%20action%20of%20chymosin%20(rennet,formation%20of%20curds%20and%20whey.)) from the original on 2023-12-10. Retrieved 2023-12-10.**Link to original publication** (https://www.sciencedirect.com/science/article/abs/pii/B9781845697587500022)
17. Donna U. Vogt and Mickey Parish. (1999) Food Biotechnology in the United States: Science, Regulation, and Issues (https://fpc.state.gov/6176.htm)
18. Gualandi-Signorini, A.; Giorgi, G. (2001). "Insulin formulations--a review". *European Review for Medical and Pharmacological Sciences*. **5** (3): 73–83. PMID 12004916 (https://pubmed.ncbi.nlm.nih.gov/12004916).
19. #Insulin aspart
20. "Insulin human" (https://go.drugbank.com/drugs/DB00030). *go.drugbank.com*. Retrieved 2023-12-10.

21. Mills, Joshua (2022-05-16). "HSC Biology Recombinant technology: Insulin production" (<http://edzion.com/2022/05/16/hsc-biology-recombinant-technology-insulin-production/>). *Edzion*. Retrieved 2022-12-26.
22. Von Fange, T.; McDiarmid, T.; MacKler, L.; Zolotor, A. (2008). "Clinical inquiries: Can recombinant growth hormone effectively treat idiopathic short stature?". *The Journal of Family Practice*. **57** (9): 611–612. PMID 18786336 (<https://pubmed.ncbi.nlm.nih.gov/18786336/>).
23. Fernandez, M.; Hosey, R. (2009). "Performance-enhancing drugs snare nonathletes, too". *The Journal of Family Practice*. **58** (1): 16–23. PMID 19141266 (<https://pubmed.ncbi.nlm.nih.gov/19141266/>).
24. "Somatotropin" (<https://go.drugbank.com/drugs/DB00052>). *go.drugbank.com*. Retrieved 2023-12-10.
25. Manco-Johnson, M. J. (2010). "Advances in the Care and Treatment of Children with Hemophilia". *Advances in Pediatrics*. **57** (1): 287–294. doi:10.1016/j.yapd.2010.08.007 (<http://doi.org/10.1016%2Fj.yapd.2010.08.007>). PMID 21056743 (<https://pubmed.ncbi.nlm.nih.gov/21056743/>).
26. "Hepatitis B Vaccine Information from Hepatitis B Foundation:" (https://web.archive.org/web/20110628230253/http://www.hepb.org/hepb/vaccine_information.htm). *web.archive.org*. 2011-06-28. Retrieved 2023-12-10.
27. Narang, Aarti (2022). "Recombinant Antibodies: Next level in antibody technology" (<https://www.evitra.com/journal/recombinant-antibodies/recombinant-antibodies-next-level-antibody-technology/>).
28. Paine, J. A.; Shipton, C. A.; Chaggar, S.; Howells, R. M.; Kennedy, M. J.; Vernon, G.; Wright, S. Y.; Hinchliffe, E.; Adams, J. L.; Silverstone, A. L.; Drake, R. (2005). "Improving the nutritional value of Golden Rice through increased pro-vitamin a content". *Nature Biotechnology*. **23** (4): 482–487. doi:10.1038/nbt1082 (<https://doi.org/10.1038%2Fnbt1082>). PMID 15793573 (<https://pubmed.ncbi.nlm.nih.gov/15793573/>). S2CID 632005 (<https://api.semanticscholar.org/CorpusID:632005>).
29. DHNS. "Foreign group roots for 'golden rice' in India" (<https://www.deccanherald.com/india/foreign-group-roots-golden-rice-2118105>). *Deccan Herald*. Retrieved 2023-12-10.
30. Funke, T.; Han, H.; Healy-Fried, M.; Fischer, M.; Schönbrunn, E. (2006). "Molecular basis for the herbicide resistance of Roundup Ready crops" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1559744>). *Proceedings of the National Academy of Sciences*. **103** (35): 13010–13015. Bibcode:2006PNAS..10313010F (<https://ui.adsabs.harvard.edu/abs/2006PNAS..10313010F>). doi:10.1073/pnas.0603638103 (<https://doi.org/10.1073%2Fpnas.0603638103>). PMC 1559744 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1559744>). PMID 16916934 (<https://pubmed.ncbi.nlm.nih.gov/16916934/>).
31. Mendelsohn, M.; Kough, J.; Vaituzis, Z.; Matthews, K. (2003). "Are Bt crops safe?" (<https://zenodo.org/record/1233343>). *Nature Biotechnology*. **21** (9): 1003–1009. doi:10.1038/nbt0903-1003 (<https://doi.org/10.1038%2Fnbt0903-1003>). PMID 12949561 (<https://pubmed.ncbi.nlm.nih.gov/12949561/>). S2CID 16392889 (<https://api.semanticscholar.org/CorpusID:16392889>).
32. Lear, J. (1978). *Recombinant DNA: The Untold Story*. New York: Crown Publishers. p. 43.
33. Jackson, D.; Symons, R.; Berg, P. (1972). "Biochemical method for inserting new genetic information into DNA of Simian Virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC389671>). *Proceedings of the National Academy of Sciences of the United States of America*. **69** (10): 2904–2909. Bibcode:1972PNAS...69.2904J (<https://ui.adsabs.harvard.edu/abs/1972PNAS...69.2904J>). doi:10.1073/pnas.69.10.2904 (<https://doi.org/10.1073%2Fpnas.69.10.2904>). PMC 389671 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC389671>). PMID 4342968 (<https://pubmed.ncbi.nlm.nih.gov/4342968/>).

34. Mertz, J. E.; Davis, R. W. (1972). "Cleavage of DNA by R 1 restriction endonuclease generates cohesive ends" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC389773>). *Proceedings of the National Academy of Sciences of the United States of America*. **69** (11): 3370–4. Bibcode:1972PNAS...69.3370M (<https://ui.adsabs.harvard.edu/abs/1972PNAS...69.3370M>). doi:10.1073/pnas.69.11.3370 (<https://doi.org/10.1073%2Fpnas.69.11.3370>). PMC 389773 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC389773>). PMID 4343968 (<https://pubmed.ncbi.nlm.nih.gov/4343968>).
35. Lobban, P.; Kaiser, A. (1973). "Enzymatic end-to end joining of DNA molecules". *Journal of Molecular Biology*. **78** (3): 453–471. doi:10.1016/0022-2836(73)90468-3 (<https://doi.org/10.1016%2F0022-2836%2873%2990468-3>). PMID 4754844 (<https://pubmed.ncbi.nlm.nih.gov/4754844>).
36. Cohen, S.; Chang, A.; Boyer, H.; Helling, R. (1973). "Construction of biologically functional bacterial plasmids in vitro" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC427208>). *Proceedings of the National Academy of Sciences of the United States of America*. **70** (11): 3240–3244. Bibcode:1973PNAS...70.3240C (<https://ui.adsabs.harvard.edu/abs/1973PNAS...70.3240C>). doi:10.1073/pnas.70.11.3240 (<https://doi.org/10.1073%2Fpnas.70.11.3240>). PMC 427208 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC427208>). PMID 4594039 (<https://pubmed.ncbi.nlm.nih.gov/4594039>).
37. Hughes, S. (2001). "Making dollars out of DNA. The first major patent in biotechnology and the commercialization of molecular biology, 1974-1980" (https://dukespace.lib.duke.edu/dspace/bitstream/10161/8125/1/Hxdocs_makingdollarsoutofdna.pdf) (PDF). *Isis; an International Review Devoted to the History of Science and Its Cultural Influences*. **92** (3): 541–575. doi:10.1086/385281 (<https://doi.org/10.1086%2F385281>). hdl:10161/8125 (<https://hdl.handle.net/10161%2F8125>). PMID 11810894 (<https://pubmed.ncbi.nlm.nih.gov/11810894>). S2CID 22823711 (<https://api.semanticscholar.org/CorpusID:22823711>).
38. Johnson, I. S. (1983). "Human insulin from recombinant DNA technology". *Science*. **219** (4585): 632–637. Bibcode:1983Sci...219..632J (<https://ui.adsabs.harvard.edu/abs/1983Sci...219..632J>). doi:10.1126/science.6337396 (<https://doi.org/10.1126%2Fscience.6337396>). PMID 6337396 (<https://pubmed.ncbi.nlm.nih.gov/6337396>).
39. Wang, Xing; Hunter, Alan K.; Mozier, Ned M. (2009-06-15). "Host cell proteins in biologics development: Identification, quantitation and risk assessment" (<https://doi.org/10.1002%2Fbit.22304>). *Biotechnology and Bioengineering*. **103** (3): 446–458. doi:10.1002/bit.22304 (<https://doi.org/10.1002%2Fbit.22304>). ISSN 0006-3592 (<https://www.worldcat.org/issn/0006-3592>). PMID 19388135 (<https://pubmed.ncbi.nlm.nih.gov/19388135>). S2CID 22707536 (<https://api.semanticscholar.org/CorpusID:22707536>).
40. Bracewell, Daniel G.; Francis, Richard; Smales, C. Mark (2015-07-14). "The future of host cell protein (HCP) identification during process development and manufacturing linked to a risk-based management for their control" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4973824>). *Biotechnology and Bioengineering*. **112** (9): 1727–1737. doi:10.1002/bit.25628 (<https://doi.org/10.1002%2Fbit.25628>). ISSN 0006-3592 (<https://www.worldcat.org/issn/0006-3592>). PMC 4973824 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4973824>). PMID 25998019 (<https://pubmed.ncbi.nlm.nih.gov/25998019>).

Further reading

- *The Eighth Day of Creation: Makers of the Revolution in Biology*. Touchstone Books, ISBN 0-671-22540-5. 2nd edition: Cold Spring Harbor Laboratory Press, 1996 paperback: ISBN 0-87969-478-5.
- Micklas, David. 2003. *DNA Science: A First Course*. Cold Spring Harbor Press: ISBN 978-0-87969-636-8.
- Rasmussen, Nicolas, *Gene Jockeys: Life Science and the rise of Biotech Enterprise*, Johns Hopkins University Press, (Baltimore), 2014 (https://books.google.com/books?id=tTE_AwAA)

QBAJ). ISBN 978-1-42141-340-2.

- Rosenfeld, Israel. 2010. *DNA: A Graphic Guide to the Molecule that Shook the World*. Columbia University Press: ISBN 978-0-231-14271-7.
- Schultz, Mark and Zander Cannon. 2009. *The Stuff of Life: A Graphic Guide to Genetics and DNA*. Hill and Wang: ISBN 0-8090-8947-5.
- Watson, James. 2004. *DNA: The Secret of Life*. Random House: ISBN 978-0-09-945184-6.

External links

- Recombinant DNA fact sheet (<https://web.archive.org/web/20100923031225/http://www.unh.edu/ehs/pdf/Recombinant-DNA.pdf>) (from University of New Hampshire)
 - Plasmids in Yeasts (<http://www.sci.sdsu.edu/~smaloy/MicrobialGenetics/topics/plasmids/yeast-plasmid.html>) (Fact sheet from San Diego State University)
 - Animation illustrating construction of recombinant DNA and foreign protein production by recombinant bacteria (<http://sciencestage.com/v/151/ampicillin-dna-bacteria-protein-recombinant.html>)
 - Recombinant DNA research at UCSF and commercial application at Genentech (<https://archive.org/stream/dnaresearchucsf00boyerich#page/n0/mode/1up>) Edited transcript of 1994 interview with Herbert W. Boyer, Living history project. Oral history.
 - Recombinant Protein Purification Principles and Methods Handbook (<http://www.gelifesciences.com/handbooks>)
 - Massachusetts Institute of Technology, Oral History Program, Oral History Collection on the Recombinant DNA Controversy (<https://archivesspace.mit.edu/repositories/2/resources/658>), MC-0100. Massachusetts Institute of Technology, Department of Distinctive Collections, Cambridge, Massachusetts
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