transformed bacteria, it is stimulated to express the cloned gene and to make useful quantities of the protein. To make a particular protein by expressing its cloned gene in bacteria (referred to as 'a recombinant process')[92] several steps must be performed.[93] First, the gene coding for the particular protein has to be isolated. Next, the isolated genes must be transferred to the host bacterium. This is typically performed by incorporating the gene into a cloning vector. A cloning vector is a portion of DNA that can be integrated into bacteria and that replicates itself each time the bacteria divide. A frequently used type of cloning vector is referred to as plasmid. A plasmid is a small circular loop of DNA originating in bacteria, which exists separately from the chromosome. Due to their small size, they can easily be isolated. Recombinant DNA technology can be used to modify plasmids. Such a modified plasmid can then be introduced into bacteria, where it replicates as the bacteria grows.[94] Even after a cloned heterologous gene has been introduced into a bacteria and replicated, it is not guaranteed that the gene will be expressed and encode for a protein. E. coli, for example, consists of genetic information for several thousand proteins. Often, a great number of those genes are not expressed at all. Thus, methods that 'turn on' the cloned gene are necessary. Many biotechnological inventions are directed to this field of research.[95]

E. Proteomic research

The determination of the genome changed the entire emphasis of protein studies of the past. It is now possible to comprehend the concrete impact that genetic information has on protein composition and structure. Moreover, it was discovered that different genes neither generate the same amount of proteins nor reveal the precise determination of circumstances under which protein synthesis is initiated. This specification of the total cellular protein output is therefore an important focus of current research efforts.[96]

92 Jollès, Pierre/Jörnvall, Hans, Proteomics in Functional Genomics, Protein Structure Analysis, Basel et al. 2002, XI.

93 Watson, James D., Molecular Biology of the Gene, Menlo Park, California 1987, 208. As for inventions in the field of recombinant technologies see Vossius, Volker/Jaenichen, Hans-Rainer, Zur Patentierung biologischer Erfindungen nach Europäischem Patentübereinkommen und Deutschem Patentgesetz - Formulierung und Auslegung von Patentansprüchen, GRUR 1985, 821, 821.

94 Whitford, David, Proteins: Structure and Function, Chichester, West Sussex, U.K., 315-316.

95 Fernandez, Dennis/Chow, Mary, Intellectual Property Strategy in Bioinformatics and Biochips, Journal of Patent and Trademark Office Society June 2003, 465, 470; Biochip companies, such as Affymetrix and Hyseq, are involved in developing assays, tools, and computational techniques for the disclosure and modification of gene expression profiles; In re O'Farell, 853 F.2d 894, 898.

96 Jollès, Pierre/Jörnvall, Hans, Proteomics in Functional Genomics, Protein Structure Analysis, Basel et al. 2002, XI.

Advances in proteomic science now allow for analyses of known protein forms with regard to their binding regions, the results of which are used for screening against database entries. An important challenge is the need for a simultaneous evaluation of many proteins in highly complex mixtures. Not only must single proteins be identified in order to understand their functional interactions, but also the global output of gene products from essentially all tissues must be determined. This determination has to be carried out under healthy as well as pathogenic conditions, and in developmental and other special states. With the 3-D geometry of proteins being critical to their function, it is important and challenging to preserve this geometry through all research steps. Furthermore, one must take into account that the proteome is constantly changing. One organism will have radically different protein expression in different parts of its body and in different stages of its life cycle.[97]

I. Proteome initiatives

Proteome research is already highly organized on an international level. The "Human Proteome Project" (HPP) has been founded as an analogue to the "Human Genome Project" (HGP).[98] It aims to consolidate national and regional proteome organizations into a worldwide network. Moreover, it engages in scientific and educational activities to encourage the spread of proteomic technologies and to disseminate knowledge pertaining to the human proteome and that of model organisms. Finally, it assists in the coordination of public proteome initiatives. In 2001, the "Human Proteome Organization" (HUPO)[99], again an analogue to the complementary genome initiative, the "Human Genome Organization" (HUGO),[100] was founded.[101] Among other things, its goals are to promote the analysis of particular proteins or protein complexes, as well as their relationship to certain diseases. Moreover, it seeks to advance: the disclosure of biomarker proteins, which allow a diagnosis of disease shortly after its outbreak, the development of diagnostic tools that enable predictions about the course of diseases or its cure, and the disclosure of proteins

97 Jollès, Pierre/Jörnvall, Hans, Proteomics in functional Genomics, Protein Structure Analysis, Functional Genomics, Protein Structure Analysis, Basel et al. 2002, XI.

98 The Human Genome Project started in 1990 and was coordinated by the U.S. Department of Energy (DOE) and the National Institutes of Health (NIH). The project was finished in 2003, when the disclosure of the human genome was completed, see Krefft, Alexander Richard, Patente auf human-genomische Erfindungen: Rechtslage in Deutschland, Europa und den USA, München 2003, 6. For further information see Straus, Joseph, Genpatente: rechtliche, ethische, wissenschafts- und entwicklungspolitische Fragen, Basel, Frankfurt/Main 1997, 16.

99 Human Proteome Organization, available at http://www.hupo.org/, last checked on January 21, 2008.

100 Human Genome Organization, available at http://www.hugo-international.org/, last checked on January 21, 2008.

101 Hanash, Sam, Building a Foundation for the Human Proteome: The Role of the Human Proteome Organization, 3 Journal of Proteome Research 2004, 197.

that can be used as new drugs targets. HUPO encompasses five major initiatives[102], including projects on plasma[103], the liver[104], bioinformatics, and the brain[105]. One of them, the "Human Brain Proteome Project" is carried out under the supervision of the German government. Furthermore, the "Deutsche Gesellschaft für Proteome Forschung e.V." represents the German proteome research in EU, HUPO, and other international proteome organizations.[106] Protein research involves immense investments. The U.S. government, for instance, is supporting a wide range of initiatives in order to cope with challenges in the proteomics field. For example, the National Heart, Lung, and Blood Institute (NHLBI) is supporting 10 proteomic centers with more than U.S. $ 150 million for a period of seven years, and the National Institute of Neurological Disorders and Stroke (NINDS) has detailed plans for other projects.[107]

102 In addition, several sub-initiatives exist, such as for Pan-Asian proteomics, see Mason, Katherine A., As Pan-Asian Proteomics Powerhouse Emerges, Focus is on Liver Cancer, SARS, Genome Technology 2003, 47. All data obtained by the (non-profit) HUPO initiatives are available for public access.

103 Human Plasma Proteome Project, available at http://psidev.sourceforge.net/ppp/pilotPhase/, last checked on September 28, 2005.

104 Human Liver Proteome Project, available at http://www.hlpp.org/hlpp/, last checked on September 28, 2005.

105 Human Brain Proteome Project, available at http://www.hbpp.org/, last checked on January 21, 2008.

106 Deutsche Gesellschaft für Proteome Forschung, available at http://www.dgpf.org/dgpf-set.htm, last checked on January 21, 2005. The Federal Ministry of Education and Research founded the proteomics-based initiative "New efficient procedures for functional proteome analysis" in June 2000. Since then, over 75 million Euro were made available for the development of proteomics-based technologies. For further information see http://www.bmbf.de/en/1756.php, last checked on January 21, 2008. One of the projects that has been sponsored by the ministerial program is "Fighting Mycobacterium tuberculosis with structural proteomics" conducted by the European Molecular Biology Laboratory (EMBL) and several partners (Max-Planck Groups for Molecular Structural Biology, Hamburg; Max-Planck-Institute for Infectious Biology, Berlin; Technical University of Munich, Research Center Weihenstefan, Biomax, Martinsried; Combinature, Berlin; MarResearch, Norderstedt). The project aims to combat tuberculosis under a proteomics approach and has received from the ministry a 3.5 million Euro grant in support of its efforts. For a detailed description, see EMBL Hamburg, MTB-Strukturproteomik Konsortium Gesamtdarstellung, Hamburg, Berlin, München 2003, 1.

107 Lottspeich, Friedrich, Humanproteomorganisation - HUPO, in: Fäden des Lebens, Tagungsband der Münchener Wissenschaftstage im Jubiläumsjahr 2003; München, 2003; 98, 100. The "beat of the proteomic drum" also encouraged many researchers to create private research organizations for the analysis of disease-related proteins, such as the Plasma Proteome Institute, see MacNeil, John S., Like Father, like Son, Genome Technology 2003, 50, 51.

II. Proteomics Technologies

1. Protein expression, purification and characterization

As defined earlier, the major objective of methods employed in proteomics is the total characterization of the protein. A thorough examination of the protein profile requires several steps, ranging from the proteins' identification and structural determination to the study of its post-translational modifications and from its quantification to the handling of the resulting proteomic data. In order to study any protein it is necessary to obtain it in a purified form. This is often a challenging task, particularly if proteins are present within the cell in low concentration. Frequently, this involves the purification of one single protein from a cell paste encompassing over 10.0000 different proteins. Two major alternatives are employed for isolating proteins. First, proteins can be isolated conventionally by obtaining the desired protein directly from the used source, such as a cell or tissue. Second, proteins can be expressed recombinantly, e.g. by introducing the DNA-sequence into a bacterial host.[108] In recent years, there have been numerous technical advances for proteomic technologies. Most commonly used methods for protein separation and identification are 2-D gel electrophoresis for protein separation and the proteome's analysis by mass spectrometry.[109] With the study of some proteins still being difficult to accomplish, further development of these tools is needed.

a) Gel electrophoresis

2D electrophoresis aims to separate proteins according to mass and overall charge. The technology is classified as the most common method for analyzing the purity of an isolated protein.[110] The principle of electrophoresis is the separation of proteins according to molecular mass by their movement through a polyacrylamide gel of closely defined composition under the influence of an electric field. The mobility of a protein through polyacrylamide gels is determined by a combination of overall charge, molecular shape, and molecular weight. The method is conducted by introducing a protein mixture to the top of a gel that proceeds through the matrix because of the electric field, with lighter components migrating faster than 'heavier' molecules. Over time, the component proteins are separated and the resolving power of

108 Whitford, David, Proteins: Structure and Function, Chichester, West Sussex, U.K., 313.

109 Another frequently employed method fort the purification of proteins is chromatography, see Whitford, David, Proteins: Structure and Function, Chichester, West Sussex, U.K., 326. There exist a number of different chromatographic methods.

110 See Gorg, Angelika/Weiss, Walter/Dunn, Michael J., Current two-dimensional electrophoresis technology for proteomics, 4 Proteomics 2004, 3665, 3665. The author considers two-dimensional gel electrophoresis (2-DE) with immobilized pH gradients (IPGs) combined with protein identification by mass spectrometry (MS) 'the workhorse' of proteomics.