Chapter 1

Introduction

BIOPHARMACEUTICAL MANUFACTURING (BIOMANUFACTURING)

Biomanufacturing, a specialization within biotechnology, is an advanced-technology manufacturing industry responsible for making biopharmaceuticals (biologics). Biopharmaceuticals are any biotechnology-based therapeutics that structurally mimic components found in a living organism. These can include:

- hormones
- growth factors
- blood proteins
- clotting factors
- enzymes
- antibodies
- DNA and RNA
- stem cells

The application of biopharmaceuticals in health and medicine are numerous:

- therapeutic proteins for treatment of disease
- vaccines to prevent disease
- protein or DNA-based diagnostics
- regenerative medicine technology
- gene therapy

Production of the first biopharmaceutical

Modern biomanufacturing began when recombinant human insulin was first commercially produced and marketed in the United States in 1982. The effort leading up to this landmark event began in the early 1970s when research scientists developed protocols to construct DNA vectors. The scientists cut out pieces of DNA then pasted them into small circular DNA molecules known as plasmid DNA to create a new piece of DNA (recombinant DNA). This recombinant DNA could be inserted into the bacterium *Escherichia coli* by the process of transformation. If one of the pieces of the new DNA included a gene that produced an enzyme that broke down a particular antibiotic, the bacterium containing the introduced gene would be resistant to that antibiotic. This provides a means of selection for the bacteria that take up the plasmid since the bacteria can now grow in a medium containing it the antibiotic (Figure 1.1).

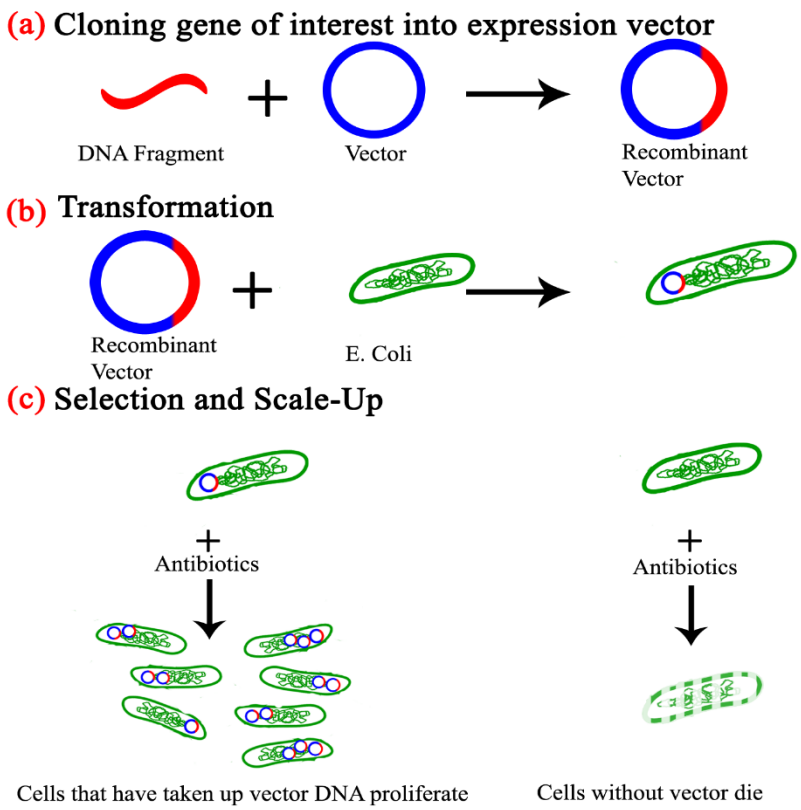

Figure 1-1. Expression vector, transformation and cloning

The human insulin gene was inserted into such a DNA vector alongside a promoter to drive the expression of the gene and a gene encoding a protein that would confer antibiotic resistance. *E.coli* transformed with this recombinant DNA were antibiotic resistant so the bacteria could grow in selective medium. More importantly they produced high levels of human insulin mRNA, which was translated into large quantities of human insulin protein. This story of the beginning of modern biomanufacturing illustrates the discovery research phase, discussed later in this chapter.

Eli Lilly and Company in Indianapolis, Indiana was the biomanufacturer of this first recombinant therapeutic protein, insulin, called humulin. Eli Lilly was already the world's number one maker of insulin from pig and cow pancreas, but patients were getting immune responses from insulin and other proteins produced by the animal pancreas. Thus, the prospect of human recombinant insulin was very attractive. In 1976 the proof of concept was published illustrating that recombinant insulin could be expressed in *E.coli* and the drug became available to the public in 1982 (6-year lead time!).

To demonstrate how biopharmaceutical proteins are produced within an academic setting, Green Florescent Proteins is expressed in E. coli. First, the plasmid expression vector for GFP is genetically engineered (Figure 1-2).

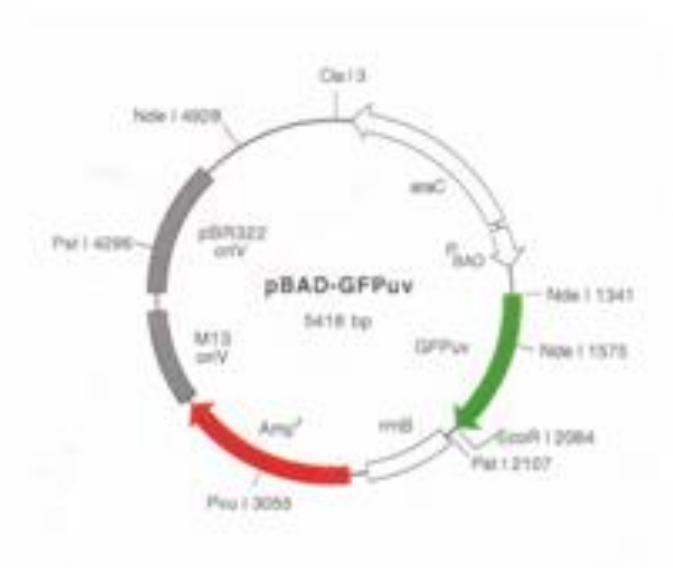

Figure 1-2. GFP Expression Vector

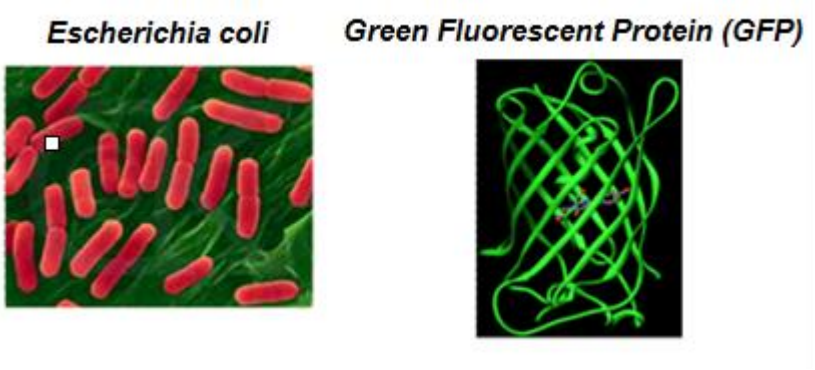

Figure 1-3. E. coli and E. coli GFP

Next the expression vector is used to transform *E. coli* and make GFP (Figure 1-3). The transformed cells can be grown in increasing quantities. As the cells grow, the DNA of the expression vector is transcribed into messenger RNA. The messenger RNA is translated into protein. The fundamental process of transcribing DNA into RNA and translating RNA into protein is referred to as the Central Dogma of Biology (Figure 1-4).

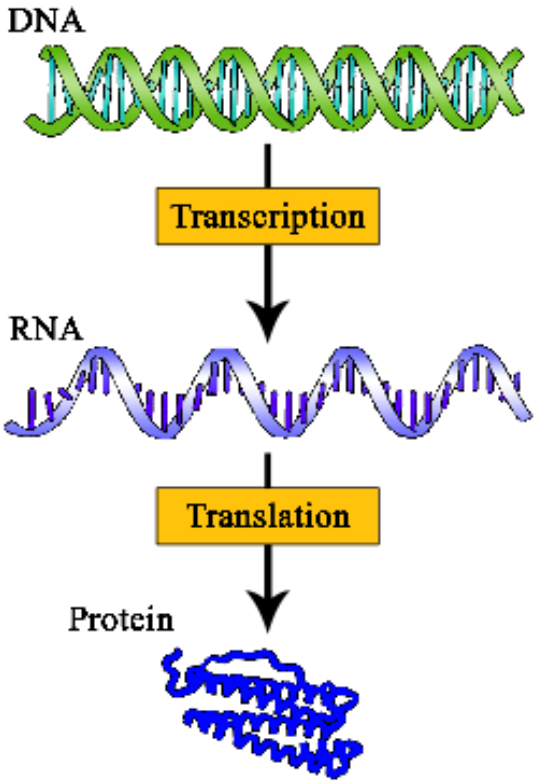

Figure 1-4. Central Dogma of Biology

Transcription and translation occur within the cell. Specifically, biopharmaceutical proteins are manufactured on the ribosomes of the cell.

Production of biopharmaceuticals from 1982 to the present

Since 1982, many recombinant biopharmaceutical proteins have been manufactured using the same basic methodology as was developed for insulin production. Although the cellular factory chosen is usually a mammalian cell, such as the Chinese Hamster Ovary (CHO) cell, some biopharmaceuticals are produced within the mammary glands of a living goat, and many other cell types are being used as factories, including yeast (humanized Pichia pastori), and plant (duck weed or tobacco).

Table 1.1 lists the early biopharmaceuticals, including their date of commercialization, indication, and biomanufacturer. These biopharmaceuticals represent a variety of hormone, blood, and enzyme recombinant therapeutic human proteins.

Table 1-1. List of early biopharmaceuticals

Biopharmaceutical Protein	Date of Commercial Production and Indication	Company
Humulin (human insulin)	1982, diabetes	Eli Lilly and Company
tPA (tissue plasminogen activator)	1987, myocardial infarction	Genentech
Humatrope (human growth hormone)	1987, human growth hormone deficiencies	Eli Lilly and Company
Epogen (hormone that builds red blood cells)	1989, anemia	Amgen
Energix-B	1989, hepatitis B vaccine	SmithKline Beecham
Betaseron (interferon□□)	1989, multiple sclerosis	Berlex Laboratories/Chiron
Ceredase (alglucerase)	1991, Type I Gaucher's disease	Genzyme
Proleukin (IL-2)	1992, kidney carcinoma	Chiron
AHF (recombinant anti-hemolytic factor)	1992, hemophilia A	Baxter Health Care
Pulmozyme (DNAase)	1993, cystic fibrosis	Genentech

A wave of fully human antibodies is in development by biopharmaceutical manufacturers. Many of the newer biologics are therapeutic antibody proteins (monoclonal antibodies or mAbs). Therapeutic mAbs targeted to cancer and inflammation represent almost one-third of all biological proteins undergoing clinical trials and are the second largest class of biopharmaceuticals after vaccines.

Table 1-2 lists some mAbs, including their date of commercialization, indication, and biomanufacturer.

Table 1-2. List of monoclonal Antibodies (mAbs)

Biopharmaceutical Protein	Date of Commercial Production/ Indication	Company
Herceptin (HER2/neu receptor antibody)	1998 metastatic breast cancer and other cancers	Genentech
Avastin (angiogenesis inhibitor antibody)	2004 colon cancer and non-small cell lung cancer	Genentech
Vectibix (epidermal growth factor receptor antibody)	2006 metastatic colorectal cancer	Amgen
Erbitux (epidermal growth factor receptor antibody)	2008 colorectal, head and neck cancer	Imclone

Drug Discovery, Clinical Trials, and Commercial Production

The following section examines drug discovery, drug development, and the approval process for biopharmaceuticals (biologics). The chart in Figure 1-11 illustrates the time (more than 15 years) and money ($1.2 billion) used in the process of moving a potential therapeutic protein from the research bench to the patient.

DISCOVERY		DEVELOPMENT			LAUNCH	
Testing Phase	**Discovery Research and Preclinical Testing**	**Clinical Trials**			**File Application**	**Phase IV**
		Phase I	**Phase II**	**Phase III**		
Test Population	Laboratory (in vitro) and animal (in vivo) studies	20 to 100 healthy volunteers	50 to 100 patient volunteers	1,000 to 5,000 patient volunteers	Review process and approval	Additional post-marketing testing required by FDA
Purpose	Assess safety, biological activity, and formulations	Determine safety and dosage	Evaluate effectiveness, look for side effects	Confirm effectiveness, monitor adverse reaction from long-term use		
Success Rate	5,000 compounds evaluated	5 enter clinical trials			1 approved	
Manufacturing Activities	**Cell line construction, cell banking**	**Process development, assay development, process optimization, scale-up, cGMP manufacture**			**Commercial manufacture**	
Years	6.5	1.5	2	3.5	1.5	=15
Approximate Cost	$350M	$70M	$100M	$200M	$80M	=$800M

File IND at FDA

File NDA at FDA

Figure 1-5. The biopharmaceutical discovery, development, and launch process

Discovery research

The discovery research process identifies a potential human therapeutic protein using recombinant DNA technology. Insulin was the first protein produced using the new technology, which involved the genetic engineering of a circle of plasmid DNA. The basic steps for the process are illustrated in Figure 1-6:

- the DNA (gene) for a particular protein of interest is identified
- a promoter gene that will produce a protein and in turn cause the DNA of the protein of interest to be transcribed into messenger RNA is added along with
- an antibiotic resistant gene that allows one to identify cells that contain the gene of interest

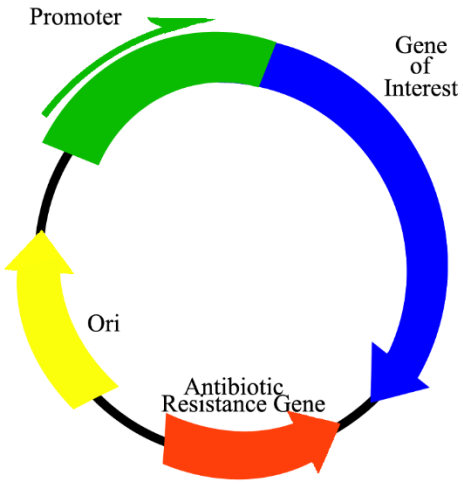

Figure 1-6. Expression vector: the basis of modern biomanufacturing

Many biopharmaceuticals continued to be made in *E. coli*, which is a prokaryote, a single cell organism that lacks a cell nucleus. However, many biopharmaceuticals are now made in eukaryotes, which have a clearly defined nucleus. An important fundamental difference between prokaryotes and eukaryotes is that the vast majority of prokaryotes lack the ability to make glycoproteins. Glycoproteins are polypeptides (proteins) with covalently-attached oligosaccharide chains. These sugars are added by enzymes as the protein is synthesized. The enzymes are located within the endoplasmic reticulum of the eukaryotic cell through which the nascent protein passes (figure 1-7). The pattern of sugars added differs between eukaryotic cell types. For example, plant cells produce a different pattern as compared to mammalian cells and are usually less complex in nature which is the principal reason why mammalian cells are used for complex mAbs.

In addition, there are differences in the extent of glycosylation of the individual molecules of a single protein. Furthermore, different protein molecules have different unique patterns of glycosylation. Finally, the sugars that are added have various physiological functions that are still not fully understood. One of the advantages of using eukaryotes is that they can produce glycoproteins. They also secrete the biopharmaceutical protein, making purification of the protein during downstream processing far easier.

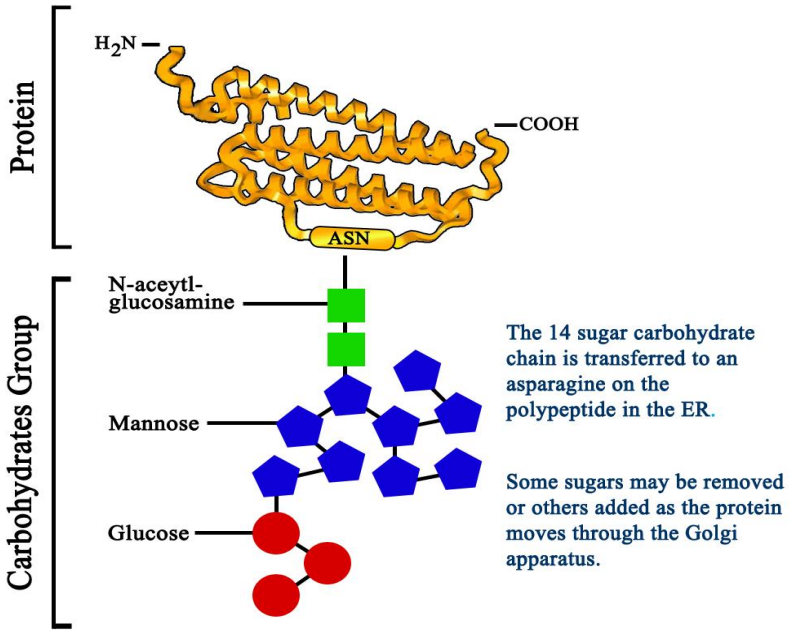

Figure 1-7. Glycosylation

Today Chinese Hamster Ovary (CHO) cells are the most popular type of eukaryotic cell deployed in the bioprocessing and production of biologics. Many of today's biologics are human therapeutic antibodies. Figure 1-8 depicts CHO cells and Figure 1-9 illustrates an expression vector for a therapeutic antibody. When inserted into a cell, its circle of recombinant DNA produces a therapeutic antibody using the protein synthetic machinery of the cell. The protein of interest is glycosylated in the endoplasmic reticulum and the Golgi apparatus of the CHO cell. The glycoprotein antibody therapeutic is then secreted by the CHO cells into the medium.

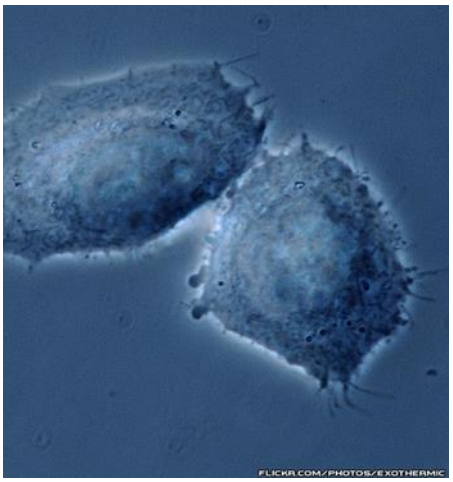

Figure 1-8. CHO cells

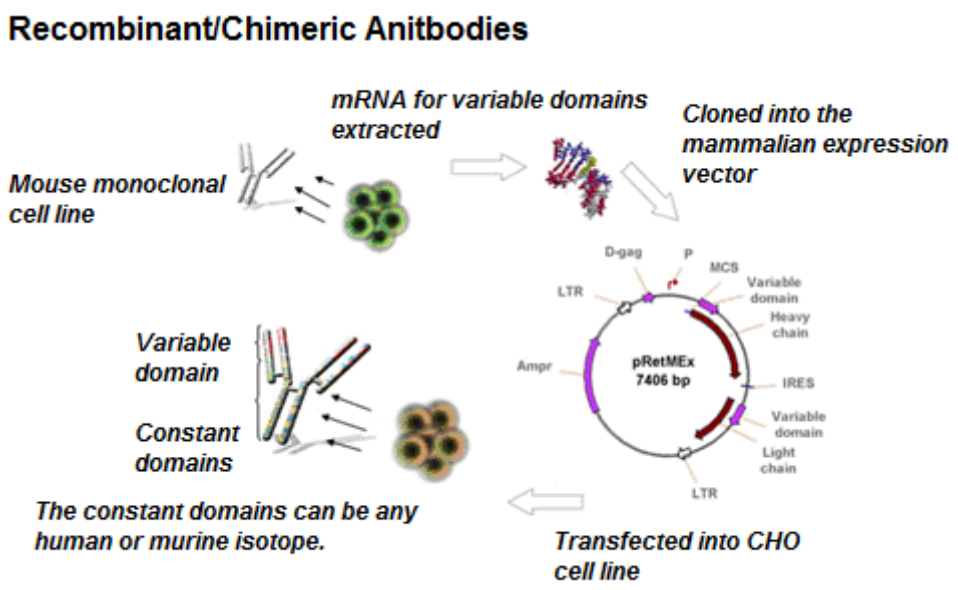

Figure 1-9. Expression vector for therapeutic antibodies in CHO cells

Process development and scale-up

The next step in the biotechnology process of protein production is called process development. During process development, the best growth conditions are identified to produce the most protein as efficiently as possible. This best process is scaled up to produce the quantities of human protein that are needed for pre-clinical and clinical trials and then ultimately at far greater levels for the manufacture of the commercial product. Process development also includes the development and securing of resources, materials, and tools for: the growth of recombinant cells (upstream processing), the isolation and purification of the recombinant protein (downstream processing), and tests to insure that both the upstream and downstream processes are proceeding in a predictable manner (quality control). This includes media, buffers, reagents, solutions, and assays. It also includes the selection of bioreactors, filtration equipment, liquid chromatography equipment, and other necessary equipment and tools.

Toward the end of the process, a master cell bank is created. Ordinarily the master cell bank is a large quantity of vials, each containing 1ml of concentrated cells. Each vial contains about one million recombinant cells (1,000,000 cells/ml) and is stored frozen in liquid nitrogen. From the master cell bank, working cell banks are then made and also stored frozen in liquid nitrogen. The master cell bank is sized to last as long as the manufacture of the product will take place.

Manufacturing

The next step in the process is manufacturing. This begins with thawing a vial from the working cell bank and adding it to a small amount of medium prepared in the media and buffer preparation area of the manufacturing facility. The cells are then grown under the conditions determined during process development and tested at the manufacturing facility during

process validation. Cells are transferred into a larger volume of growth medium when they reach certain predetermined conditions (i.e., when they are in a particular place on the log phase of their growth curve as determined through optical density (OD) readings using a spectrophotometer and through live-cell counts using a microscope). This process repeats itself (called “scale up”) until the final bioreactor volume is reached. Some of the largest final bioreactor volumes are 20,000 liters and greater (figure 1-10). All of this occurs during upstream processing. Because of the huge costs associated with scale-up, it is vital to have an experienced technical staff that understands the consequences of process variables such as temperature and pressure.

Figure 1-10. A 20,000 liter bioreactor suite

After upstream processing, the cells are separated from the media in which they are growing. The protein is isolated from the cells or the media by a combination of techniques that include filtration, chromatography, and concentration. This process is termed downstream processing.

The protein characteristics and purity must conform to certain conditions determined during process development and must be tested at the manufacturing facility during process validation. Both upstream and downstream processing are monitored by the **Quality Control (QC)** department of the manufacturing facility. Quality control also handles environmental monitoring during production of the protein. The **Quality Assurance (QA)** department handles all paperwork generated by the various departments of the manufacturing facility. The biomanufacturing facilities operate in compliance with current Good Manufacturing Practices (cGMPs) determined by the appropriate regulatory agencies, such as the United States Food and Drug Administration (FDA). The FDA's mission is to protect the public health by assuring the efficacy and security of human and veterinary drugs, biological products, medical devices, the United States food supply, cosmetics, and products that emit radiation. Once the protein is manufactured, it must be formulated. For example, excipients must be added to the purified protein to modify its activity or its storage qualities. If the protein is a therapeutic protein or a vaccine, the formulated preparation is filled into glass ampoules, lyophilized (freeze-dried), sealed, and labeled once excipients are added. This process is known as aseptic

formulate/fill/finish and is also regulated by agencies such as the FDA under its cGMP regulations. Some examples of excipients are illustrated in Figure 1-11.

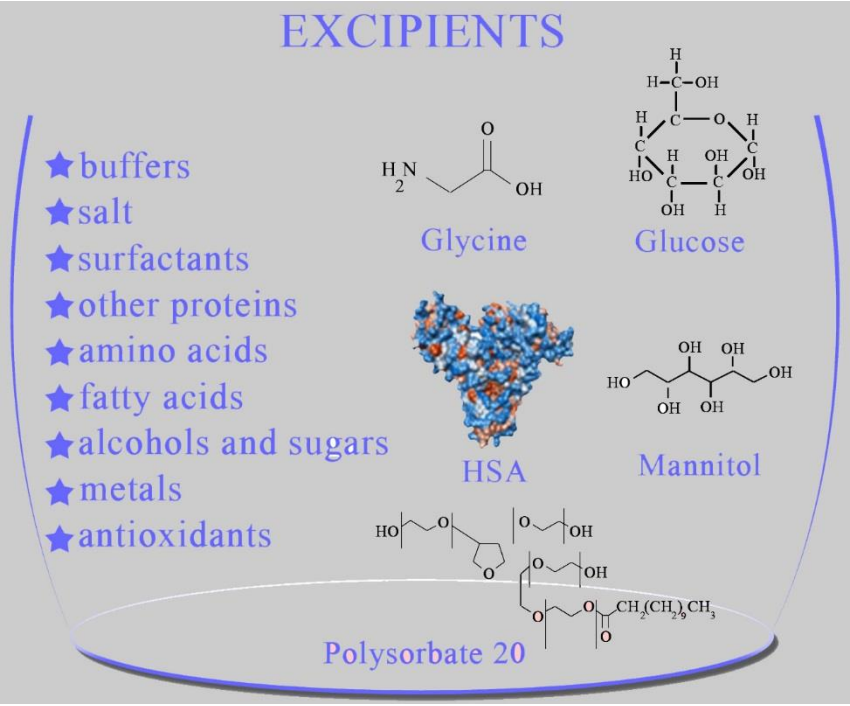

Figure 1-11. Types of excipients

Building and Staffing a Biopharmaceutical Production Facility

In order to manufacture the amount of a biopharmaceutical needed to satisfy the yearly needs of a large patient pool, it may become necessary at times to construct a new facility or reconstruct an existing one. In either case the facility must include a clean room area. This area must be kept aseptic, as biopharmaceuticals are parenteral drugs. Typically parenteral drugs are those that must be delivered through the blood by injection rather than through ingestion by the stomach, as is the case with oral dosage medications.

Figure 1-12. Biopharmaceuticals are parenteral drugs

During early clinical trials, the protein of interest may be manufactured in a smaller facility known as a pilot plant. Figure 1-13 illustrates the overall flow plan for a small pilot plant with clean room areas for media/buffer preparation, the seed stock area where the recombinant cells are banked, and the cell culture and purification areas where upstream and downstream processing technicians grow cells and purify their proteins.

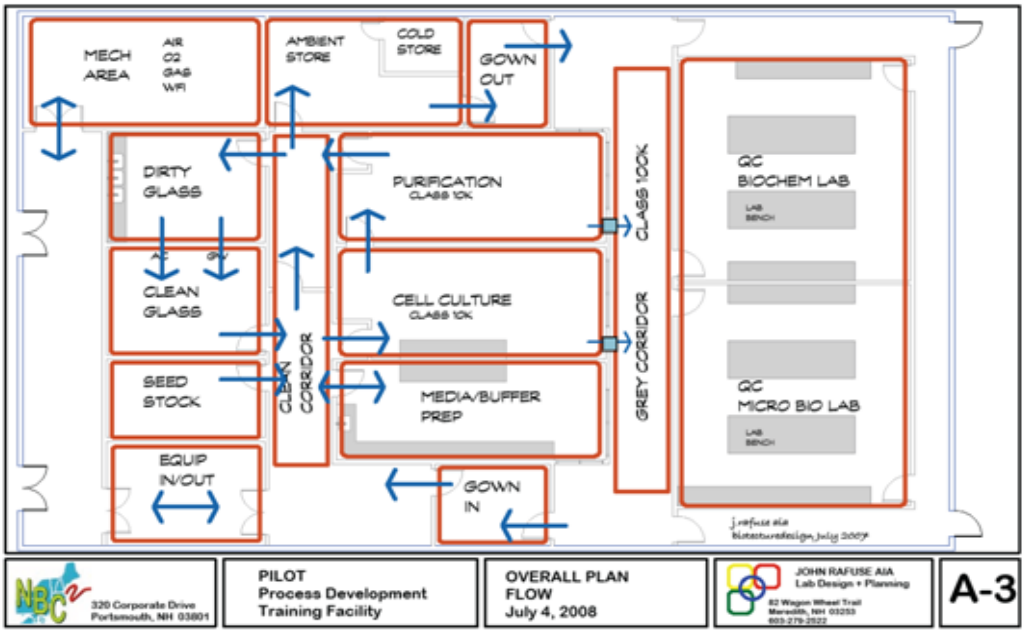

Figure 1-13. Pilot plant overall flow plan

Adjacent to the clean room area (in the grey spaces shown in the illustration) are quality control laboratories. Pass-throughs along the hallway allow for samples from cell culture and

purification to be picked up for analysis by quality control microbiology and biochemistry technicians.

Figure 1-14. A Quality Control technician prepares samples for testing

A larger facility designed for commercial production will also have a central utilities area or building managed by facilities and metrology technicians (Figure 1-15). Increasingly, these jobs are combined into a single facilities/metrology technician position. These technicians:

- maintain clean room status
- store and/or deliver potable de-ionized water, purified water (USP water), and WFI (Water For Injection) for the facility
- assist in providing utilities such as clean steam (made with WFI) and gases (air, oxygen, carbon dioxide, nitrogen, helium, etc.)
- calibrate, operate, and maintain equipment
- maintain the Heating/Ventilation/Air Conditioning (HVAC) system in clean rooms and throughout the facility
- dispose of wastes, such as sludge (waste cells) and liquids (media and buffers)

Figure 1-15. Facilities and Metrology technicians

Validation technicians are responsible for ensuring the equipment in the facility is operating in accordance with specifications and within the parameters of the process (Figure 1-16). They perform the following tasks to make certain all equipment is operating according to a corresponding Standard Operating Procedure (SOP), a document for the facility that describes step-by-step operations:

- Installation Qualification (IQ)
- Operation Qualification (OQ)
- Process Qualification (PQ)

Validation technicians are either employed as permanent staff or hired as consultants.

Figure 1-16. Validation technicians locate temperature probes in a process using drawings called Piping & Instrumentation Diagrams (P&IDs)

Finally, the Environmental Health and Safety (EHS/SHE/HSE) department is responsible for maintaining the safety and health of the workers at the facility, the products, processes, and equipment in the facility, and the surrounding environment and community.

To ensure that the protein is produced in the correct manner, QA technicians (also referred to as lot analysis technicians) check the paperwork for each batch (or lot) of biopharmaceutical proteins manufactured at the facility. They ensure that all SOPs necessary to make the particular biopharmaceutical have been followed, batch records have been filled out properly, and process deviations have been appropriately investigated and documented. The Quality Assurance department approves and keeps on file the following documents for a batch of biopharmaceutical protein:

- raw material specifications
- SOPs
- master batch production records
- production batch records
- deviation forms
- numbering system
- validation records
- equipment use and cleaning log books
- component, container, and closure records
- distribution records
- complaint files

There is an adage among those in Quality Assurance that goes: “If it wasn’t documented, it wasn’t done.” Documentation is required not only to maintain overall quality and quality practices at a facility but also to meet the requirements of the law. In countries where the biomanufacturing organizations distribute and/or produce biopharmaceuticals, the applicable regulatory agency enforces that country's laws related to those products. In the United States the FDA regulates biopharmaceuticals and pharmaceuticals. QA verifies that the Code of Federal Regulations (CFR) on cGMPs is followed:

> *“21 CFR Parts 210-211 contain the minimum current good manufacturing practice for methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing, or holding of a drug to assure that such drug meets the requirements of the act as to safety, and has the identity and strength and meets the quality and purity characteristics that it purports or is represented to possess.”*

Textbook Organization

Now that the process for producing biopharmaceuticals and the related jobs and tasks have been described, the final section of this chapter explains how the remainder of the textbook is organized.

The textbook is divided into three units addressing topics related to engineering, quality, and production.

The **Engineering Unit** includes:

Facilities (Chapter 2): the physical building, layout, equipment, and utilities required to manufacture biopharmaceuticals

Metrology (Chapter 3): the fundamental methods by which objects and phenomena are measured, the means for assigning values to measurements, and the certainty of those assigned values. Correct measurements and data are required to produce safe, effective, pure, and high-quality biopharmaceuticals

Validation (Chapter 4): the practices and procedures for checking and verifying that the equipment, process, and methods used in biopharmaceutical production meet all specifications

Environmental, Health, and Safety (Chapter 5): the practices and procedures for ensuring the safety and health of all workers in a biomanufacturing facility, along with the safety of the products, equipment, and other resources in the facility as well as the practices and procedures for protecting the environment in and around the facility (i.e. surrounding community)

Operational Excellence (Chapter 6): the approaches and systems used to manufacture quality products

The **Quality Unit** includes:

Quality Assurance (Chapter 7): the role and tasks of QA staff to systematically monitor and evaluate the various activities carried out during the biomanufacturing process to verify that appropriate quality and regulatory standards are attained and that the resulting products are of the quality required for their intended use

Microbiological Control (Chapter 8): the role and tasks of QC staff related to the identification and control of microbes that can potentially contaminate biomanufacturing processes and products. Microbes are controlled at a facility using a variety of techniques, protective clothing for personnel, equipment, and tools

Quality Control: Biochemistry (Chapter 9): the role and tasks of QC staff as related to performing tests and/or measurements on the biochemistry of product samples then drawing conclusions about the corresponding batch of product. A variety of tests and measurements are used to make sure that the biopharmaceutical product meets the standards set for its safe use, effectiveness, purity, and quality.

The **Production Unit** includes:

Upstream Processing (Chapter 10): the phase of the biomanufacturing process that involves creating the environment necessary for cells to make the target protein and then growing the recombinant cells

Downstream Processing (Chapter 11): the phase of the biomanufacturing process that starts with the isolation of the cell culture bioreactor harvest containing the expressed drug product. This phase ends with a highly purified and appropriately concentrated recombinant protein that is ready for final formulation and packaging

Process Development (Chapter 12): the methods and approaches used when a potential biopharmaceutical product is first expressed in a cell and is proven to have the correct identity, including the best growth conditions to produce the most protein product as efficiently as possible. This process continues through the evaluation and optimization of various steps followed by scale-up to manufacturing