Chapter 1
Convergence of Aquaculture and Molecular Biology

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Introduction

More than 1 billion people rely on fish as their main protein source. Of the world's food fish supply for consumption, more than 48% is supplied by aquaculture. Currently, more than 240 diverse species are produced by aquaculture; however, within aquaculture only 10 species constitute approximately 69% of the production, while 25 species account for approximately 90%. On a weight basis, finfish account for 85% of all aquaculture production (FAO 2005). Most farmed finfish are of the class Teleostei (teleosts), which contains 96% of all fish species, and are some of the most diverse, which is exhibited in their behavior, diet, reproduction, and habitat (Nelson 1994). Finfish also play a significant role in research and have become more prominent as a research subject during the past three decades.

The human population has continually developed methods for the increase of aquatic products for food production. However, the roots of aquaculture, as illustrated by the writings of Fan Li, go back to more than 4,000 years ago when the Asian emperors maintained stocks of their favorite fish in ponds. Until the late nineteenth century aquaculture research mainly consisted of domestication and rearing of wild stocks in captivity. Initially, rearing species harvested from the wild was the main form of aquaculture. Closing the life cycle of certain fish species allowed them to be maintained, grown, and spawned without having to constantly capture fish from the wild. As the production of fish products moved beyond the provisional rearing of fish for individuals and into product marketing, demand and economics of production led to intensification of aquaculture. Today, aquaculture occurs in multiple countries around the world and accounts for more than US$65 billion in trade. Also, when considering fish for human consumption and wild fish harvested for use in aquaculture diets, aquaculture is responsible for more than half of the world's fishery production (FAO 2005).

The scope of early aquaculture studies was greatly influenced by the production systems in use. Preliminary studies in fish culture began with the evaluation of intended improvements in the growing environment and the type of feed that would either reduce loss to disease, enhance growth, or facilitate ease in rearing the animals. These types of studies mainly depended on the species and the area in which rearing occurred and were usually performed by private entities to improve production for their specific case. Rearing typically occurred in ponds; this limited the chance of escape and also established boundaries for the rearing of freshwater fish species. The concept of cage culture arose next, whereby existing bodies of water could be utilized while still maintaining a safe and secure enclosure. For practical and economical reasons, most

of the original work was performed in freshwater while all marine fish used for food were harvested from the wild.

The course and development of aquaculture has obviously varied widely throughout the world, according to location, environment, and population. Historically, with the intensification of aquaculture began the initial stages of research, with individuals or groups attempting to qualitatively experiment to determine the optimal water conditions, stocking densities, and diets for the available species that performed best under local conditions. However, it was not until the early nineteenth century that aquaculture research could essentially be considered an applied science. Some scientific studies related to aquaculture were undertaken in government and academic laboratories prior to this period; however, they were relatively limited in number and scope (Stickney 1996). Nevertheless, progress was made through trial and error and through careful observation of cultured animals while attempting to re-create optimal natural conditions in captive environments.

As was typical for the time, most of the knowledge that was gained in early studies was never written and published in the research journals or the popular press. Information on the practices for the rearing and care of fish was passed down by traditional methods. In the late 1800s, state and federal government agencies had developed the technology for the production of millions of eggs and small fish for stocking into marine, coastal, and freshwaters. Yet, the technology to spawn some of the species mentioned in the literature has only recently been redeveloped (Stickney 1994). Still there are several species where little or no breeding of captive broodstock has occurred and all broodstock or fry are obtained from the wild. This is still a problem that is being researched for new species, especially when considering larval diets and rearing conditions.

The development of a vitamin-free purified diet that supported growth but allowed for the testing of the qualitative and quantitative requirements for vitamins was a landmark discovery by John Halver in the 1950s in aquaculture research on the production of diets for the maintenance and study of commercial and research species (Halver 1957). Before this discovery, diets needed to be supplemented with multiple nutrient factors to ensure animal health from fry stages to fully mature individuals. Modification of dietary formulations has been necessary to study amino and fatty acid requirements. With the development of complete diets, initial studies involving selection for improvement of performance, chromosomal manipulation, and sex reversal started. Dietary research has now expanded tremendously with ongoing studies, evaluating the nutritional requirements of several different species at all life stages, improving immune performance, developing diets composed of material from sustainable sources, and modifying formulations now specific for the development of newer species for aquaculture (Amar et al. 2000; Cahu and Infante 2001; Twibell et al. 2003).

Until recently, aquaculture was not a distinct scientific discipline. Rather, it was the application of discrete scientific disciplines such as nutrition, genetics, physiology, and health management to aquatic species. Culturists and researchers alike obtained information from studies involving other agricultural animals or scientific species and then integrated this information into traditional practices and research studies and applied it to aquaculture research. To some extent this practice is still followed today. However, with the growth and economic development of aquaculture has come increased funding, fueling the development of research programs and departments and even entire institutes devoted specifically to aquaculture research. The rapid

advancement of scientific research found today in aquaculture is due to the rapid application of technology, which is often adapted from human or medical research. As this technology becomes available for agriculture research and as the methodology becomes developed and refined, it is then incorporated into studies involving aquaculture species. Since 2000, the number of published scientific articles related to aquaculture research is fivefold greater than the number of articles published in the entire previous decade. And typically most of the articles now being published are more of a scientific nature, whereas some of the earlier literature related to fishery conditions and were not specifically dealing with research in aquaculture. Unlike traditional agriculture where a limited number of species are under study, in aquaculture more than 240 diverse species are being evaluated, cultured, and studied in multiple different environments. The diversity of aquaculture species defies a united front to the scientific advancement of studies for these species, as each is in a different phase of development. Also, the value of the product and its potential usefulness as either a research animal or a food animal impacts research funding as has been seen in recent years. However, the use of some fish species as models for the analysis of development and so forth has enhanced our general knowledge of fish anatomy and physiology. In terms of research, fish are the third most commonly used experimental animal after mice and rats in countries such as the UK (Ostrander 2000). This increase is a result of the rapid development of the aquaculture industry, regulatory requirements for testing involving fish as indicators of environmental change, and the use of fish as a replacement for mammals in biomedical, pharmacological, and genetic research. Several aquatic species such as zebrafish, fugu, rainbow trout, catfish, Atlantic salmon, tilapia, and bivalves are exclusively used in several scientific and agricultural research programs.

Of these species, the zebrafish is the most extensively studied fish species. Although not reared commercially as a food animal, zebrafish are nevertheless economically important, with specific stocks and species being reared and sold around the world. Studies with zebrafish began in the 1970s by Oregon scientist George Steisinger (Detrich et al. 1999). The identification and study of mutations in zebrafish has been extremely successful in providing an understanding of early embryonic development. Mutagenesis screens have provided proof of principle that classical forward genetics can be used to understand vertebrate development. In other vertebrate research species, embryogenesis is difficult to study as it occurs within the uterus. In the late 1970s, researchers began to use zebrafish as an organism for study since it was readily available, had a relatively short generation time, and produced large clutches of embryos, as well as since all its developmental stages could be easily visualized. Furthermore, the zebrafish embryo possessed a simple organization, containing fewer cells than other vertebrate species, and the embryo possessed transparent cells that are accessible for manipulative studies, which can be injected with tracer dyes to track emerging cell lineages. Molecular, cellular, and developmental studies of mutant zebrafish collections have yielded a wealth of knowledge regarding vertebrate development. Currently, there is zebrafish research on the genetics of behavior and the generation of conditional mutants contributing to the dissection of gene function. Other related important genomic research species are the fugu or pufferfish (*Tetraodon nigroviridis*) and medaka (*Oryzias latipes*).

Rainbow trout have been a popular species for research. More is known about the physiology and biology of rainbow trout than of any other aquatic species. Rainbow

trout excel as a physiological and genetic model organism. The fact that they are large in size allows for isolation and harvesting of large amounts of specific tissues and cell types for biochemical, immunological, and molecular biological analyses. Much is known about rainbow trout from research involving their natural populations, established clonal and transgenic lines, and extensive generated sequence information (Thorgaard et al. 2002). Furthermore, regarding salmonid genomics research, several countries (including Norway and Canada) have generated salmonid libraries and microarrays, mainly geared toward Atlantic salmon, for research use. Currently, a wealth of information is being generated for a number of other species, including cod, flounder, and tilapia.

In early studies using fish, as early as 1863, a majority of experiments involved fish as monitors for toxicity in aquatic environments or as a readily available source of tissue (Hunn 1989). During the middle of the last century, toxicity tests with fish increased, mainly due to concerns regarding the widespread use of pesticides. Since then, fish have remained as a standard for use in bioassays for acute toxicity monitoring and in the evaluation of chemical toxicity. With increasing concerns regarding industrial contamination, fish are actively used as environmental biomarkers in monitoring the environmental status of both fresh and marine water systems (Rand and Petrocelli 1985). By the seventeenth century, biologists throughout Europe were beginning to refine and expand their scientific observations. In the late 1600s, Anton van Leeuwenhoek used microscope to observe and describe sperm from fish and other organisms. In 1668, the court physician Francesco Redi used the fish and other animal tissue to refute the idea that flies could develop spontaneously from putrefying flesh. And then in 1686 in England, Francis Willughby published a manuscript of detailed fish drawings sufficient for the identification of several species. The studies by Borelli on the mechanics of muscular action and swimming action led to models on how animals moved and how fish swam (Ostrander 2000).

Nigrelli (1953) in an overview on the "utilization of fish in biological research" offers a chronological synopsis of fish as experimental animals. In this overview, Nigrelli asserts that at one time most important researchers have probably used fish in their research. The fish mummichog (*Fundulus heteroclitus*) was used in a significant number of studies dated to the late 1900s by researchers at Woods Hole, Maine. Many experiments were conducted on mummichog genetics, pigmentation, and endocrinology. Perhaps one of the earliest known species worked with was *Carassius auratus*, or the common goldfish. Because of their size, availability, and early domestication, tropical fish were a favored species for researchers. Early research dealt with egg or gross anatomical development, physiology, viability under different environmental conditions, and the effects of chemicals on development. Beginning in the 1970s and extending to the present day, the concept of fish as experimental models has taken hold, and they have proven to be an indispensable asset to research advancement.

Until recently, in most scientific studies involving fish or their eggs, fish were used as components for monitoring an effect, typically of waterborne toxins, and the studies were not specifically fish related. However, with the burgeoning economic importance of aquaculture and development of fish models, some aquatic specimens have come to the forefront of modern research, and this information is now being used to improve aquaculture.

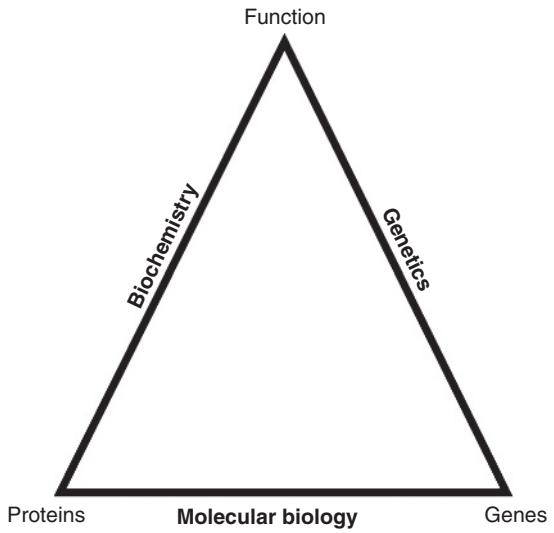

Figure 1.1. Interrelationship between scientific disciplines and studies on how genes and proteins function.

Molecular Technology

Although fish farming and aquaculture research has been practiced for several thousand years, the advent of research on the molecular level is a more recent occurrence. The history of molecular biology began around 1930 with the convergence of various previously distinct biological disciplines including biochemistry, genetics, and virology (Figure 1.1). However, the basics of molecular research was provided even earlier with studies in chemistry, physics, and microbiology. Researchers in these areas began integrating their research with the hope of obtaining an understanding of life at its most basic level. The combination of research goals in these areas of science led to what is now known as molecular biology. In an article in the journal *Nature*, Astbury (1961) once described molecular biology as not so much a technique as an approach for studying physical manifestations in form, and from this determining their development from biological molecules and their function (Bernal 1963). James Crick in 1957 coined the term "the Central Dogma," which he used to describe the biological flow of sequence information from nucleic acids, including DNA replication, RNA transcription, and the translation of proteins and their processing. Basically, this premise translates into a working description for the replication of cellular DNA, transcription of DNA to RNA, translation of RNA to protein, and the action of translated proteins on cellular and physiological levels relating to development and whole-body traits. The term "molecular biology" most likely originated in 1938, when Warren Weaver used it to explain the working of particles involved with life. These particles, we now know, consist of nucleic acids and proteins. In its modern sense, molecular biology attempts to explain the phenomena of life, starting with the biological components that contain the information necessary to replicate and give rise to organisms. The current definition of molecular research involves studies involving DNA, RNA, and protein,

as well as research involving their tissue-specific levels, activities, pathway linkages, function, and related involvements with physiological changes seen in an organism. A brief chronological description of the historical events that occurred involving nucleic acids, proteins, and genetics provides a better understanding of the current status of molecular research.

The characterization of chemical molecules that determine the physiological makeup of living organisms gained momentum with the birth of physiological chemistry in the nineteenth century and biochemistry at the beginning of the twentieth century. When the molecular revolution is evaluated within the context of biological history, it is easy to note that it is the culmination of a long process that began with the first observations through a microscope in the eighteenth century. The aim of early biologists was to deduce the functions of living organisms by describing their organization at the microscopic level, while chemists were obviously more interested in studying the chemical compounds found in organisms. Between the molecules that make up chemical compounds studied by chemists and the tiny structures visible under the optical microscope, such as the cellular nucleus or the chromosomes, there was an obscure zone, "the world of the neglected dimensions," as it was called by the chemist Wolfgang Ostwald. This zone was populated by colloids, biopolymers, and chemical compounds whose structures and properties were not well defined. Most of the important early scientific advancements involved working with materials that were too small to visualize with a microscope yet possessed the ability to transfer information from parents to offspring in a mysterious manner. This line of research required the generation of techniques for the indirect study of the experimental samples. Eventually, through the culmination of related information gained from biochemistry, genetics, and physics, our current understanding of the molecular components controlling inheritance, development, and physical traits was pieced together.

Nucleic acids were first isolated in 1869 by Friedrich Miescher, when he discovered a weak acid in white blood cells that he referred to as "nuclein." Miescher isolated a pure sample of this substance from salmon sperm, and in 1889 Richard Altmann, a student of Miescher, termed the isolate "nucleic acid." At that time, this substance was found to exist only in the chromosomes of cell nuclei, and biochemists initially isolated DNA and RNA concurrently from cell nuclei. Researchers soon discovered that the nucleic acids isolated had a polymorphic nature and it was later realized that there were two distinct types: RNA which contains ribose and DNA which contains deoxyribose.

In 1929, Phoebus Levene at the Rockefeller Institute identified the basic components of DNA, which consisted of four bases, sugar, and a phosphate chain, and then determined how they were linked. He called each of these units a nucleotide and suggested that a molecule of DNA was composed of a string of nucleotide units attached together by phosphate groups. These phosphate groups provided the structural support for the molecule. However, Levene thought that the chain was short and that the bases were repeated in the same fixed order. In 1937, William Astbury produced the first X-ray diffraction patterns from DNA. He was not able to propose the correct structure, but the patterns showed that it was regular and repetitious, suggesting that it might be possible to deduce the structure.

The structure of DNA was finally elucidated in the 1950s, when three groups made it their goal to determine its cellular assembly. By using different methods, Maurice Wilkins and Rosalind Franklin at King's College London, Linus Pauling at Caltech, and Francis Crick and James Watson at Cambridge attempted to generate

an accurate structure of the DNA molecule. Piecing together the information from all three groups, which included the discovery of helical shapes in proteins by Pauling and the X-ray diffraction information from Wilkins, Watson and Crick attempted to build a physical model of the helical structure using the chemical structure of the nucleotides and their linkages. A final crucial piece of information came from the work of Erwin Chargaff, who had reported that although the proportion of the four nucleotides varied among different DNA samples, the proportion of pairs of the nucleotides were always the same. Restricting themselves to the development of a model that they considered as chemically and biologically reasonable, Watson and Crick in 1953 developed the first accurate model of the molecular structure of DNA (Figure 1.2).

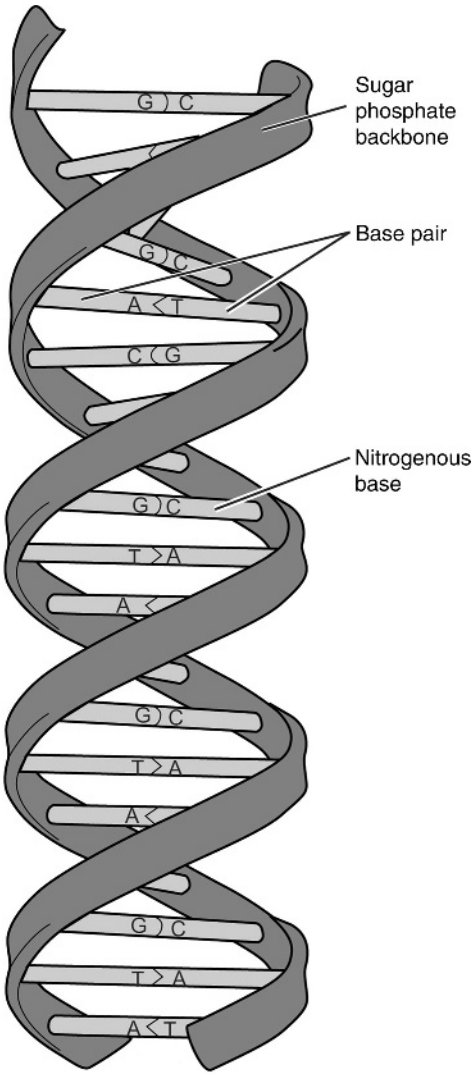

Figure 1.2. Diagram of the helical form of DNA, detailing the position of certain chemical components.

In 1962, Watson, Crick, and Wilkins jointly received the Nobel Prize for determining the structure of DNA.

Experiments by Meselson and Stahl in 1958 proved that DNA was semiconservatively replicated. This information helped to further confirm the double-helical model proposed by Watson and Crick, who later showed that the genetic code consisted of triplicate bases, termed codons. Later, Har Gobind Khorana interpreted the genetic code and its function in protein synthesis. In 1964, Howard Temin demonstrated, by using RNA viruses, that the direction of DNA to RNA transcription could be reversed.

Molecular Biology and Genetics

Prior to the successful characterization of the structure of DNA with new technologies developed by chemists and physicists, such as X-ray diffraction, electron microscopy, ultracentrifugation, and electrophoresis, parallel research was being performed in genetics by studying how genetic traits were physically transferred from parents to offspring. These studies went beyond evaluating the structure and function of the macromolecules. Scientists were attempting to link the action of unknown biological compounds with biological function. In 1865, Gregory Mendel published "Experiments in Plant Hybridization." Through the careful crossbreeding of pea plants, Mendel was able to determine how specific traits were passed from generation to generation. During the late 1800s Walter Flemming and coworkers showed that chromosomes divide and are distributed equally during cell division, and in 1903 Walter Sutton hypothesized that since chromosomes appear to segregate in Mendelian fashion, they might function as hereditary units.

After the rediscovery of the work of Mendel through the studies of Hugo de Vries, Carl Correns, and Erich von Tschermack in 1900, the study of inheritance and how it was passed on moved forward rapidly with the work of Thomas Hunt Morgan, who in 1910 used the fruit fly, *Drosophila*, as a model organism for genetic studies. Morgan showed that genes are localized on chromosomes. Following this discovery, he continued working with *Drosophila* and, along with numerous other research groups, confirmed the importance of genes in the development and physiology of organisms. On the basis of the work of Morgan and his own research, Alfred Sturtevant in 1913 was able to produce the first genetic map of a chromosome and demonstrate the linear arrangement of genes. It was not until 1931, however, that Jean Brachet demonstrated that chromosomes were the cellular components that contained DNA and that RNA was present in the cytoplasm of all living cells. Despite these discoveries, the chemical nature of genes, their structures, and their mechanisms of action remained elusive. Researchers from multiple disciplines committed themselves to determining the structure and elucidating the complex relations between genes and proteins.

Max Delbrück, Nikolai Timofeeff-Ressovsky, and Karl Zimmer published results in 1935 suggesting that chromosomes were very large molecules whose structures could be changed by exposure to X-rays and that by so altering their structure it was possible to change the heritable characteristics governed by those chromosomes. In 1928, Frederick Griffith demonstrated the potential for nonpathogenic bacteria to acquire traits from dead pathogenic bacteria when cocultured in mice. Unfortunately, Griffith was killed at work during an air raid. However, in 1943 Oswald Theodore Avery and a team of scientists were able to duplicate some of Griffith's research results and discovered that traits associated with one form of the bacteria pneumococcus could be

transferred to another form of the same bacteria merely by making biological material from a killed form available to living bacteria. Then, quite unexpectedly, it was found that these transferred traits were heritable. Avery identified DNA, and not protein, as the material responsible for the transformed bacteria and called the transfer of traits the transforming principle.

Also during the early 1940s, George Beadle and Edward Tatum were able to demonstrate the existence of a relationship between coded genes and expressed proteins within an organism. Beadle and Tatum switched from using *Drosophila* as their genetic animal model to a more appropriate model organism, the fungus *Neurospora*. By constructing mutant strains that required specific amino acids or vitamins, they verified, by means of gene mutations, that individual genes were responsible for specific steps in the metabolism and synthesis of vital nutrients. The culmination of this work in 1941 led to the proposal of the "one gene–one enzyme hypothesis," in which the concept is that a single gene specifies a single enzyme or protein rather than a complex set of characteristics. In 1944, Oswald Avery, working alongside Alfred Mirsky at the Rockefeller Institute of New York, demonstrated that genes were composed of DNA. In 1952, Alfred Hershey and Martha Chase, in what is now termed the Hershey–Chase experiment, confirmed that the genetic material of the T2 bacteriophage, a virus that infects bacteria, was made up of DNA. In 1961, Francois Jacob and Jacques Monod demonstrated how certain specific proteins, called regulative proteins, latch onto DNA at the edges of the genes and control the transcription of these genes into messenger RNA. A milestone during the process of deciphering the link between DNA and protein was provided by the work of Linus Pauling who for the first time linked a specific genetic mutation in patients with sickle cell disease to a demonstrated change in an individual protein, the hemoglobin in the erythrocytes of heterozygous or homozygous individuals.

Between 1961 and 1965, researchers were able to determine the relationship between the information contained in DNA and the structure of protein. They found that the nucleotide arrangement of DNA on chromosomes provides a "genetic code" which is followed in order to make a complementary sequence of the nucleic acid RNA. This code then corresponds to a chain of amino acids that are linked together by ribosomes during translation of the RNA sequence to generate a protein.

Thus, several of the key discoveries of molecular biology took place in a period of only about 25 years. Over the next 20 years, new and more sophisticated technologies allowed for the isolation and characterization of genes and their function. This effort continues today.

Links between Molecular Biology and Genetics and Biochemistry

Biochemistry and genetics also had significant impacts on the development of molecular biology. Although a large amount of research had already been done in regard to protein chemistry since the late 1700s, during the first half of the twentieth century significant progress was made in our understanding of the role of proteins in metabolism and even in genetics. Prior to the 1900s biologists and chemists studied fermentation, the liquefaction of meat when exposed to stomach secretions, and the conversion of starch to sugars, but had yet been unable to determine the mechanisms causing these changes, the link being each of these processes is catalyzed by a specific enzyme. As a consequence, the study of proteins, their structures, and syntheses became one of the principal objectives of biochemists.

Protein Biochemistry

In the late 1830s, the Dutch chemist Gerhardus Johannes Mulder began elemental analyses of common animal and plant proteins. Unexpectedly, he discovered that all proteins had nearly the same empirical formula. Mulder's professor Jons Jakob Berzelius proposed the term "protein" for these isolated substances. Mulder went on to identify amino acids as degradation products of proteins and even determined the correct molecular weight of several amino acids. Mulder's analysis of a pure isolated product suggested a weight that was much greater than that for other known molecules under study. Working against skepticism of the scientific community that such long macromolecules would be stable in solution, in 1902 Franz Hofmeister and Emil Fischer concurrently proposed the idea that proteins were linear polymers of amino acids linked by peptide bonds. It was not until 1920 that Theodor Svedberg was finally able to demonstrate, by using analytical ultracentrifugation, that proteins were macromolecules of well-defined composition. Later within the same decade, James Sumner was able to demonstrate, by using the enzyme urease, that proteins are not merely carriers but are responsible for enzymatic function. Sumner's method to isolate and crystallize proteins was extremely important because it eventually proved essential to determining their structures by X-ray crystallography. Early research with proteins was extremely difficult because most proteins were difficult to purify in more than milligram quantities, even using the most modern methods. Hence, early studies focused on proteins that could be purified in the largest quantities available, such as those found in blood, egg whites, and digestive/metabolic enzymes obtained from slaughterhouses. Several techniques of protein purification were developed by Edwin Joseph Cohn during World War II in an attempt to purify blood proteins for use in treating wounded soldiers. Then in an exceptionally alturistic gesture during the late 1950s, the Armour Hot Dog Co. purified 1 kg (=1 million milligrams) of pure bovine pancreatic ribonuclease A (RNase A) and made it freely available in 10-mg batches to scientists around the world. This generous act made RNase A the model system for protein basic research for the next several decades.

Studies in protein formation and folding began around 1910, when Henrietta Chick and C.J. Martin showed that the flocculation of a protein was composed of two distinct processes: First, during denaturation the protein becomes less soluble, enzymatically inactive, and more chemically active; then, the protein begins to precipitate from solution. In 1929, Tim Anson and Alfred Mirsky proposed in a paper that denaturation was a reversible process, a hypothesis that was widely ridiculed at the time. Anson later published an article with Linus Pauling detailing the energy states of proteins and suggested that denaturation was an all or none process in which the same changes occur that were documented by Chick and Martin. Around this time, Hsien Wu hypothesized that denaturation involved changes in the folded state of the protein, a purely conformational change that resulted in the exposure of amino acid side chains to solvents. According to Wu, exposure of side chains to solvent rendered the protein less soluble and more reactive, whereas the change in conformation was the reason for loss of enzymatic activity. In the early 1960s, Chris Anfinsen developed what he called his "thermodynamic hypothesis" of protein folding to explain the native conformation of amino acid structures. He theorized that the native or natural conformation occurs because this particular shape is thermodynamically the most stable in the intracellular environment. Anfinsen demonstrated that the three-dimensional state of the enzyme

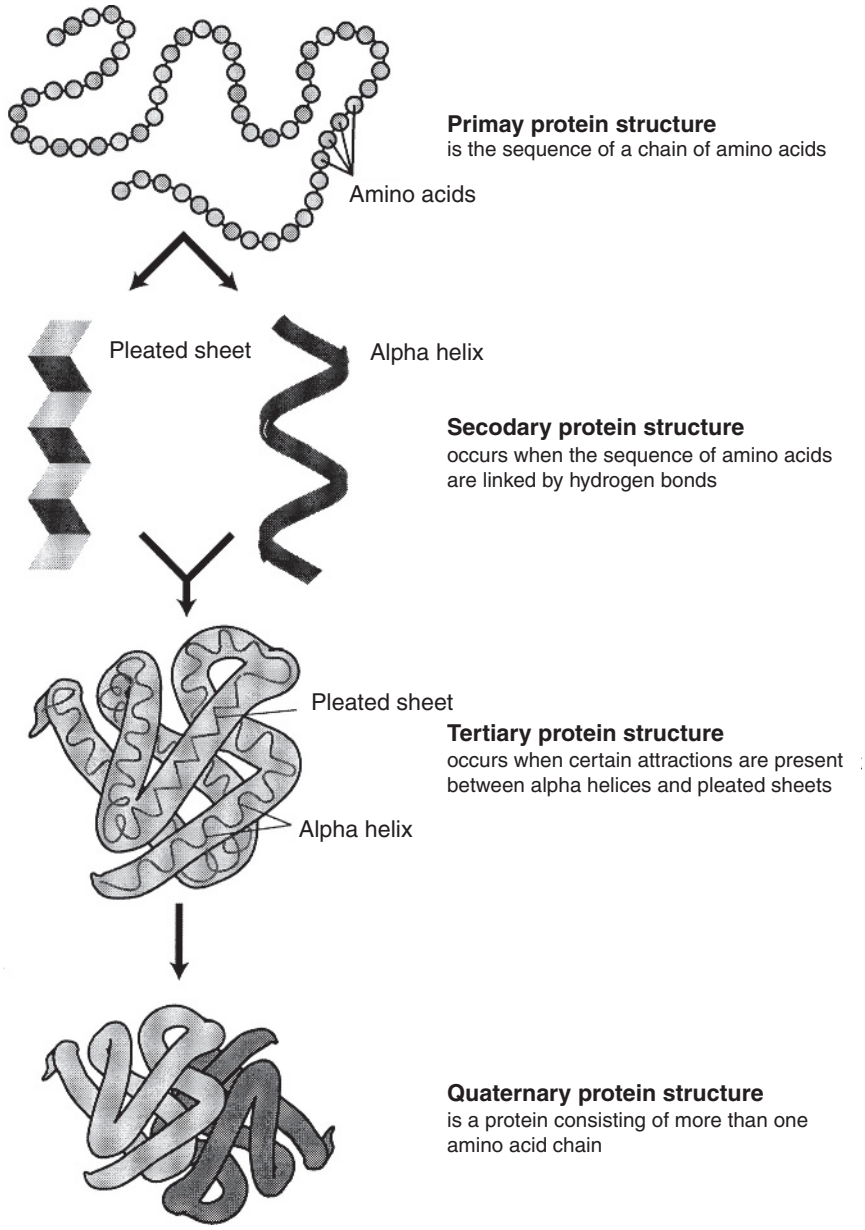

Figure 1.3. Protein folding structures. Examples of four different types of protein structures and their relative complexities.

RNase A was fully reversible with no external cofactors needed, verifying that the folded state represents the lowest free energy for a protein.

Linus Pauling was the first to correctly determine the secondary structure forms of the alpha helix and beta sheet of proteins (Figure 1.3). The stability of hydrophobic interaction for maintaining protein stability was first proposed in the late 1920s but

refuted until publication of an article on denaturation in 1959 by Walter Kauzman, based partly on work by Kaj Linderstrom-Lang. Arne Tiselius and associates were the first to demonstrate the ionic nature of proteins; however, Kaj Urik Linderstrom-Lang established that these charged bonds were accessible to solvent and not stringently bonded to each other.

The secondary and low-resolution tertiary structure of globular protein was investigated initially by hydrodynamic methods such as analytical ultracentrifugation and flow birefringence. The primary structure of protein was an extremely active area of research, when in 1949 Fred Sanger developed sequencing techniques for proteins and was able to sequence insulin. However, by the 1960s the first atomic-resolution structures of proteins were determined by X-ray crystallography and further clarified by the NMR method in the 1980s. As of 2009, the Protein Data Bank has over 55,000 atomic-resolution structures of proteins. Currently, cryoelectron microscopy of large macromolecular assemblies and computational protein structure prediction of small protein domains are two methods used that approach atomic resolution.

Research from these different scientific disciplines culminated in the 1970s and 1980s, when DNA sequencing allowed for (a) the separation and identification of different gene sequences along with the isolation and understanding of restriction enzymes for site-specific cleavage of DNA, (b) the development of cloning vectors for cloning and amplification of isolated sequences, and finally (c) the development of polymerase chain reaction for rapid amplification of nucleic acids. These and other techniques have opened up vast and remarkable techniques for determining physiological differences or changes that either are undetectable or cannot be discreetly measured by physical determination. In this book several of the methods or techniques most used or that are coming into common practice in current aquaculture research are discussed along with their practical applications to aquaculture.

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