MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic：Cloning and biological information analysis of alpha interferon from Yangzhou white goose

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
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Completed:
student of group BEBT-20
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Scientific supervisor
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Dr. Sc., Prof.
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APPROVE

Head of Department of Biotechnology,

Leather and Fur, Professor,

Doctor of Technical Science

Olena MOKROUSOVA

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«___»_____2024

ASSIGNMENTS

FOR THE QUALIFICATION THESIS

Cheng Peng

1. Thesis topic **Cloning and biological information analysis of alpha interferon from Yangzhou white goose**

scientific supervisor Tetiana Shcherbatiuk, Dr. Sc., Prof.

approved by the order of KNUTD "____"_____2024, №____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

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EXECUTION SCHEDULE

	The name of the stages of the qualification thesis	Terms of performance of stage	Note
	Introduction	From 01 April 2024 to 11 April 2024	
	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
	Conclusions	From 07 May 2024to 12 May 2024	
	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	

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I am familiar with the task:

Student _____Cheng PENG

Scientific supervisor _____ Tetiana SHCHERBATIUK

ABSTRACT

Cloning and biological information analysis of alpha interferon from Yangzhou white goose. - Manuscript.

Since ancient times, our country is a big country of poultry breeding, and our poultry breeding industry has a long history. At the same time, with the continuous development of China's livestock and poultry breeding industry in recent years, the national government has issued a series of relevant policies to support and regulate the development of the poultry industry, providing a good development environment for the poultry industry. However, with the development of large-scale intensive breeding industry, there are more and more diseases of geese, among which viral infectious diseases are the main cause of death of geese. Yangzhou white goose is the first local breed bred with domestic goose breed resources in China, and is known as the first new goose breed in China. Yangzhou White goose is an ideal goose breed because of its high reproductive rate, stable genetic performance, rapid initial growth of goslings for meat, and high muscle protein content. However, the breeding process of white geese in Yangzhou is often affected by a variety of infectious diseases, especially viral diseases such as bird flu, paramyxovirus disease and bursal disease of geese, which threaten the development of goose breeding industry. If a large number of chemical agents are used in the aquaculture industry, it will not only threaten people's health, but also lead to increased drug resistance of the virus, and interferon has the advantages of wider spectrum, high efficiency and safety than chemical agents.

In this experiment, type I interferon alpha with strong antiviral activity was selected for the experiment, and the sequence-designed primer on goose interferon alpha published in Gen Bank was referred to. The interferon alpha gene was amplified and cloned by RT-PCR technology, and named yz goose IFNα-1 and yz goose IFNα-2. The nucleic acid and amino acid sequences of

interferon alpha gene were analyzed by bioinformatics software and on-line analysis method. In this study, we obtained a fragment of 576bp size encoding 191 amino acids with a molecular weight of about 21.6kDa, which is consistent with the expected size. The obtained alpha-interferon sequences of two Yangzhou white geese were much more similar to those of chicken and duck than to those of human and other animals. The two sequences obtained above were imported into the online software NetPhos 2.0 for the prediction of phosphorylation sites. When the threshold was set at 0.5, the two amino acid sequences each contained 17 potential phosphorylation sites. The hydrophobicity of the known yz goose IFNα-1 and yz goose IFNα-2 derived peptide chains was analyzed by online ProtScale software. According to the hydrophobicity map, the maximum value of hydrophobicity of the two genes was 2.856, and the minimum value was -2.656. The first hydrophobic zone is located at 10-30aa and is the most hydrophobic.

Keywords: Biological information; Interferon; Yangzhou white goose; Cloning; analyze

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INTRODUCTION

Waterfowl breeding industry has a special economic significance in our country. But at present, the existence of various viral diseases in waterfowl has seriously hindered the orderly development of waterfowl industry. In particular, geese infectious diseases (gosling plague, goose virus paramyxosis, bird flu, etc.) affect the production performance of geese and cause huge economic losses. Goose alpha interferon can be effectively used in the prevention and control of goose infectious diseases, promote the development of goose raising industry, and have important significance in enhancing goose immunity and optimizing breeding environment. Therefore, it is the top priority to develop biological agents that can effectively prevent and control goose viral diseases.

Interferon is an important cytokine, which has broad-spectrum antiviral activity, good inhibition of cell division, anti-tumor activity and efficient immune regulation.

Recently, the research on avian interferon is continuing to deepen, especially for chicken and duck interferon, but there are few reports on goose interferon.

Interferon is a class of cytokines that can induce a variety of antiviral small molecules. When the host cell detects the molecular pattern associated with foreign pathogens, the body will recognize specific pathogen pattern molecules (including nucleic acids, proteins, lipopolysaccharides, glycans and other components of the antigen) through pattern recognition receptors, and then induce the host innate immune system through the action of joint molecules and regulatory factors. The production of terminal signaling factors (interferons) to resist the invasion and replication of pathogens, the initial interferon produced by the attacked host cell and the specific subtype of interferon receptor after specific binding, and then through the intracellular

antiviral signaling pathway, Activation of specific interferon activation response element sequence regions to initiate the transcription of specific anti-viral, anti-bacterial, anti-tumor, anti-parasitic and other immune genes, producing more interferons and effector proteins that help the host clear pathogens so far, the study of interferon immune function in birds has only been partially explained by scientists. In addition, the exploration of signal mechanism and function lags behind that of mammalian studies. Nowadays, in the era of frequent international exchanges and trade and frequent animal diseases, many viruses carried by birds themselves not only endanger the modern animal industry, but also pose a great threat to future food safety and even human health. Based on the current research, the basic and applied research of avian immune cytokines is not systematic enough. In the process of virus and host immune regulation, interferon participates in the first immune storm of immune defense. The functional analysis of this antiviral protein will become one of the breakthrough points in the study of avian antiviral immune mechanism. Therefore, the study of the regulation of host interferon and its related proteins in the state of viral infection W and the rule of virus invasion and regression is helpful to further understand the specific process of body recognition and elimination of 'non-self'. The basic research of this subject can provide theoretical support for the development of more efficient animal antiviral agents.

CHAPTER 1
LITERATURE REVIEW

Cytokines are soluble extracellular proteins or glycoproteins that are usually produced by immune cells and some non-immune cells in response to harmful stimuli and are secreted in varying amounts by different cells. According to the structure and homology of their receptors, cytokines can be divided into different families, such as interleukin, growth factor, colony-stimulating factor, interferon, chemokines, tumor necrosis factor superfamilies, etc.

Interferon (IFN) is mainly produced by monocytes and other immune cells. It is a multipotent cytokine with antiviral, anti-tumor and immunomodulatory properties that is universally expressed in cells, and is known as the central coordinator of immune response. Interferon is a kind of broad-spectrum antiviral agent, but it does not directly kill or inhibit the virus, but mainly by binding with the receptor on the cell surface to produce antiviral protein, thus inhibiting the replication of the virus. At the same time, interferon can also enhance the activity of Natural Killer cells (NK cells), macrophages and T lymphocytes, thus playing an immunomodulatory role, and further enhance the antiviral ability of immune cells. The molecular structure of interferon consists of three parts: the coding region at both ends, the peptide coding region, and the signal peptide coding region [1] (Figure 1.1).

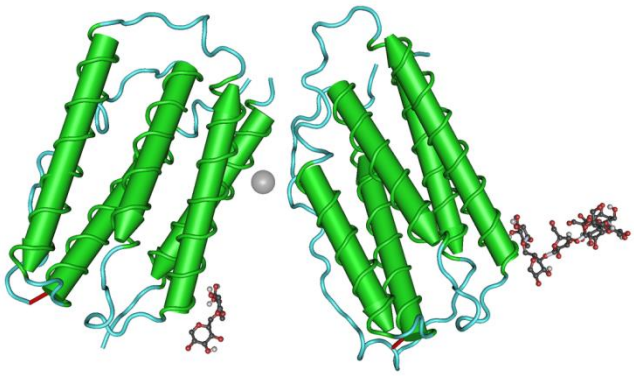


Figure 1.1 – interferon structure

Interferon is a special glycoprotein synthesized and secreted by the receptor cells under the action of virus infection or induction of interferon inducers. Scientists through the study of interferon gene sequence, found that interferon sequence as early as 100 million years ago has existed in the genetic sequence of biological cells, is an ancient protective factor in biological body, this factor not only exists in mammals, birds, reptiles and even most bony fish have interferon in the body. As an important factor regulating cell function, interferon not only has extensive antiviral activity and good immunomodulatory function, but also plays an important role in the study of cell gene expression regulation, the pathogenesis and prevention of viral diseases. Interferon has become a research hotspot in cytology, virology, immunology, clinical medicine, molecular biology, oncology and other related disciplines.

1. Classification of interferon

Interferon is secreted by fibroblasts, macrophages, vascular epithelial cells and osteoblasts. According to the location on the chromosome, the difference in the nucleotide sequence of interferon, receptor and protein structure, interferon can be generally divided into four categories and three types: four categories include leukocyte interferon (interferon alpha), fibroblasts interferon (interferon beta), immune interferon (interferon gamma) and interferon λs; The three main types are

type I, type II and type III. Type I interferon is not only an antiviral cytokine, but also an inducer of tumor cell apoptosis and anti-angiogenesis, and has many important roles in the immune system. Type II interferon is a cytokine with both anti-tumor and pro-tumor activity, which can be used as a link in immunotherapy response. Type III interferon is a new type of interferon discovered in recent years. Its induction process and biological function are similar to that of type I interferon, and it plays an important role in antiviral, immune regulation, anti-tumor, inhibition of autoimmune diseases, inhibition of allergic asthma, and anti-bacteria and fungi [2].

1.1.1 Type I interferon

Type I interferons are the product of a relatively conserved polygenic family of evolution, and most cells can produce type I interferons. Type I interferons, also known as viral interferons, include eight types of alpha, beta, and delta in eukaryotes. These different types of interferons have similar biological activities and bind to the same cellular receptors when they act, so they are collectively called type I interferons. The main structural feature of type I interferons consists of five alpha helices labeled A to E, which are interconnected by one long ring and three short rings (see Figure 1.2). Studies have shown that all type I interferon genes are located adjacent to each other on the same chromosome (human chromosome 9 and mouse chromosome 4), are derived from a common ancestor gene, and type I interferons have similar spatial structure and activity and enjoy the same class of receptors [2].

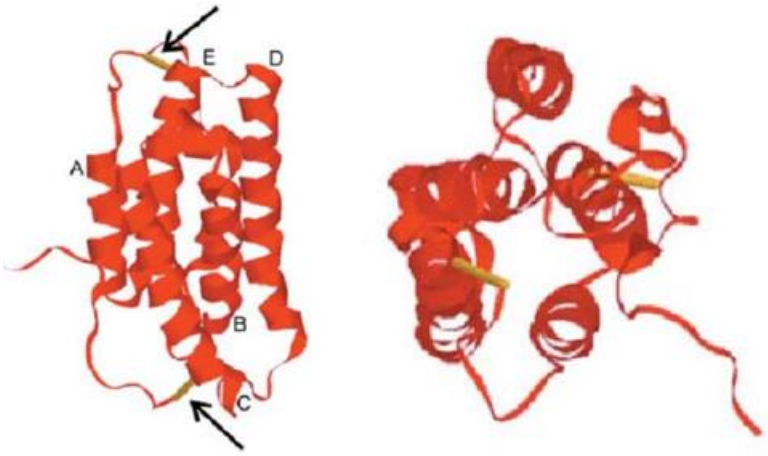


Figure 1.2 – Typical interferon alpha stereoconformation

Alpha interferon and beta interferon are produced by leukocytes and fibroblasts, respectively. Alpha and beta interferons have similar physical and chemical properties and biological functions, are stable in acidic environment, have antiviral activity, and bind to the same receptor. As a cytokine secreted by the human body, interferon alpha has antiviral, anti-tumor and immunomodulatory effects. Alpha interferon protein is modified by 4 N glycosylation sites, 5 O glycosylation sites and 20 phosphorylation sites, mainly composed of alpha helix and random coil. There are more than 20 subtypes of alpha interferons and rich in leucine and glutamic acid. The amino acid sequence alignment of each interferon in the same genus has 80% homology, and there is only one subtype of interferon beta. The physiological activity of the above types of type I interferons and the cell receptors they require are similar, and most of the genes do not contain introns, and most of the signals are transmitted through the receptor composed of IFNAR-1 and IFNAR-2. Beta interferon upregates and downregulates a variety of genes through the signaling pathways STAT1 and STAT2, most of which are involved in antiviral immune responses. In addition, beta interferon plays an important role in inducing a wide range of non-specific antiviral infections, even affecting cell proliferation and regulating immune responses.

ω interferon comes from white blood cells, and its amino acid sequence is more than 60% homology with the amino acid sequence of alpha interferon, which is

far more than other interferons, so it is also called interferon alpha type II. Therefore, it also has the function of promoting the expression of MHC class I molecules and enhancing the activity of phagocytes and cytotoxic T cells. Natural interferon alpha is often a mixture of interferon alpha and interferon omega functional gene expression products. At present, it has been found that interferon ω contains 5-6 gene loci, but except for 1 gene loci expression, the rest gene loci are not expressed.

Interferon τ is a specialized interferon produced only in trophoblast cells, hence the name trophoblast interferon, which is composed of 172 amino acids. Interferon τ is an interferon with common characteristics of typical type I interferons, but has its own characteristics, such as: it is only expressed in embryonic trophoblast cells and does not require virus induction; High concentration interferon τ showed less cytotoxicity than other type I interferons. As a new type I interferon, interferon τ has the biological functions of anti-virus, anti-luteolysis, maintenance of normal pregnancy and immune regulation, especially the high specificity of retrovirus inhibition, low immunomodulatory activity and weak cytotoxicity to autoimmune diseases. These characteristics will make it a new molecular drug [2].

Interferon κ b has a strong hydrophobicity, so its expression form in the prokaryotic expression system is basically in the form of inclusion bodies. Composed of 166 amino acids, it can induce the body to produce antiviral proteins.

Interferon delta and ζ are found in pigs and mice, respectively, and are not found in humans.

1.1.2 Type Ⅱ interferon

Only one type II interferon has been found, interferon gamma, also known as immune interferon, which is an immune cytokine. Interferon gamma is mainly secreted by T lymphocytes, is unstable to acids, and binds to

receptors different from the previous two, and its immunostimulative activity is the strongest among the three. It has been suggested that the different expression of interferon alpha and interferon gamma depends not only on the cell type that produces them, but also on viral stimulation and intracellular transcription factors. Interferon gamma can exist in the form of an extracellular matrix and can control cell growth in a neighboring manner, so it can be distributed on almost all cell surfaces except mature red blood cells. Human and mouse interferon gamma have about 40% homology at the amino acid level and about 65% homology at the DNA level. Human interferon gamma mature molecules exist in the form of homologous dimers, consisting mainly of 143 amino acids, and glycoproteins are structurally unstable at pH 2.0 and less resistant to heat and acids. [5].

1.1.3 Type Ⅲ interferon

In 2003, scientists first identified the interferon type III family from the human genome sequence, including interferon λ1, interferon λ2, and interferon λ3. Human interferon λ4, first reported in 2013, is produced by a frameshift mutation in a gene upstream of interferon λ3. Interferon λ consists of 166 amino acids, and unlike type I interferon, which has no introns, interferon λ1 contains five exons and four introns, and interferon λ2 and interferon λ3 contain six exons and five introns. Like type I interferons, type III interferons are produced by viral infection and are also cytokines that act on cell surface receptors to perform their biological functions. Same as type I interferon, interferon λs can transmit ligand binding signals by activating JAK-STAT signaling pathway. Therefore, interferon λs shows similar biological activities such as antiviral, tumor cell growth inhibition and immunomodulatory function as interferon α. However, due to its bone marrow suppression and few toxic side effects, the biological effects are long-lasting.

1.2 Molecular structure of interferon

As far as the gene structure of interferon is concerned, it is composed of three parts: the polypeptide coding region, the signal peptide coding region and the coding region at both ends. Similar to the molecular structure of most secreted proteins, interferon can be secreted out of the cell through the guidance of signal peptides, and the further decomposition of protein peptides promotes the formation of mature interferon proteins. All type I interferons have a similar molecular structure and a similar three-dimensional structure, are composed of tightly packed alpha-helical domains, and exhibit similar physiological and biochemical characteristics.

Generally mature alpha interferon is composed of 165-166 amino acids, and the length is about 495-498bp. In the interferon family, alpha interferon has the most prominent antiviral activity, and it has at least 13 subtypes, different subtypes contain 166-172 amino acids. Their structures are similar, and the molecular weight is mostly 19kDa, and the homology between different species can reach about 70%. Alpha interferon contains rich leucine and glutamic acid, and the amino acid sequence homology of each interferon in the same genus is as high as 80%. Interferon beta contains 166 amino acids, while interferon ω has between 172-174 amino acids, and the three-dimensional structure has a tightly arranged α-helix domain, which presents roughly the same physiological and biochemical characteristics. Interferon alpha generally has a spherical molecular structure in space (Figure 1.3), and this conformation plays a crucial role in its biological function. The amino acid homology of alpha interferon and beta interferon is between 26-30 percent, beta interferon is encoded by a single gene, and the amino acid number of mature beta interferon is 166, while the cysteine residue is only 3, and the cysteine thiol group is free in the 17th position. When Cys17 on beta interferon was replaced by serine, the biological activity was not affected but the conformation stability of the molecule was enhanced. The mature ω interferon

contains 172-174 amino acids, the number of amino acids on the C-terminal is 6 more than alpha interferon and beta interferon, and has 4 conserved cysteine residues. The amino acid sequence of ω interferon accounts for about 60% of the homologous part of alpha interferon and about 30% of the homologous part of beta interferon.

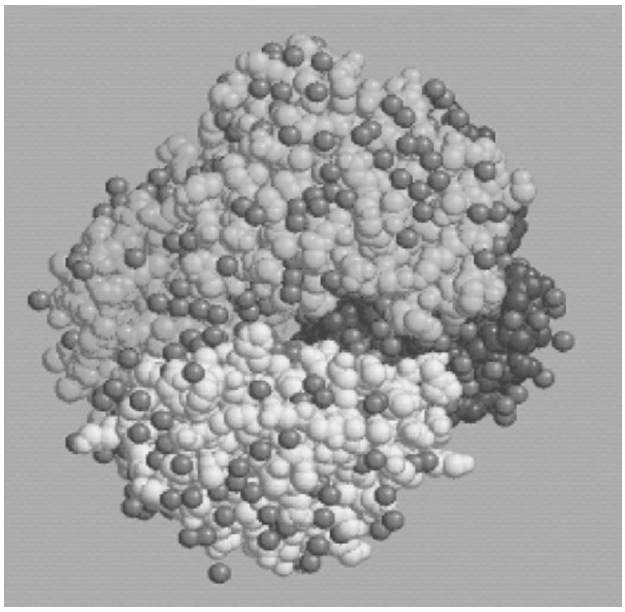


Figure 1.3 – Spherical spatial structure of alpha interferon

Type II interferon gamma Interferon is also encoded by a single gene, but contains introns, and n-glycosylated homologous dimers are its natural active form. The mature interferon gamma molecule consists of 143 amino acids, does not contain cysteine residues, and contains two n-glycosylation sites at aspartic acid positions 25 and 97. Normally, interferon gamma in space is composed of six alpha helices, A-F,A-D on one side of the dimer, and E and F participate in the connection of the dimer. Homologous dimer is the main form of interferon γ , the monomer has a mass of 17.1kDa, and the dibody has about 40kDa. The amino acid sequences at positions 86-90 and 128-132 are Lys-Lys-Lys-Arg and Lys-Arg-Lys-Arg, respectively. The arrangement of the above sequences may cause the instability of gamma interferon in the presence of acid. All gamma interferons are spatially similar to interleukin IL-10, whereas type I interferons are different from interleukin [1].

For type III interferon, the mass of the unit protein encoded by the interferon λ s gene is about 20kDa, and the disulfide bond formed in the structure is very

important for the correct folding of interferon λs and the realization of biological function. Interferon λ1 consists of a signal peptide containing 22 amino acids and a mature peptide containing 178 amino acids, containing two disulfide bonds and a potential n-glycoylation site at amino acid residues 65-67. The sequence composition of interferon λ2 and interferon λ3 is very similar, both consisting of a signal peptide containing 22 amino acids and a mature peptide containing 174 amino acids, including three disulfide bonds. The amino acid homology of λ1 and λ2 interferons is 81%, while that of λ2 and λ3 interferons can reach 96%.

1.3 Production and action characteristics of interferon

Interferon is a special glycoprotein produced by cells after virus infection, which has antiviral, cell proliferation inhibition, immune regulation and anti-tumor effects. When the body begins to defend against viruses from the outside world, interferon is often regarded as the body's first line of immunity. Currently known interferons do not kill viruses directly, but induce host cells infected by viruses to produce a variety of enzymes that interfere with the transcription of viral genes or the translation of viral protein components [4]. However, the body can not spontaneously synthesize interferon, the interferon gene inhibitor of the cell's DNA will bind to the interferon gene, inhibiting the production of interferon, so the interferon gene is usually in a suppressed static state in normal cells. When foreign substances such as bioinducers act on the cell membrane, interferon genes can be released from inhibition, interferon operons can also be transcribed to synthesize mRNA, and mRNA that is rapidly transferred to the cytoplasm will be translated into precursors on the ribosome, and mature interferon can be secreted outside the cell after signal removal. After more detailed research on the induction process of interferon, it can be found that the production of

interferon needs three stages: receptor recognition, signal transduction and gene regulation. There are many substances that can induce the body to produce interferon, and people usually refer to them as interferon inducers, mainly including:

(1)Live viruses, inactivated viruses and their products, such as double-stranded RNA;

(2)other pathogenic microorganisms and their products, such as bacteria and bacterial lipopolysaccharides;

(3) mitogen etc.;

(4) Specific immune inducers [2].

Interferon is a class of cytokines with antiviral, anti-tumor and immunomodulatory activities, and is an important part of innate immunity. After secreted from virus-infected cells, interferon binds specifically to cell surface receptors, and then induces cells to synthesize antiviral proteins to exert biological activities. Since the approval of interferon products in the 1980s, they have been widely used in clinical practice, used to treat dozens of diseases, and the market demand for interferon has increased significantly in recent years. Interferon has also played an important role in the response to the current COVID-19 pandemic.

1.4 Research progress of mammalian interferon

In 1976, Greenberg and other scientists first reported the successful treatment of four hepatitis B patients with human leukocyte interferon therapy, but because of the low development of genetic engineering technology at that time, human interferon could not be mass-produced, so interferon therapy could not enter the routine clinical treatment program.

In the late 1970s, Tsai et al. conducted the first study using dogs as experimental animals to evaluate interferon production after animal infection with viruses.

In 1986, Lefevre et al. expressed porcine alpha 1 interferon in E. coli for the first time and determined that its antiviral activity was much higher than that of natural porcine alpha interferon.

In 1990, Dijkmans successfully cloned porcine interferon gamma for the first time, and subsequently related research on porcine interferon gamma gamma was gradually carried out.

In 2004, Cao Ruibing et al. [6] expressed porcine beta interferon in prokaryotes, and concluded that porcine beta interferon had a significant inhibitory effect on porcine epidemic diarrhea virus. In the same year, Shi Xiju et al. [18] cloned the alpha interferon gene of Jinnan yellow cattle and constructed the recombinant plasmid of cattle.

In 2007, Zhang Yonghong et al. [7] cloned the alpha interferon gene of Luxi cattle and successfully expressed the alpha interferon active protein with the help of expression vector.

1.5 Research progress of avian interferon

In 1957, the British viral biologist Alick Isaacs and the Swiss researcher Jean Lindenmann, studying influenza interference using the chor allantoic membrane of chicken embryos, learned that virus-infected cells produce a factor that interferes with viral replication when it acts on other cells. Hence the name interferon.

1966-1971, scientist Friedman discovered the antiviral mechanism of interferon, which aroused people's attention to the antiviral effect of interferon, and then the antiviral effect of interferon, immune regulation, anti-proliferation effect and anti-tumor effect were gradually recognized.

In 1980-1982, scientists obtained interferon in E. coli and yeast cells using genetic engineering methods, and 20-40ml interferon could be obtained from every 1 liter of cell culture. Since 1987, interferon produced by genetic engineering methods has entered industrial production and been put on the market in large quantities.

In 1994, Sekellick et al. cloned the chicken type I interferon gene for the first time, and transfected chicken interferon alpha mRNA into mouse cells to successfully express a protein with antiviral activity.

In 1995, Schuits et al. first recombinant expression of duck IFN-α, and in 1999 recombinant expression of IFN-γ was also successful. In the same year, Schultz et al. confirmed that chicken interferon alpha is a glycoprotein; Digby et al. cloned chicken gamma interferon gene by RT-PCR method.

In 2005, Mi Jingwei et al. [8] extracted total RNA from goose peripheral blood white blood cells and successfully cloned goose IFN-α and γ genes using RT-PCR. The following year, Li et al. cloned the IFN-α gene of geese, and successfully expressed it in Escherichia coli and Sf9 cells.

In 2008, Zheng Xiaoling et al. cloned lion-head goose interferon alpha gene and successfully achieved high expression in Escherichia coli. In the same year, Gong Yongqiang, Cheng Anchun et al. successfully expressed duck alpha-interferon maturation protein gene using pET-32a.

In 2010, Hu Liping et al. cloned the Guangxi goose IFN-γ gene. The experimental results showed that the nucleotide sequence homology of the cloned Guangxi goose IFN-γ gene was higher than 99.2% and the amino acid homology was higher than 98.2% with that of the goose IFN-γ gene published in GenBank. Compared with the nucleotide and amino acid sequences of IFN-γ gene in other animals, the more distant the relationship, the lower the homology.

In 2011, Liu Fei [2] et al. successfully cloned the alpha interferon gene of Tianfu meat goose and submitted it to GenBank for registration (NM-HQ115583), and the cloned complete interferon gene was 576 nucleotides in length.

In 2014, He Jing, Zhang Panpan et al. [9] fused chicken interferon alpha and IL-18 genes, and the recombinant protein played an effective inhibitory role in vesicular stomatitis virus and Newcastle disease virus.

In 2021, Yang Min [4] et al. successfully used the prokaryotic expression system and baculovirus expression system to successfully express recombinant goose

interferon alpha with good biological activity, and the recombinant goose interferon alpha prepared by them could effectively inhibit the proliferation of goose parvovirus in goslings.

Summary of the chapter I

1.Classification and structure of interferon

2.The development process of interferon at home and abroad

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

China is a large country of waterfowl breeding, with the adjustment of industrial structure, goose breeding industry has become a bright spot and a new growth point of aquaculture in China. Yangzhou white goose is an excellent goose breed newly cultivated in China, mainly produced in various cities in Jiangsu province, because of its good genetic characteristics, such as: large egg production, strong disease resistance, delicious meat and other characteristics, so it is promoted to Shandong, Henan, Hunan, Shanghai and other places. However, with the development of large-scale intensive breeding industry, there are more and more diseases of geese, among which viral infectious diseases are the main cause of death of geese. Once the disease occurs, it will not only cause large-scale death, but also affect the production performance of geese, and even cause the decline of immune function, which is easy to secondary or concurrent infection with other pathogens, bringing huge economic losses to the goose industry. Therefore, in the development of goose raising industry, infectious diseases have become the bottleneck restricting the further development of goose raising industry.

At present, the prevention and control of goose diseases mainly rely on vaccines and antibiotics, but the use of antibiotics is easy to lead to the enhancement of drug resistance of pathogenic bacteria, the spread of drug resistance and drug residues in poultry products. At the same time, some of the virus serotypes infected geese tend to be complicated, various factors lead to virus mutation and other problems, and most importantly, there are still several viruses that are not protected by vaccines, which indicates that conventional vaccines and antibiotics can no longer guarantee the healthy and efficient development of modern goose farming industry.

Interferon is a kind of trace glycoprotein with high biological activity, which is produced when human and animal cells are properly stimulated. It is a kind of non-specific antiviral substance, and it is a defensive substance for the body to maintain

the stability of the body or cell function when the virus invades. As effector molecules that affect viral replication, interferon can inhibit the synthesis of viral nucleic acid and protein, induce the expression of MHC molecules, activate natural killer cells, selectively kill infected cells and play the role of immune regulation, etc. Compared with the use of chemical agents, interferon has the advantages of wider spectrum, high efficiency and safety.

Because there is only a very small amount of interferon in animals, it is difficult to prepare and purify by traditional methods. With the rapid development of molecular biology, interferon research has become increasingly in-depth, and the use of genetic engineering to produce interferon has become an effective way to solve this problem. In this paper, we cloned α-interferon, which has strong antiviral function, and analyzed the sequence of bioinformatics, so as to further study the molecular biological characteristics of Yangzhou white goose α-interferon.

EXPERIMENTAL PART

2.1 material

2.1.1 Experimental animals

5 Yangzhou white geeses

2.1.2 Instruments and equipment

Autoclave, ultra-clean workbench, electronic balance, PCR amplification instrument, ultra-micro spectrophotometer, constant temperature water bath, refrigerator, centrifuge, constant temperature horizontal shaking table

2.1.3 Main reagents

T4 DNA linker, TaqDNA polymerase, restriction enzyme, sodium citrate, PBS buffer, D-Hanks buffer, DEPC water, Buffer, MixTaq DNA polymerase, ultra-pure water, etc

2.2 experimental method

2.2.1 RT-PCR amplification of interferon alpha gene in Yangzhou white geese

2.2.1.1 Primer design and synthesis

Based on the goose alpha-interferon gene sequence (HQ115583) published in the GenBank database, Primer Premier 6.0 was used to design a pair of primers, namely F1 and R1, to amplify the full sequence of the interferon alpha gene.

F1：5’-ATGCCTGGGCCATCAGCCCCAC-3’
R1：5’-TTAGCGCATGGCGCGGGTGAGG-3’

2.2.1.2 Total RNA was extracted from peripheral blood lymphocytes of Yangzhou White geese

A sterile syringe was used to take 2ml of goose wing venous blood and add 0.22ml of blood anticoagulant 3.8% sodium citrate for anticoagulation. Isolate lymphocytes according to the instructions of the lymphocyte separation solution from Beijing Solaibao Technology Co., LTD. : The peripheral blood with anticoagulant was diluted with equal volume of PBS buffer, then 10ml of lymphocyte separation solution was added, and centrifuged at room temperature at 1500rpm/min for 20min. Then the lymphocyte layer was absorbed into the EP tube with a pipette. After that, the extracted lymphocyte layer was re-suspended with D-Hanks buffer, and then centrifuged at 1500rpm/min for 5min, and the supernatant was discarded. After repeated washing for 2-3 times, total RNA was extracted according to Trizol instructions purchased from Thermo Fisher Technology, and its purity and concentration were determined.

2.2.1.3 Whole-length interferon α gene of Yangzhou white goose was amplified by RT-PCR

①Reverse transcription of goose interferon alpha gene (RT)

Take the above RNA suspension 5 μ l, according to the reverse transcriptase instructions, add RNA5 μ L, specific primer 1 μ L, DEPC water to the PCR tube to 12.5 μ L, ice shock in 65℃ water bath for 5min, and then add 5×buffer4 μ L to it. 10mM dNTP Mix 2 μ L,RNA inhibitor 1 μ L, reverse transcriptase 0.5 μ L, a total of 20 μ L. Finally, reverse transcription was performed according to the following reaction procedures: 60min at 42℃, 10min at 25℃ and 10min at 70℃.

② PCR amplification of goose interferon α full-length gene

2 μ L of the above product was taken as the cDNA template, 25 μ L of MixTaq DNA polymerase was added successively, and the upstream and downstream primes (F1/R1) were 1 μ L each, supplemented with ultra-pure water to 50 μ L. After mixing, PCR amplification was performed according to the following reaction procedures.

Reaction procedure: pre-denaturation 95℃/3min → (denaturation 95℃/30s→ annealing 68℃/30s→ extension 72℃/1min) ×28cycles→ final extension 72℃/10min.

After the reaction, the fragment size of the 5 μ LPCR product was preliminarily identified by 1.0% agarose gel electrophoresis.

2.2.1.4 Purification of amplified products

The interferon α full-length gene PCR products of Yangzhou goose identified by gel electrophoresis were purified and recovered according to the DNA gel recovery kit of Aisygen.

2.2.1.5 Sequencing

The PCR products were purified and recovered, then linked with pGEMT-easy vector, and then used to transform receptive cells. Then, 10 single white colonies of uniform size were randomly selected from the petri dish and inoculated into the liquid LB medium containing Amp resistance, and were oscillated at 37℃ for more than 12h and labeled. After culture, the suspected positive strains were preserved for enzyme digestion identification, and then 100V electrophoresis in agarose gel for 45-60min. Finally, the positive recombinant clones identified were sent to the company for sequencing.

2.2.1.6 Sequence analysis

① Evolutionary tree analysis: MEGA6 software was used to construct phylogenetic trees of interferon sequences of different species by adjacency connection method, and the analysis parameters were set as: random number generator seed value was 111; The number of bootsrap trials is set to 1000; The confidence percentage of each branch of the evolutionary tree is represented by the number on that branch.

② Homology analysis: The blast function provided by ncbi website was used for homology comparison of sequences.

③ Signal peptide analysis: The online software SignalP V3.0 World Wide Web Server was used to predict the signal peptide cutting site of cloned goose interferon alpha sequences.

④ Glycosylation site analysis: Online analysis software NetNGlyc 1.0 and YinOYang 1.2 were used to analyze the amino acid glycosylation site of alpha interferon in Yangzhou white goose.

⑤ Phosphorylation site prediction: Yangzhou White goose alpha interferon derived peptide chain was used to predict phosphorylation site through online software NetPhos 2.0.

⑥ Hydrophobicity analysis: Online ProtScale software was used to analyze the hydrophobicity of Yangzhou white goose alpha interferon derived peptide chain.

Summary of chapter II

1.Experimental method and required bioinformatics analysis

2.RNA was extracted for analysis

CHAPTER 3
Cloning and biological analysis of interferon alpha gene in Yangzhou white goose

3.1. Extraction results of total RNA from peripheral blood lymphocytes of Yangzhou White goose

3.1.1 Centrifugal stratification of peripheral blood of Yangzhou white geese

The collected peripheral blood of Yangzhou white geese was stratified through multiple low speed centrifugation: the upper layer of light yellow transparent area was serum; The lower dark red area is red blood cells, and the thin white layer in the middle is lymphocytes. (Figure 4.1) The collected middle layer lymphocytes are used to extract the total RNA we need.

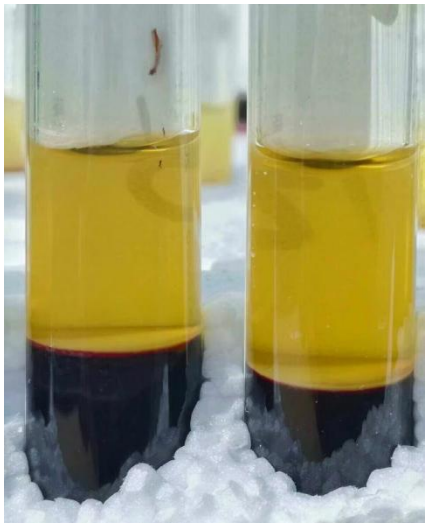


Figure 3.1 – Schematic diagram of blood centrifugation stratification

3.1.2 Determination of total RNA in peripheral blood lymphocytes of Yangzhou White geese

The purity and concentration of the newly extracted RNA were determined according to the Trizol instructions (purchased from Thermo Fisher Technologies Co., LTD.). The measured RNAOD260/OD280 values are 2.0; The OD260/OD230 value is 1.9. In the case of OD ratio standard (OD260/OD230:1.5-2.4), we found that when

the RNA concentration range was 30-1000ng/ul, the quantitative accuracy of RNA by spectrophotometer was almost the same as that of the biological analyzer. However, when the OD ratio is not up to standard (OD260/OD230<1.5) or the sample concentration range is <30ng/ul, the spectrophotometer is usually inaccurate, and the bioanalyzer is needed to re-quantify the RNA.

3.1.3 Electrophoretic identification of interferon alpha gene in Yangzhou white geese

The above mentioned interferon alpha gene of Yangzhou white goose was identified by gel electrophoresis, and the electrophoretic map as shown in the figure was obtained.

3.1.4 Sequencing results of interferon alpha gene in Yangzhou white goose

We obtained two different interferon alpha gene sequences with a total length of 576bp from five Yangzhou white geese, named yz goose IFN α-1 and yz goose IFN α-2, respectively.

yz goose IFNα-1 :

ATGCCTGGGCCCGCAGCCCCACCACCAACAGCCATCCACAGCGCCCTGGCGCTCCTGCTCCTCCTCAC
GCCTCCCGCCGACGCCTTCTCCTGCAGCCCCCTGCGCCTCCACGACAGCGCCTTCCCCTGGGACAGCC
TCCAGCTCCTCCGCGACATGGCTCCCAGCCCCACGCAGCCCTGCCCGCACCAACACGCGCCTTGCTCC
TTCCCGGACACCCTCCTGGACACCAACGACACACAGCAAGCCGCACACGCCGCCCTCCACCTCCTCCA
GCACCTCTTCGACACCCTCAGCAGCCCCAGCACCCCCGCGCACTGGCTCCACACCGCACGCCACGACC
TCCTCAACCAGCTCCAGCACCACATCCACCACCTCGAGCGCTGCTTCCCAGCCGACGCCACGCGCTTC
CACAGGCGAGGGCCCCGCAACCTTCACCTCGGCATCAACAAGTACTTCGGCTGCATCCAACACTTCCT
CCAGAACCACACCTACAGCCCCTGCGCCTGGGACCACGTCCGCCTCGAGGCTCACGCCTGCTTCCAGC
GCATCCACCGCCTCACCCGCACCATGCGCTAA

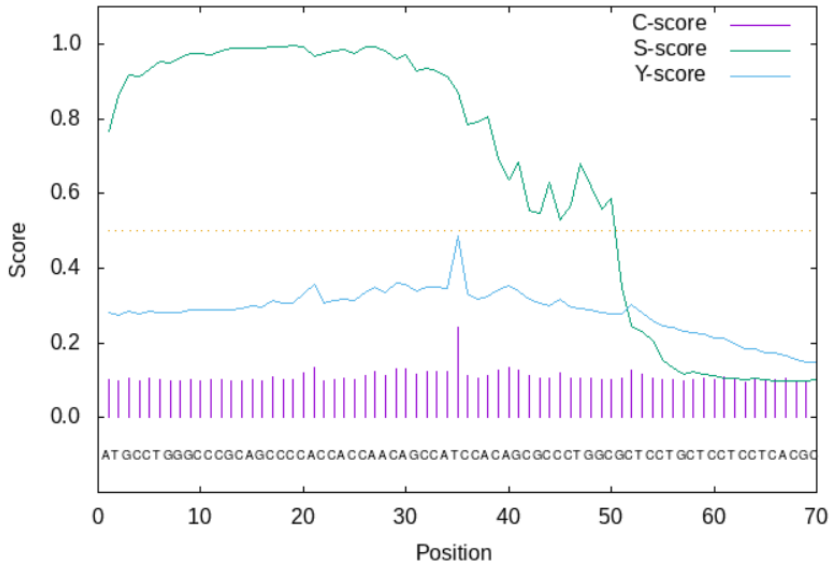
yz goose IFNα-2 :

ATGCCTGGGCCCGCAGCCCCACCACCAACAGCCATCCACAGCGCCCTGGCGCTC
CTGCTCCTCCTCACGCCTCCCGCCGACGCCTTCTCCTGCAGCCCCCTGCGCCTCCACGA
CAGCGCCTTCCCCTGGGACAGCCTCCAGCTCCTCTGCAACATGGCTCCCAGCCCCACAC
AGCCCTGCCCGCAGCAACATGCACCTTGCTCCTTCCCGGACACCCTCCTGGACACCAAC
GACACACAGCAAGCCTCACACGCCACCCTCCACCTCCTCCAACACCTCTTCGACACCCT
CAGCAGCCCCAGCACCCCCGCGCACTGGCTCCACACCGCACGCCACGACCTCCTCAAC
CAGCTCCAGCACCACATCCACCACCTCGAGCGCTGCTTCCCAGCCGACGCCACGCGCTT
CCACAGGCGAGGGCCCCGCAACCTTCACCTCGGCATCAACAAGTACTTCGGCTGCATC
CAACACTTCCTCCAGAACCACACCTACAGCCCCTGCGCCTGGGACCACGTCCGCCTCG
AGGCTCACGCCTGCTTCCAGCGCATCCACCGCCTCACCCGCACCATGCGCTAA

3.2 Biological analysis of interferon alpha gene in Yangzhou white geese

3.2.1 Analysis of signal peptide

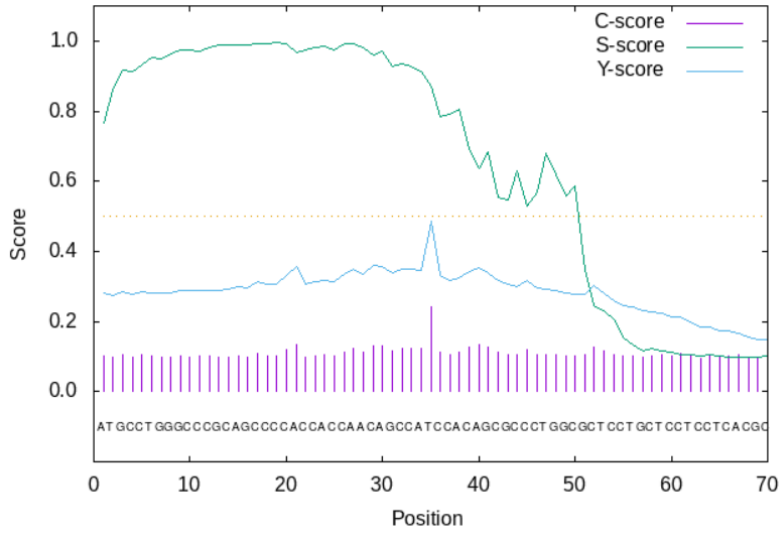
SignalP V3.0, an online application software, was used to predict the signal peptide site of yz goose IFN-α-1 (Figure 4.2). It can be seen from the figure that the signal peptide for predicting yz goose IFN-α-1 is located at the position of 1-33bp, and the shear site is between amino acids No. 34-35.



# Measure	Position	Value	Cutoff	signal peptide?
max. C	35	0.243		
max. Y	35	0.486		
max. S	19	0.995		
mean S	1-34	0.958		
D	1-34	0.741	0.450	YES

Figure 3.2 – Prediction of yz goose IFN-α-1 signal peptide site

SignalP V3.0, an online application software, was used to predict the signal peptide site of yz goose IFN-α-2 (Figure 4.3). It can be seen from the figure that the signal peptide for predicting yz goose IFN-α-2 is located at the position of 1-33bp, and the shear site is between amino acids No. 34-35.



# Measure	Position	Value	Cutoff	signal peptide?
max. C	35	0.243		
max. Y	35	0.486		
max. S	19	0.995		
mean S	1-34	0.958		
D	1-34	0.741	0.450	YES

Figure 3.3 – Prediction of yz goose IFN-α-2 signal peptide site

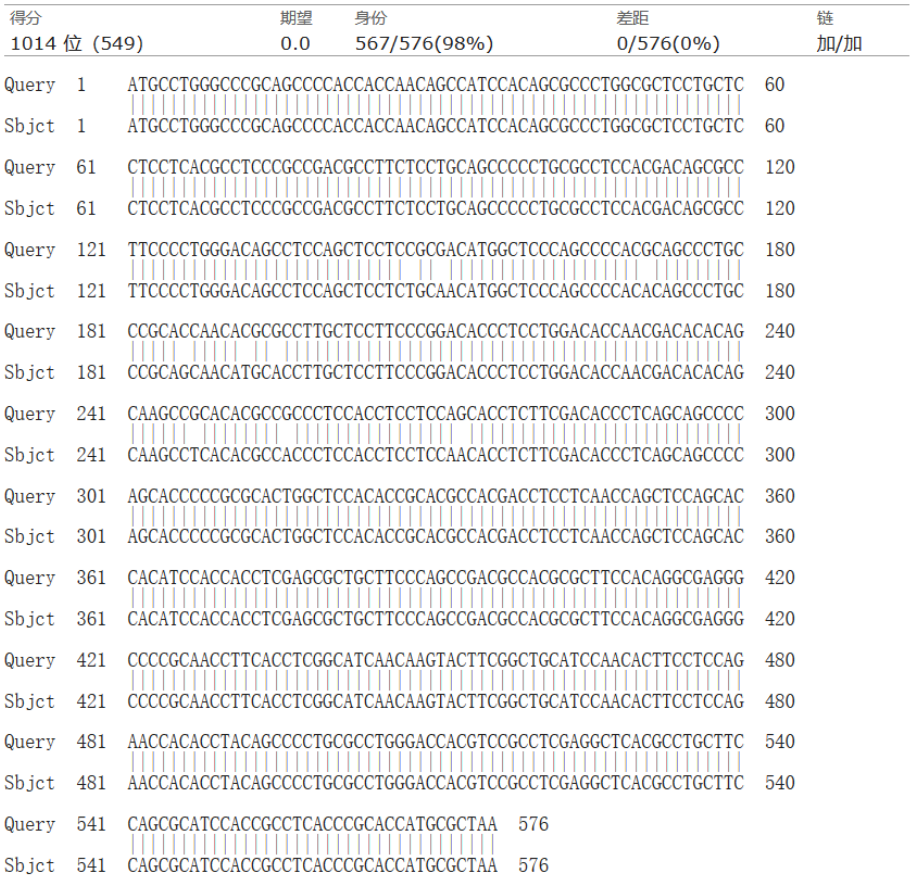
3.2.2 Homology analysis

The homology relationship between the two sequences and other goose sequences in the database was obtained by BLAST analysis in the NCBI website (Figure 4.4). As can be seen from the figure, the homology of yz goose IFNα-1 and yz goose IFNα-2 sequences with other goose interferon alpha sequences is above 90%.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
PREDICTED: Anser cygnoides interferon (LOC125180136), mRNA	Anser cygnoides	1059	1059	100%	0.0	99.83%	1913	XM_048048544.1
PREDICTED: Anser cygnoides interferon (LOC125180135), mRNA	Anser cygnoides	1059	1059	100%	0.0	99.83%	4070	XM_048048543.1
PREDICTED: Anser cygnoides interferon (LOC106045178), mRNA	Anser cygnoides	1059	1059	100%	0.0	99.83%	2427	XM_048048542.1
Anser cygnoides orientalis interferon alpha gene, complete cds	Anser cygnoides orientalis	1059	1059	100%	0.0	99.83%	576	EU022750.1
PREDICTED: Anser cygnoides interferon-like (LOC106045177), m…	Anser cygnoides	821	821	94%	0.0	93.78%	2049	XM_013195551.2

Figure 3.4 – Homology comparison between obtained sequences and other sequences in the database

The BLAST function of NCBI was also used to compare the two sequences obtained, and the homology of the two sequences could be compared (see Figure 4.5). The sequence similarity between the two sequences was 98%, with a difference of only 9bp.



得分	期望	身份	差距	链
1014 位 (549)	0.0	567/576(98%)	0/576(0%)	加/加

```
Query  1    ATGCCTGGGCCCGCAGCCCCACCACCAACAGCCATCCACAGCGCCCTGGCGCTCCTGCTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATGCCTGGGCCCGCAGCCCCACCACCAACAGCCATCCACAGCGCCCTGGCGCTCCTGCTC  60

Query  61   CTCCTCACGCCTCCCGCCGACGCCTTCTCCTGCAGCCCCCTGCGCCTCCACGACAGCGCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CTCCTCACGCCTCCCGCCGACGCCTTCTCCTGCAGCCCCCTGCGCCTCCACGACAGCGCC  120

Query  121  TTCCCCTGGGACAGCCTCCAGCTCCTCCGCGACATGGCTCCCAGCCCCACGCAGCCCTGC  180
            |||||||||||||||||||||||||| || ||||||||||||||||||| ||||||||||
Sbjct  121  TTCCCCTGGGACAGCCTCCAGCTCCTCTGCAACATGGCTCCCAGCCCCACACAGCCCTGC  180

Query  181  CCGCACCAACACGCGCCTTGCTCCTTCCCGGACACCCTCCTGGACACCAACGACACACAG  240
            ||||| ||||| || ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCGCAGCAACATGCACCTTGCTCCTTCCCGGACACCCTCCTGGACACCAACGACACACAG  240

Query  241  CAAGCCGCACACGCCGCCCTCCACCTCCTCCAGCACCTCTTCGACACCCTCAGCAGCCCC  300
            |||||| |||||||| |||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  241  CAAGCCTCACACGCCACCCTCCACCTCCTCCAACACCTCTTCGACACCCTCAGCAGCCCC  300

Query  301  AGCACCCCCGCGCACTGGCTCCACACCGCACGCCACGACCTCCTCAACCAGCTCCAGCAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AGCACCCCCGCGCACTGGCTCCACACCGCACGCCACGACCTCCTCAACCAGCTCCAGCAC  360

Query  361  CACATCCACCACCTCGAGCGCTGCTTCCCAGCCGACGCCACGCGCTTCCACAGGCGAGGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CACATCCACCACCTCGAGCGCTGCTTCCCAGCCGACGCCACGCGCTTCCACAGGCGAGGG  420

Query  421  CCCCGCAACCTTCACCTCGGCATCAACAAGTACTTCGGCTGCATCCAACACTTCCTCCAG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CCCCGCAACCTTCACCTCGGCATCAACAAGTACTTCGGCTGCATCCAACACTTCCTCCAG  480

Query  481  AACCACACCTACAGCCCCTGCGCCTGGGACCACGTCCGCCTCGAGGCTCACGCCTGCTTC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  AACCACACCTACAGCCCCTGCGCCTGGGACCACGTCCGCCTCGAGGCTCACGCCTGCTTC  540

Query  541  CAGCGCATCCACCGCCTCACCCGCACCATGCGCTAA  576
            ||||||||||||||||||||||||||||||||||||
Sbjct  541  CAGCGCATCCACCGCCTCACCCGCACCATGCGCTAA  576
```

Figure 3.5 – Homology comparison of the two obtained sequences

3.2.3 Evolutionary tree analysis

Through MEGA software, the sequences obtained in the experiment were compared with other sequences of geese, ducks, chickens and other birds with similar homology, and the evolutionary tree map was made to know the distance of the relatives between organisms (Figure 4.6).

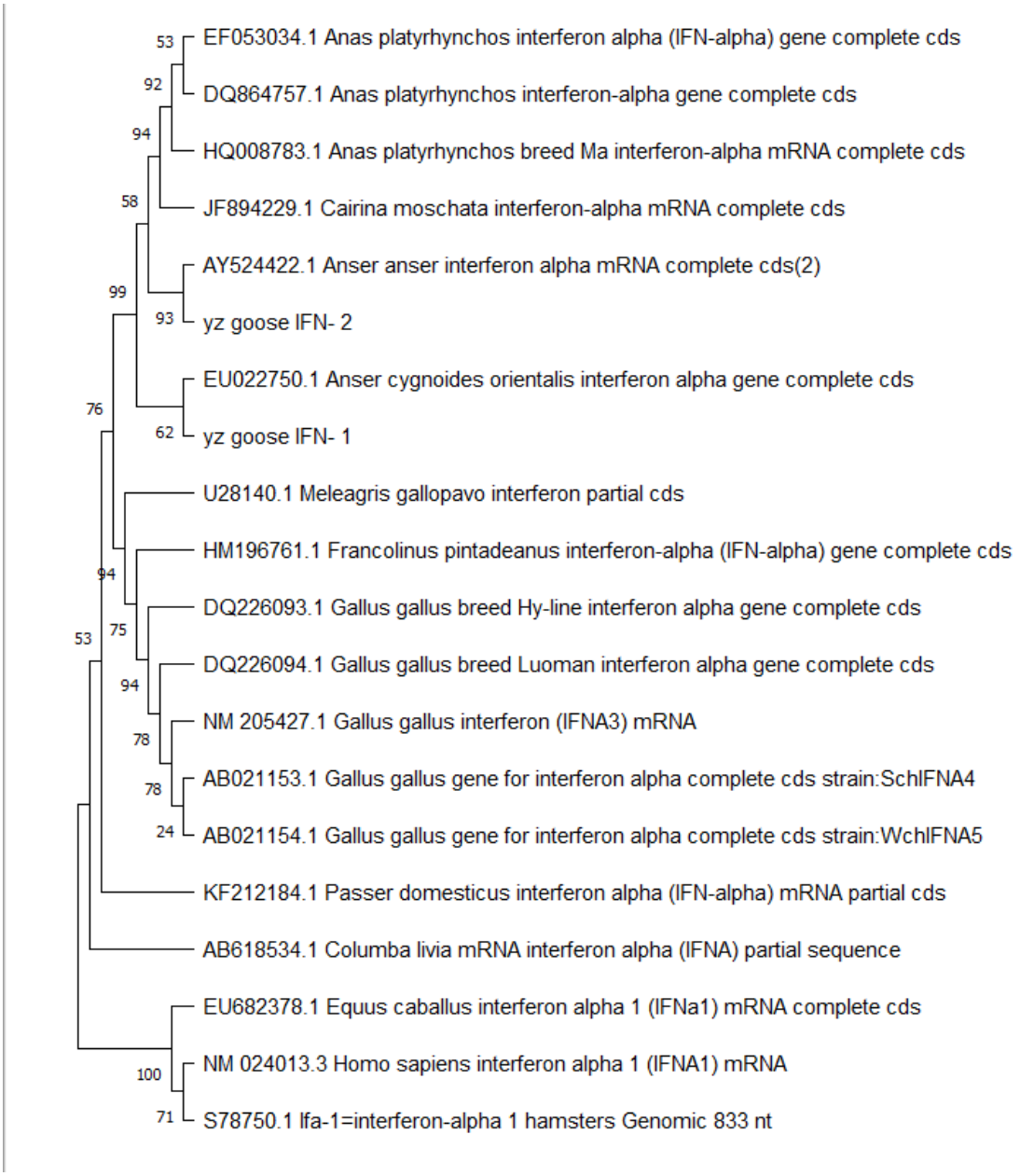


Figure 3.6 – Evolutionary tree

The sequence involving tree building is as follows:

We obtained two interferon alpha gene sequences of Yangzhou white goose: yz goose IFN α -1 and yz goose IFN α -2.

There are other published interferon alpha gene sequences: Chicken (DQ226094.1, U28140.1, DQ226093.1, NM_205427.1, AB021153.1, AB021154.1), duck (JF894229.1, EF053034.1, DQ864757.1, HQ008783.1), goose (AY5 24422.1, EU022750.1), other birds (HM196761.1, KF212184.1, AB618534.1) Mammals (NM_024013.3, S78750.1, EU682378.1)

3.2.4 Phosphorylation site prediction

The yz goose IFN- α -1 sequence obtained above was imported into the online software NetPhos 2.0 to predict the phosphorylation site, as shown in Figure 4.7. When the threshold is 0.5, the amino acid sequence of yz goose IFN- α -1 contains 17 potential phosphorylation sites.

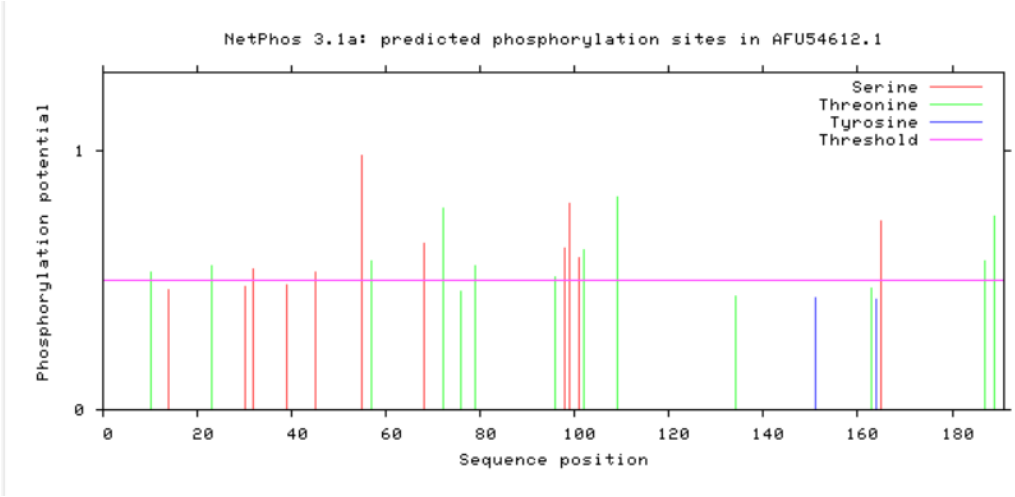


Figure 3.7 – Prediction of yz goose IFN- α -1 phosphorylation site

The yz goose IFN- α -2 sequence obtained above was imported into the online software NetPhos 2.0 to predict the phosphorylation site, as shown in Figure 4.8. When the threshold is 0.5, the amino acid sequence of yz goose IFN- α -2 contains 17 potential phosphorylation sites.

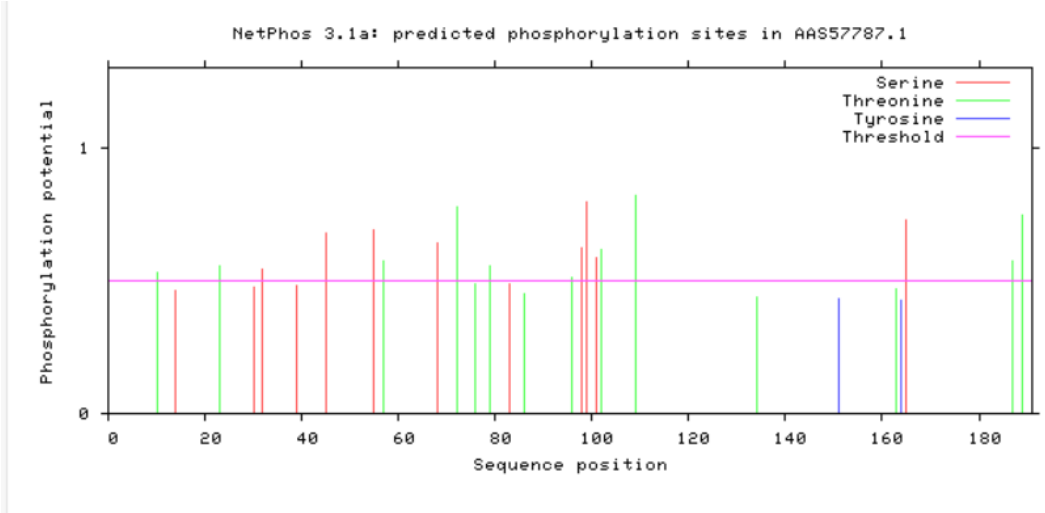


Figure 3.8 – Prediction of yz goose IFN- α -2 phosphorylation site

3.2.5 Hydrophobic analysis

The hydrophobicity of the known yz goose IFN-α-1 derived peptide chain was analyzed by online ProtScale software. The hydrophobicity phase measurement diagram (Figure 4.9) shows that the maximum value of hydrophobicity of yz goose IFN-α-1 gene protein is 2.856, and the minimum value is -2.656. The first hydrophobic zone is located at 10-30aa and is the most hydrophobic.

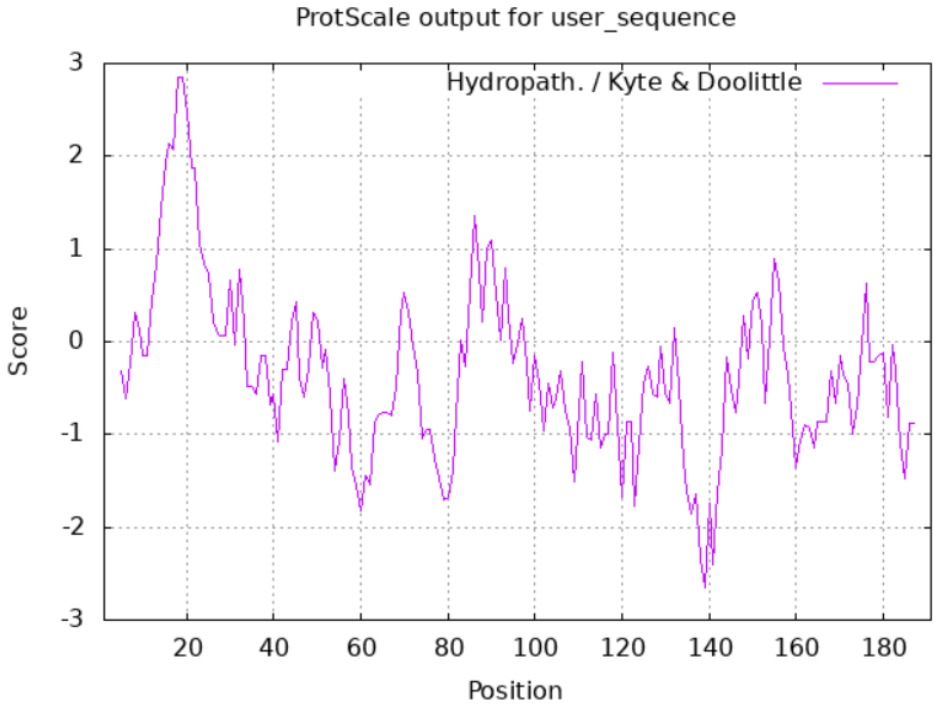


Figure 4.9 – Prediction of yz goose IFN-α-1 amino acid hydrophobicity

The hydrophobicity of the known yz goose IFN-α-2 derived peptide chain was analyzed by online ProtScale software. The hydrophobicity phase diagram (Figure 4.10) shows that the maximum value of hydrophobicity of yz goose IFN-α-2 gene protein is 2.856, and the minimum value is -2.656. The first hydrophobic zone is located at 10-30aa and is the most hydrophobic.

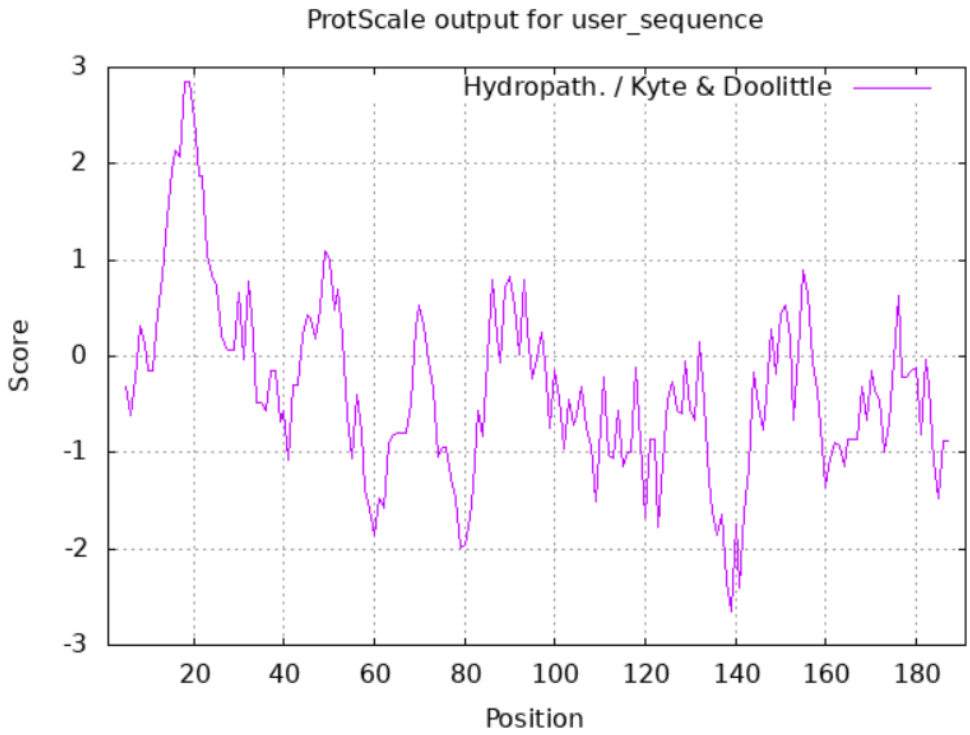


Figure 3.10 – Prediction of yz goose IFN- α -2 amino acid hydrophobicity

Summary of chapter III

1. In this study, we obtained a fragment of 576bp size encoding 191 amino acids with a molecular weight of about 21.6kDa, which is consistent with the expected size.

CONCLUSIONS

Domestic and foreign scholars in the cloning of interferon-based cytokines, canavanthin, plant hemagglutinin, etc. to induce and stimulate lymphocytes, and then further isolate, purify, collect cells, so as to clone the required genes. This experiment preliminarily tried to use different methods to extract RNA from lymphocytes, that is, the total RNA was successfully extracted from peripheral blood lymphocytes of Yangzhou White goose without cell induction culture, which greatly shortened the experiment time and improved the experiment efficiency.

On the basis of the extraction of high quality lymphocyte RNA, we performed RT-PCR, that is, the F1/R1 cDNA product was cloned and amplified by primers, and the interferon α gene sequence of Yangzhou White goose was obtained. According to the analysis, the successful cloning of interferon α gene of Yangzhou White geese may be due to the fact that the 5 Yangzhou white geese used in the experiment were vaccinated with some necessary vaccines during the breeding process, resulting in a large amount of interferon α mRNA expression. Thus, this uninduced culture approach saves time for RNA extraction, reduces the possibility of cell contamination during cell culture, and also provides a reference for cloning other types of interferons.

In this study, we obtained a fragment of 576bp size encoding 191 amino acids with a molecular weight of about 21.6kDa, which is consistent with the expected size. The obtained alpha-interferon sequences of two Yangzhou white geese were much more similar to those of chicken and duck than to those of human and other animals, which indicated that the alpha-interferon gene was evolutionally-conserved and specific between species, and the closer the genetic

relationship was, the more significant the homology relationship of the alpha-interferon sequence was.

Phosphorylation is one of the most important covalent modifications in vivo. When proteins are phosphorylated, they become charged, which changes the structure and further changes the activity of proteins. The yz goose IFN α-1 and yz goose IFN α-2 sequences obtained above were imported into the online software NetPhos 2.0 for the prediction of phosphorylation sites. When the threshold was set at 0.5, the two amino acid sequences each contained 17 potential phosphorylation sites.

Hydrophobicity analysis has important guiding significance for the analysis of transmembrane region, secondary structure and spatial structure of globulin. Hydrophobicity helps proteins fold inward to form secondary structures, and further form domains and tertiary structures. At the same time, hydrophobicity also helps the protein form α helix, ensuring its stability. The hydrophobicity of the known yz goose IFN α-1 and yz goose IFN α-2 derived peptide chains was analyzed by online ProtScale software. The hydrophobicity of yz goose IFN α-1 and yz goose IFN α-2 gene protein was 2.856, and the minimum value was -2.656. The first hydrophobic zone is located at 10-30aa and is the most hydrophobic.

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