 DOI: 10.5114/ceji.2014.42118

Molecular cloning and sequencing analysis of the interferon β from *Coturnix*

BEI ZHENG, WEI-SHAN CHANG

Shandong Agriculture University, Taian, PR China

Abstract

One pair of primers was designed according to Gallus and Meleagris gallopavo interferon β (IFN-β) sequences published in GenBank. The primers and RNA extraction from the spleen of Coturnix were used to amplify Coturnix IFN-β cDNA by real-time polymerase chain reaction (RT-PCR). The product was cloned into pEasy-T1 vector. Evaluating recombinant plasmid by PCR and restriction enzyme digestion. Sequence the cloning sequences, comparing the sequencing results by NCBI. We successfully got a Coturnix IFN-β partial sequence. The sequence was subtyped and put to homologous analysis. The results suggested the homology of IFN-β gene of Coturnix and chicken (88.7%), the homology of IFN-β gene of Coturnix and Anas platyrhynchos (72.5%), the homology of IFN-β sequence registered in GenBank. The analysis of the genetic tree showed that the relationship of Coturnix and chicken IFN-β had a high homology. It can be seen that in this study we successfully got a partial sequence of IFN-β of quail.

Key words: *coturnix, IFN-β, gene cloning, gene sequence analysis.*

(Centr Eur J Immunol 2014; 39 (1): 25-29)

Introduction

The immune response against viral pathogens includes specific and non specific mechanisms. Cytokines are peptides which can play a role in the non specific immunity [1]. Interferon (IFN) is a kind of highly active multifunctional glycoprotein, and it is an important cytokine with a broad range of biological activities [2]. Interferon is a secretory glycoprotein produced by the biological cells when it is subjected to the influence of the virus or other inducing agent [3]. Interferon has many kinds of bioactivity, such as antiviral activity, immune regulation and so on [4]. Interferon is an important part of the body's defense system. Interferon has broad-spectrum resistance [5]. When interferon acts on the body's organic tissue cells, it can make it obtain the ability to resist a variety of viruses and microbes [6]. Interferon has strict selectivity for a somatic cell, and has a relative species specificity. But the specificity is relative, not absolute [7]. Interferon can be divided into type I and type II [8]. Interferon of type I is a product of many gene families, including 14-20 interferon α genes, 1 kind of interferon β gene; interferon of type α contains only one family member, namely the interferon γ [9]. Type I interferons are the most effective antiviral cytokines. Type I interferon genes are located on chromosome 9, and are segregated in a "modern" and an "ancestral" group with distinctive effects on the cells. Interferons α are represented by a large family of structurally related genes while the IFN-β is encoded by a single gene [1, 10, 11].

The meat and eggs of *Coturnix* were delicious in China and known as "animal's ginseng". Currently, the number of *Coturnix* raised is about 200 million in China, which is 1/5 of the world production. *Coturnix* is an important part of the economic animal production in China. But the occurrence of avian influenza and New-castle diseases posed a serious threat to the production of *Coturnix*. Establishing the *Coturnix* immune mechanism, looking for a new and efficient security immune route makes it necessary to breed *Coturnix*. By contrast, the research on animal interferon lags behind. There/This is still the main stay/subject? in basic research and clinical trials, and most concentrating on a few animals such as pigs, chicken, fish. But a big progress has been made in recent years. There are commercial interferon of pigs, dogs, chicken and recombinant interferon product coming to the market. However, there are very few research reports about the *Coturnix* interferon.

Material and methods

Sample

Spleen was collected from *Coturnix*. *Coturnix* was brought as a live mature *Coturnix* from a common farm and kept under inspection for 5 days to be sure that it is

Correspondence: Bei Zheng, Shandong Province, Taian, Daizong Street No.61, 271000 Taian, PR China, e-mail: 302981954@qq.com

free from any clinical infection, then the sample was collected and stored at –20°C.

Primer design

We downloaded the complete IFN-β mRNA sequences of the *Gallus* (NM_001024836) and *Meleagris gallopavo* (U28140) from GenBank. The sequence was aligned using the ClustalW application (Dnastar software) where we designed primers match with the alignment of the sequences.

Amplification of cDNA sequence

Total RNA from *Coturnix* spleen was extracted by Trizol (TransGen) according to the manufacturer's protocol and was reverse transcribed to cDNA. Reverse transcription is processed with a Prime script real-time (RT) reagent kit (TaKaRa) according to the manufacturer's instructions in 10 μl reaction system. The reagent was blended and put in the polymerase chain reaction (PCR) Amplifier at 37°C for 15 min and inactivated at 85°C for 5 s.

The PCR using the reverse transcription product was performed to amplify the target gene using specific primers. Polymerase chain reaction mixtures contained 50 pmol for each forward and reverse primer, 1 μl template cDNA, 200 μM dNTP mix and 0.2 μl 5 U Ex Taq polymerase (TransGen) in 1× Ex buffer up to 25 μl with ddH_2O. The PCR was processed in the PCR Amplifier as follows: initial denaturation at 94°C for 5 min, 35 cycles at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, final extension at 72°C for 10 min. The PCR amplicons were verified by 0.8% agarose gel electrophoresis at 120 mA for 30 min and products were visualized by staining with DuRed (Fanbo Biochemicals), we checked the band and got the images with GelDoc™ XR+system (Bio-RAD, USA).

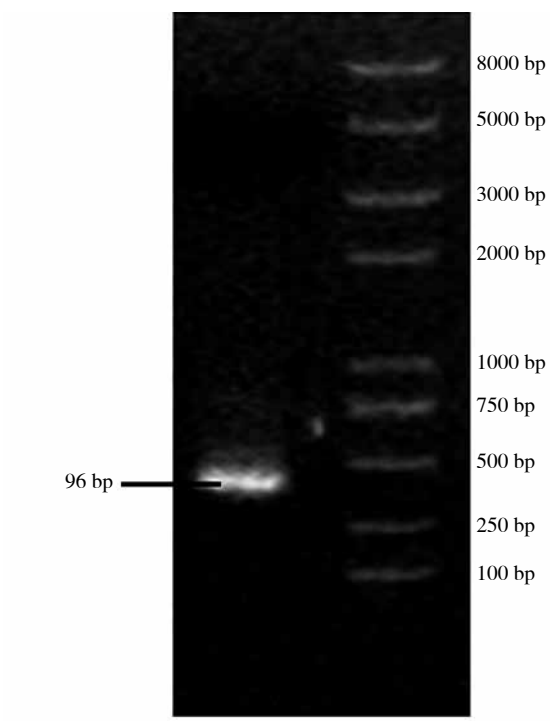

Fig. 1. Real-time PCR amplification of interferon β

Construction and identification of recombinant plasmid with interferon β

The Gel Extraction Kit was used to purify and recycle the PCR products. With reference to the instruction of cloning vector pEasy – T1, in order to join the components in the 10 μl reaction system, the following steps were taken. After gently blending, we put it in the PCR instrument to connect 25°C 10 min for conversion. Getting 5 μl connecting product into fresh preparative DH5a cells, coating converted products on the LB/Amp plate evenly under aseptic conditions. Putting the tablet at 37°C thermostat in 12 h and picking a single colony after culturing, preparing and evaluating plasmid DNA. Picking a single colony from the above overnight culture tablet randomly, vaccinating in 5 ml LB liquid medium containing Amp. Shaking culture overnight in 37°C, preparing plasmid DNA using GenEluteTM High Performance Plasmid Kits. Evaluating recombinant plasmid by PCR and restriction enzyme digestion. In order to further determine the resulting clone, recombinant plasmid identified as positive clones after the above steps was sent to Shanghai Sangon Biological Company for DNA sequencing. Sequencing results and the nucleotide sequence registered in GenBank was subtyped and put to homologous analysis.

Interferon-β nucleotide sequence homology comparison and the analysis of the phylogenetic tree

The sequence of the *Coturnix* IFN-β mRNA was blast in the GenBank using nucleotide blast and the translated amino acids were also blast by protein blast to check whether the new sequence is related to any other cloned gene.

The sequence of *Coturnix* IFN-β was compared with the known IFN-β mRNA sequences from different species which were downloaded from the GenBank and aligned by Dnastar software. Sequencing results of *Coturnix* IFN-β and the nucleotide sequence of *Gallus* (NM_001024836), *Meleagris gallopavo* (U28140), *A. platyrhynchos* (X84764), *Mus musculus* (NM_010510), *Callithrix jacchus* (AB571244), *Ovis aries* (NM_001009392), *Danio rerio* (NM_001261449), *Papio anubis* (NM_001173536), *Macaca mulatta* (NM_001042733), *Homo sapiens* (NM_000600), *Mus musculus* (NM_010510), *Canis lupus familiaris* (NM_001135787), *Sus scrofa* (NM_001003923), *Oryctolagus cuniculus* (NM_001082064) registered in GenBank was subtyped and put to homologous analysis. The phylogenetic tree was constructed from the gene alignments and the amino acid alignments.

Results

Interferon gene cloning results

Total RNA was extracted from *Coturnix* spleen cells, then amplified to products by RT-PCR. The PCR products underwent electrophoresis in 0.8% agarose gel. There was a specific band in 400 bp with expectations (Fig. 1).

Interferon β sequence analysis

The partial mRNA sequence of *Coturnix* IFN-β consists of 396 nucleotides. The homology of IFN-β gene of *Coturnix* and *Gallus* was 88.7%. The consensus cDNA sequence showed 72.5% identity with *A. platyrhynchos* and 71.5% identity with *Meleagris gallopavo* as compared with the nucleotide sequence registered in GenBank (Figs. 2, 3). The

	1	2	3	4	5	6	7	8	9	10	11	12	13		
1		93.1	34.0	33.6	33.0	33.9	31.3	92.8	33.7	64.9	93.7	36.3	38.0	**1**	Callithrix jacchus seq.
2	7.3		34.6	34.8	34.9	34.8	32.2	99.2	35.5	64.1	97.5	36.3	38.0	**2**	Macaca mulatta seq.
3	165.3	158.6		70.8	43.8	44.6	62.1	34.6	35.7	35.5	35.5	44.0	45.4	**3**	Ovis aries seq.
4	171.5	157.6	37.6		41.4	43.8	64.8	34.9	37.7	34.2	35.7	43.3	43.4	**4**	Canis lupus familiaris seq.
5	168.3	151.9	103.9	114.3		41.4	38.2	34.9	29.7	29.9	30.1	67.0	72.5	**5**	A. platyrhynchos IFN gene seq.
6	165.3	156.5	100.9	103.7	34.3		41.7	34.6	34.9	36.5	34.8	72.0	71.5	**6**	Meleagris gallopavo interferon, partial
7	203.4	186.2	54.4	49.1	130.5	113.9		32.2	34.0	33.3	31.7	41.7	43.9	**7**	Mus muculus seq.
8	7.6	0.8	159.6	156.7	151.8	157.9	197.7		35.5	64.1	97.7	36.3	38.0	**8**	Papio anubis seq.
9	178.4	160.1	152.9	137.1	213.7	151.9	169.7	160.1		35.6	33.8	33.4	35.2	**9**	Danio rerio seq.
10	48.9	50.5	149.8	161.8	204.0	141.3	166.8	50.3	148.2		66.9	35.0	35.1	**10**	Oryctolagus cuniculus seq.
11	6.7	2.5	150.1	149.2	203.0	155.3	193.0	2.4	178.1	44.6		36.6	38.3	**11**	Homo sapiens seq.
12	149.9	152.7	103.1	106.4	43.8	35.4	113.8	151.0	168.3	154.8	148.1		88.7	**12**	Gallus seq.
13	133.4	134.6	97.5	105.5	34.6	36.2	103.8	134.2	150.2	150.2	132.0	12.5		**13**	Coturnix seq.
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**		

Fig. 2. Homology comparisons of nucleotide sequence among chickens, quails, ducks and other animals interferon β from GenBank

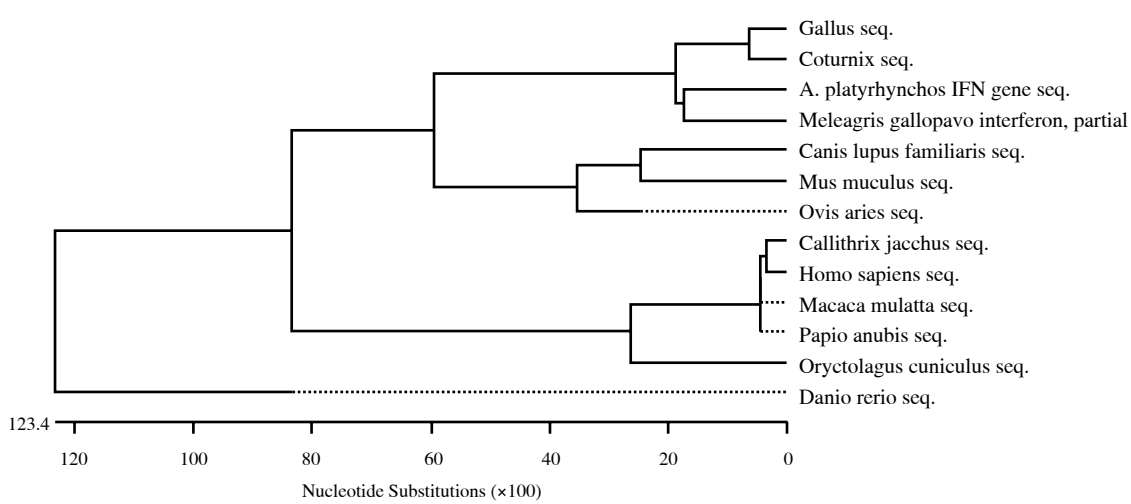

Fig. 3. The evolutionary tree of quail compared with other animals from GenBank

analysis of the genetic tree confirmed that the new sequence is probably a homolog to gallus IFN-β while the relationship of *Coturnix* and *Gallus* IFN-β had high homology.

Interferon β amino acid sequence analysis

The predicted protein encoded by *Coturnix* IFN-β mRNA sequence is composed of 132 amino acids. The predicted amino acid sequence of *Coturnix* IFN-β was compared with the IFN-β of *Gallus* and *Meleagris gallopavo* using DNAMAN (Fig. 4). The homology of IFN-β amino acid of *Coturnix* and *Gallus* was 78.8%, the homology of IFN-β amino acid of *Coturnix* and *Meleagris gallopavo* was 56.8% as compared with the amino acid sequence (Figs. 5, 6). The analysis of the phylogenetic tree

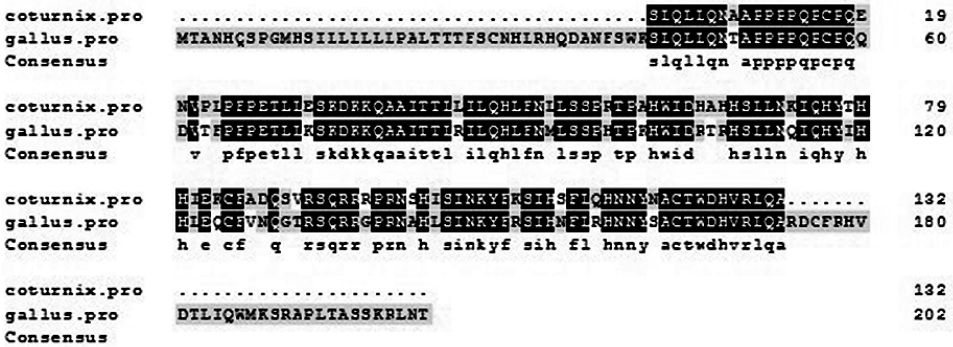

Fig. 4. Results of multiple alignments of amino acid sequences

	1	2	3	4	5	6	7	8	9		
1		10.7	13.9	11.2	15.0	98.6	10.2	12.2	9.8	**1**	Papio anubis pro.
2	476.0		20.7	62.6	9.8	10.7	49.7	19.6	19.1	**2**	Canis lupus familiaris pro.
3	357.0	238.0		19.0	10.0	13.9	21.7	53.4	78.8	**3**	Gallus pro.
4	447.0	51.5	258.0		10.4	11.2	62.0	17.4	19.8	**4**	Sus scrofa pro.
5	328.0	524.0	517.0	493.0		15.5	8.2	7.4	10.2	**5**	Danio rerio pro.
6	1.4	476.0	357.0	447.0	317.0		10.2	12.2	9.8	**6**	Macaca mulatta pro.
7	504.0	80.6	226.0	52.5	688.0	504.0		20.1	22.1	**7**	Ovis aries pro.
8	407.0	252.0	71.1	284.0	819.0	407.0	244.0		56.8	**8**	Meleagris gallopavo pro.
9	524.0	258.0	25.0	248.0	504.0	524.0	221.0	63.3		**9**	Coturnix pro.
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		

Fig. 5. Homology comparisons of amino acid sequence among *Gallus*, *Coturnix*, and *Meleagris gallopavo*

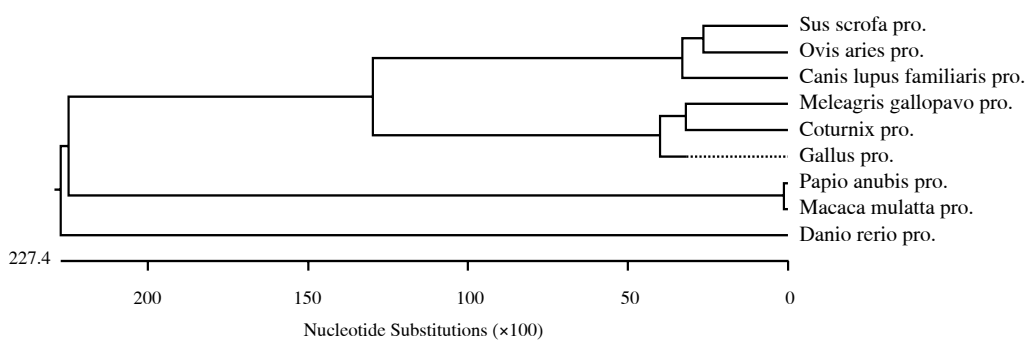

Fig. 6. The evolutionary tree of quail as compared with other animals

showed that the relationship of *Coturnix* and *Gallus* IFN-β had a high homology.

Conclusions

Temporarily quail β interferon gene sequence is not published at home and abroad. This is the first study to focus on the sequence of *Coturnix* IFN-β. We successfully got a *Coturnix* INF-β partial sequence. The sequence was subtyped and put to homologous analysis. Our results proved that the relationship of *Coturnix* and *Gallus* IFN-β had a high homology.

The predicted protein encoded by *Coturnix* IFN-β mRNA sequence has a high homology to *Gallus*. This may have a role in the difference of the immune response against pathogen between *Coturnix* and *Gallus*. The result can be a reference for further research and practical application.

Authors declare no conflict of interest.

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