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Phylogenetic and topological analyses of the bovine interferon-induced transmembrane protein (IFITM3)

YONG-CHAN KIM^{1,2} and BYUNG-HOON JEONG^{1,2*} 

¹ Korea Zoonosis Research Institute, Jeonbuk National University, 820-120 Hana-ro, Iksan, Jeonbuk 54531, Republic of Korea

² Department of Bioactive Material Sciences and Institute for Molecular Biology and Genetics, Jeonbuk National University, Jeonju, Jeonbuk 54531, Republic of Korea

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RESEARCH ARTICLE



ABSTRACT

Interferon-induced transmembrane protein 3 (IFITM3) plays a pivotal role in antiviral capacity in several species. However, to date, investigations of the IFITM3 protein in cattle have been rare. According to recent studies, interspecific differences in the IFITM3 protein result in several unique features of the IFITM3 protein relative to primates and birds. Thus, in the present study, we investigated the bovine IFITM3 protein based on nucleotide and amino acid sequences to find its distinct features. We found that the bovine *IFITM3* gene showed a significantly different length and homology relative to other species, including primates, rodents and birds. Phylogenetic analyses indicated that the bovine *IFITM3* gene and IFITM3 protein showed closer evolutionary distance with primates than with rodents. However, cattle showed an independent clade among primates, rodents and birds. Multiple sequence alignment of the IFITM3 protein indicated that the bovine IFITM3 protein contains 36 bovine-specific amino acids. Notably, the bovine IFITM3 protein was predicted to prefer inside-to-outside topology of intramembrane domain 1 (IMD1) and inside-to-outside topology of transmembrane domain 2 by Tmpred and three membrane embedding domains according to the SOSUI system.

KEYWORDS

cattle, interferon-stimulated gene, IFITM3, topology

INTRODUCTION

Interferon-induced transmembrane protein 3 (IFITM3) is a kind of small interferon stimulated gene (ISG) and a member of the IFITM protein family (Everitt et al., 2012; Allen et al., 2017; Kim and Jeong, 2017; Yanez et al., 2020). The IFITM3 protein is a potent viral restriction factor, and expression of the IFITM3 protein is strongly elevated by type I and II interferons, which are mediated by viral sensors including RIG-I and MDA5 or the detection of viral genetic materials by members of the Toll-like receptor family (Feeley et al., 2011; Bedford et al., 2019). The IFITM3 protein potently inhibits a number of viruses, such as influenza A viruses (IAVs), Ebola virus (EBOV), Marburg virus (MARV), severe acute respiratory syndrome coronavirus (SARS-CoV), dengue virus (DEV), West Nile virus (WNV), Zika virus (ZIKV) and foot-and-mouth disease virus (FMDV) (Perreira et al., 2013; Zani and Yount, 2018).

The human IFITM3 protein is composed of 5 major domains including the N-terminal domain (NTD), intramembrane domain (IMD), conserved intracellular loop (CIL), transmembrane domain (TMD) and C-terminal domain (CTD). Among these domains, IM1 and CIL define cluster of differentiation 225 (CD225), which plays a pivotal role in the inhibition of IAV and DEV replication in the A549 cell line. In addition, the human IFITM3 protein contains the endocytic Yxx ϕ cargo sorting motif in which Y is tyrosine, X could be any

*Corresponding author. Tel.: +82 63 900 4040; fax: +82 63 900 4012
E-mail: bhjeong@jbnu.ac.kr

amino acid, and ϕ represents an amino acid residue with a bulky hydrophobic side chain; 20-YEML-23 is found in the NTD, and this signal motif in the late endosome contributes to the correct localisation of the IFITM3 protein, which protects from viral hemifusion to the host (Jia et al., 2014). Furthermore, 75-F and 78-F in IMD1 and R-87 and Y99 in the CIL, which are conserved in primates and rodents, are necessary for the antiviral defence capacity of the IFITM3 protein (John et al., 2013). However, the avian IFITM3 protein shows significantly different features relative to those of primates and rodents. Avian IFITM3 showed very low homology of the NTD and CTD domains to those of primates and rodents (Kim et al., 2019). In addition, mutagenesis of the endocytic Yxx ϕ cargo sorting motif in the duck IFITM3 protein did not affect correct localisation in the late endosome (Blyth et al., 2016). Furthermore, the avian IFITM3 protein prefers the outside-to-inside topology of IMD1 and the inside-to-outside topology of TMD2. Since

Table 1. Detailed information on the interferon-induced transmembrane protein 3 gene (*IFITM3*) sequences analysed in this study

Scientific name	Common name	GenBank numbers	
		Nucleotides	Amino acids
<i>Homo sapiens</i>	Human	10410	AFF60355.1
<i>Cercopithecus albogularis</i>	Sykes' monkey	KU570014.1	ANJ01447.1
<i>Mus musculus</i>	Mouse	66141	NP_079654.1
<i>Rattus norvegicus</i>	Rat	361673	NP_001129596.1
<i>Gallus gallus</i>	Chicken	770612	NP_001336990.1
<i>Anser cygnoides</i>	Goose	KX594327.1	AQM74179.1
<i>Bos taurus</i>	Cattle	777594	NP_863657.1

this topology of the avian IFITM3 protein shows the opposite direction compared to that of primates and rodents, the IFITM3 protein of primates and rodents prefers the inside-to-outside topology of IMD1 and the outside-to-inside topology of TMD2 (Kim et al., 2019). Information on the interspecific differences of the immune system is necessary to construct species-specific breeding and surveillance systems. Thus, investigation of the bovine IFITM3 protein will be helpful to understand unique features of the bovine immune system.

In the present study, we carried out sequence alignment of the *IFITM3* gene and IFITM3 protein among species and found bovine-specific features based on sequences of the *IFITM3* gene and IFITM3 protein using ClustalW2 (Sievers and Higgins, 2014). In addition, we analysed nucleotide sequences of the *IFITM3* gene and amino acid sequences of the IFITM3 protein by a phylogenetic approach using MEGA X (Kumar et al., 2018). Furthermore, we performed topology analysis of the bovine IFITM3 protein using TMpred and SOSUI and compared the topology of the IFITM3 protein among species (Hirokawa et al., 1998; Ikeda et al., 2003).

MATERIALS AND METHODS

Sequence alignment of the *IFITM3* gene and IFITM3 protein

Alignments of nucleotide and amino acid sequences were performed using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalo>). Nucleotide and amino acid sequences of the IFITM3 proteins in human, monkey, mouse, rat, chicken, goose and cattle were obtained from GenBank at the

Table 2. The results of sequence alignment of the *IFITM3* gene among 7 species

Species		Matches	Mismatches	Gap	Sequence identity
Cattle	Human	329	73	39	74.60%
	Sykes' monkey	319	83	39	72.34%
	Mouse	298	116	27	67.57%
	Rat	303	111	27	68.71%
	Chicken	232	182	27	52.61%
	Goose	280	146	15	63.49%
Human	Sykes' monkey	382	20	0	95.02%
	Mouse	290	100	12	72.14%
	Rat	285	105	12	70.90%
	Chicken	201	189	12	49.90%
	Goose	219	159	24	54.47%
Sykes' monkey	Mouse	290	100	12	72.14%
	Rat	285	105	12	70.90%
	Chicken	227	163	12	56.56%
	Goose	221	157	24	55.01%
Mouse	Rat	376	38	0	90.82%
	Chicken	230	184	0	55.56%
	Goose	223	179	12	53.81%
Rat	Chicken	225	189	0	54.46%
	Goose	222	180	12	53.54%
Chicken	Goose	331	71	12	79.91%

Cattle: 441 bp; human: 402 bp; Sykes' monkey: 402 bp; mouse: 414 bp; rat: 414 bp; chicken: 414 bp; goose: 426 bp.



National Center for Biotechnology Information (NCBI). Detailed information is described in Table 1.

Phylogenetic analyses

DNA sequences of the *IFITM3* gene and amino acid sequences of the IFITM3 protein in human, monkey, mouse,

rat, chicken, goose and cattle were used for phylogenetic analysis. These sequences of the *IFITM3* gene were obtained from GenBank at NCBI. Detailed information is described in Table 1. A neighbour-joining phylogenetic tree was drawn using MEGA X software. A bootstrap test from 1,000 replicates was applied to estimate the confidence level of the

Human	atgaatcacactgtccaaaaccttctctctcctgtcaacagtgccagcccccaactat	60
Monkey	atgaaccacacggtccaaaaccttctctctcctgtcaacagcgccagcccccaactat	60
Mouse	atgaaccacacttctcaagccttcatcaccgctgccagtgaggacagccccaaactac	60
Rat	atgaaccacacttctcaagccttctgtgaacgctgccactgggggacacccccaaactac	60
Chicken	atg-----gagcgggtacgctctcgggtccgggagtcaccacgtat	42
Goose	atg-----gagcggaccagcagccccgggagtttcgctgccccctac	42
Cattle	atgaaccgacatcccagccttgttcaactggggcccaaggggcgtgccccagcctat	60
	*** *	
Human	gagatgctcaaggaggacacgaggtggctgtgctgggggcccccaaacctgctccc	120
Monkey	gagattctcaaggaagacacgaggtagctgtgctgggggcccccaaacctgctccc	120
Mouse	gaaagaatcaaggaagaatatgaggtggctgagatgggggacccgacggatcggcttct	120
Rat	gaaagaatcaaggaagaatatgaggtgtctgaactgggggacccccagggatcggcttct	120
Chicken	gaaccct-----gatggcgggagtgacatggagggaagaccgcagc	87
Goose	gagcctct-----ggtggagggttagacatggagaacatgtcccgaagc	87
Cattle	gaggtgcttaaggaggacacgaggtggcgtgctgggggccccagagccagggcgccc	120
	** *	
Human	ccgacgtccaccgtgatccacatccgcagcgagacctccgtgccccaccatgtcgtctgg	180
Monkey	ccgacgtccaccgtgatccacatccgcagcgagacctccgtgccccaccatgtcgtctgg	180
Mouse	gtcagaactactgtgatcaacatgccagagaggtgctcgggtgcctgaccatgtggtctgg	180
Rat	gtcagaactaccgtgatcaacatgccagagaggtctctgtgcctgaccatgtggtctgg	180
Chicken	acggtggtgacggtggagagcgc-----cctggtgcctcctccccgacccacctggcctgg	144
Goose	gtcgtggtgccccgtggaggcccccccggtgcagcccccccccgacccacctggcctgg	147
Cattle	ctgacgaccaacggtgatcaacatccgcagcgacaccgacctgccccaccatcgtgtgg	180
	* ** *	
Human	tcctgttcaaacaccttctcatgaacacctgctgcctgggctcatagcattcgcctac	240
Monkey	tcctgttcaaacaccttctcatgaacacctgctgcctgggctcatagcattcgcctac	240
Mouse	tcctgttcaatacacttctcatgaacttctgctgcctgggctcatagcattcgcctac	240
Rat	tcctgttcaatacacttctcatgaacttctgctgcctgggctcatagcattcgcctac	240
Chicken	tcgctgtgacaccgctgtacgcaacgctctgctgcctcggcttctgggctcgtcttc	204
Goose	tcgctgtgctccacgctgtacgcaacgctctgctgcctgggcttctgggctcgtcttc	207
Cattle	tcctgttcaaacaccttctcatgaactggtgctgcctgggctcgtgattcgcctac	240
	** ** ** *	
Human	tcctggaagtctagggaaggaagatggtggcgacgtgacggggccccagggcctatgcc	300
Monkey	tcctggaagtctagggaaggaagatggtggcgacgtgactggggccccagggcctatgcc	300
Mouse	tcctggaagtctagggatcggaagatggtgggtgatgtgactggagccccagggcctacgcc	300
Rat	tcctggaagtctagggatcggaagatggtgggtgatgtgactggagccccagggcctacgcc	300
Chicken	tcctggaagtccagggatcgcaaaagtccctgggtgactacagcggggcgctcagctatggc	264
Goose	tcctgcaagtccagggatcgcaaaagtccctggcgactacagcggggcgctcagctacggc	267
Cattle	tcctggaagtctagggaacgggaagatggtcggcgacatcactggggccccagagctacgcc	300
	** *	
Human	tcaccgccaagtgcctgaacatctggccctgattctgggcatcctcatgaccattctg	360
Monkey	tcaccgccaagtgcctgaacatctggccctgatttctgctcatcctcatgaccattctg	360
Mouse	tcactgctaagtgcctgaacatcagcacttggctcctcagcattcctgatggtgttatc	360
Rat	tcactgccaagtgcctgaacatcagcacttggctcctcagcattcctcatggttatttc	360
Chicken	tcactgccaagtgcctgaacatcagcacttggctcctcagcattcctcatcattcctc	324
Goose	tcaccgccaagtgcctgaacatcagcacttggctcctcagcattcctcctcgtcattcctc	327
Cattle	tcaccgccaagtgcctgaacatctgctcctggctcctgggcatcctctgactgtcgtc	360
	***** *	
Human	ctcatcgtcatcccagtgctgatcttccaggcctatggatag-----	402
Monkey	ctcattgtcatcccagttatgctcctccaggcctatcaatag-----	402
Mouse	accattgttagtgcctcatcattgttcttaacgctcaaaaacctcactta-----	414
Rat	actattgttactgtcgtcatcattgtccttaacgctcctcgtctccagactga-----	414
Chicken	atcatcgccctggttggcagtgaccatcatggtggccaacatcctcaaccaccagcag	384
Goose	atcgtcgccctgatcgcagcggccaccatcaccatagtcacactgctcaaccaccagcag	387
Cattle	ctcatcgtcctcgtgtccaccggctcctcctgatgatcgttcaggcagctcggagctcatg	420
	* * *	
Human	-----	402
Monkey	-----	402
Mouse	-----	414
Rat	-----	414
Chicken	caacccccgaattcatt-----ggaccacttag	414
Goose	cagcagcagggcaaccaccttctcggccccacatag	426
Cattle	cagaactacggaggccactag-----	441

Fig. 1. Comparison of the *IFITM3* gene sequences in humans, monkey, mouse, rat, chicken, goose and cattle. Sequence alignment includes humans (*Homo sapiens*, Gene ID: 10410), Sykes' monkey (*Cercopithecus albogularis*, Gene ID: KU570014.1), mouse (*Mus musculus*, Gene ID: 66141), rat (*Rattus norvegicus*, Gene ID: 361673), chicken (*Gallus gallus*, Gene ID: 770612), goose (*Anser cygnoides*, Gene ID: KX594327.1) and cattle (*Bos taurus*, Gene ID: 777594). Detailed information for the *IFITM3* gene sequences is described in Table 1. Nucleotide sequences were aligned using ClustalW2. Asterisks indicate identical nucleotides among species



branching patterns of the neighbour-joining tree. The evolutionary distances were computed by using the Maximum Composite Likelihood method.

Topology prediction of the IFITM3 protein

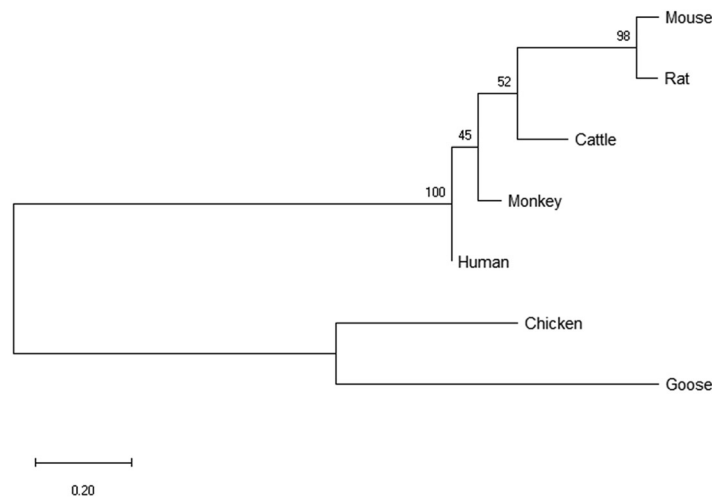
The topologies of the IFITM3 protein were predicted by TMpred (http://www.ch.embnet.org/software/TMPRED_form.html) and SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html).

RESULTS

Nucleotide sequence comparison of the *IFITM3* gene

Multiple sequence alignments of the ORF region in the *IFITM3* gene were performed in human, monkey, mouse, rat, chicken, goose and cattle. Among these 7 species, the length of the ORF region in the bovine *IFITM3* gene is the longest (human: 402 bp, monkey: 402 bp, mouse: 414 bp,

(A)



(B)

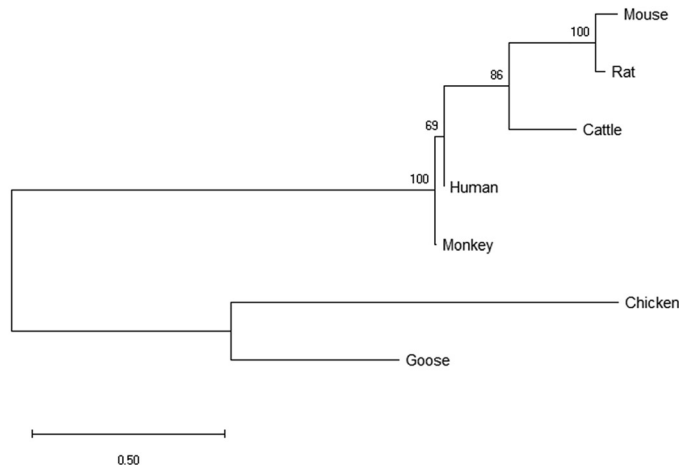


Fig. 2. Phylogenetic analyses of the *IFITM3* gene and IFITM3 protein in 7 species. (A) Phylogenetic analysis includes nucleotide sequences of humans (*Homo sapiens*, Gene ID: 10410), Sykes' monkey (*Cercopithecus albogularis*, Gene ID: KU570014.1), mouse (*Mus musculus*, Gene ID: 66141), rat (*Rattus norvegicus*, Gene ID: 361673), chicken (*Gallus gallus*, Gene ID: 770612), goose (*Anser cygnoides*, Gene ID: KX594327.1) and cattle (*Bos taurus*, Gene ID: 777594). The scale bar represents a distance of 0.20. (B) Phylogenetic analysis includes amino acid sequences of humans (*Homo sapiens*, Protein ID: AFF60355.1), Sykes' monkey (*Cercopithecus albogularis*, Protein ID: ANJ01447.1), mouse (*Mus musculus*, Protein ID: NP_079654.1), rat (*Rattus norvegicus*, Protein ID: 361673), chicken (*Gallus gallus*, Protein ID: NP_001336990.1), goose (*Anser cygnoides*, Protein ID: AQM74179.1) and cattle (*Bos taurus*, Protein ID: NP_863657.1). All phylogenetic trees were drawn by the neighbour-joining method using Molecular Evolutionary Genetics Analysis (MEGA) X software. The numbers at the branch nodes denote bootstrap confidence intervals obtained from 1,000 replicates. The branch lengths of the tree indicate evolutionary distances, which were calculated using the Poisson correction method and are expressed in units of the number of nucleotide substitutions per site

rat: 414 bp, chicken: 414 bp, goose: 426 bp and cattle: 441 bp). Notably, cattle showed very low *IFITM3* gene sequence homology with those of other species (human: 74.60%, monkey: 72.34%, mouse: 67.57%, rat: 68.71%, chicken: 52.61%, goose: 63.49%). Detailed information is described in Table 2.

Phylogenetic analyses

To investigate the evolutionary relationships of taxa, the nucleotide sequences of the *IFITM3* gene and the amino acid sequences of the IFITM3 protein in human, monkey, mouse, rat, chicken, goose and cattle were analysed by MEGA X. Evolutionary analysis was carried out using the Neighbour-Joining method. The branch lengths indicate evolutionary distances and were computed by using the maximum composite likelihood method with units consisting of the number of base substitutions per site. Notably, cattle showed closer evolutionary distances with human and monkey than with mouse and rat. Chicken and goose showed the farthest evolutionary distances from cattle. In addition, cattle constituted an independent clade among species (Fig. 2).

Comparison of the amino acid sequences of the *IFITM3* gene among species

Multiple alignments of amino acid sequences of the *IFITM3* gene were performed among human, monkey, mouse, rat, chicken, goose and cattle. IFITM3 protein lengths were different among species, and those of the bovine IFITM3 protein were the longest (human: 133, monkey: 133, mouse: 137, rat: 137, chicken: 137, goose: 141, cattle: 147). Notably,

the bovine IFITM3 protein showed a total of 36 bovine-specific amino acids including 3R, 7L, 8L, 9L, 11G, 13H, 15A, 16V, 19A, 22V, 25A, 28V, 36Q, 37S, 38Q, 41L, 51D, 53A, 66I, 70W, 76V, 93I, 109C, 110S, 120V, 126S, 127N, 129S, 130L, 136V, 137S, 138E, 140M, 142N, 143Y and 145G (Fig. 3).

Analysis of the topology of the IFITM3 protein among species

Since bovine-specific amino acids and length difference of the IFITM3 protein can induce a unique topology of the bovine IFITM3 protein, we performed topology analysis using Tmpred and SOSUI. Detailed information is described in Table 3. Strikingly, the topology prediction by Tmpred indicated that the bovine IFITM3 protein prefers the inside-to-outside topology of IMD1 and the inside-to-outside topology of TMD2. This topology of cattle is significantly different from that of primates, rodents and birds (i.e., primates and rodents prefer the inside-to-outside topology of IMD1 and the outside-to-inside topology of TMD2. In addition, birds prefer the outside-to-inside topology of IMD1 and the inside-to-outside topology of TMD2). Furthermore, the topology prediction by SOSUI indicated that the bovine IFITM3 protein prefers three membrane embedding domains (IMD1, codons 28–50; IMD2, codons 60–81; TMD3, codons 110–132). Except for cattle, the IFITM3 proteins in all other species showed two membrane embedding domains. Two models of the bovine IFITM3 protein were visualised based on topology analysis (Fig. 4). Figures 4A and B indicate the models of the bovine IFITM3 protein analysed by Tmpred and SOSUI, respectively.

Cattle	MNRTS [*] QLLLTGAHGAVPPAYEVLKEEHEVAVLGAPQSQAPLTTT ^{***} VINIRSDTAVPDHIVW ^{**}	60
Human	MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPPPTSTVIHIRSETSVDPHVWV	60
Monkey	MNHTVQTFFSPVNSGQPPNYEILKEEHEVAVLGAPHNPPPTSTVIHIRSETSVDPHVWV	60
Mouse	MNHTSQAFITAASGGQPPNYERIKEEYEVAEMGAPHGASV ^{***} TTVINMPREVSVDPHVWV	60
Rat	MNHTSQAFVNAATGGQPPNYERIKEEYEVSELGAPHGASV ^{***} TTVINMPREVSVDPHVWV	60
Chicken	-----MERVRASGPGVPPYEPLMDGMDMEGKT-----RSTVVTVETP-LVPPPRDHLAW	48
Goose	-----MERTRAPGVSLPPYEPLVEGLDMENMS-----RSVVVPVEAPPVQPPPRDHLAW	49
Cattle	SLFNTIFM [*] NW [*] CCLGFVAFAYSVKSRDRKMVGDITGAQSYASTAKCLNICS ^{**} LVLGILLTVV	120
Human	SLFNTLFMNP [*] CCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTIL	120
Monkey	SLFNTLFMNP [*] CCLGFIAFAYSVKSRDRKMVGDLTGAQAYASTAKCLNIWALILCIFMTIL	120
Mouse	SLFNTLFM [*] NFCCLGFIAAYAYSVKSRDRKMVGDVTGAQAYASTAKCLNISTLVLSILMVVI	120
Rat	SLFNTLFM [*] NFCCLGFIAAYAYSVKSRDRKMVGDVTGAQAYASTAKCLNISSLVLSILMVVI	120
Chicken	SLCTTLYANV [*] CCLGFIALVFSVKSRDRKVLGDYSGALSYGSTAKYLNITAHLLNIVFLIIL	108
Goose	SLCSTLYANV [*] CCLGFIALVFSVKSRDRKVLGDYSGALSYGSTAKYLNITALLLNIFLVL	109
Cattle	LIVLV ^{***} SNGLMIVQAVSELMQNYGGH-----	146
Human	LIVIPVL----IFQAYG-----	133
Monkey	LIVIPVL----ILQAYQ-----	133
Mouse	TIVSVII----IVLNAQNLHT-----	137
Rat	TIVTVVI----IALNAPRLQT-----	137
Chicken	IIVALVSGTIMVANI ^{***} FNHQQQH---PEFIGPT	137
Goose	IIVALIASGTITIVN ^{***} LHNHQQQQGNHPFLGPT	141

Fig. 3. Multiple sequence alignment of the IFITM3 protein among species. Protein sequences were obtained from GenBank at the National Center for Biotechnology Information (NCBI), including those of humans (*Homo sapiens*, AFF60355.1), Sykes' monkey (*Cercopithecus albogularis*, ANJ01447.1), mouse (*Mus musculus*, NP_079654.1), rat (*Rattus norvegicus*, NP_001129596.1), chicken (*Gallus gallus*, NP_001336990.1), goose (*Anser cygnoides*, AQM74179.1) and cattle (*Bos taurus*, NP_863657.1). Protein sequences were aligned using ClustalW2. Colours indicate the chemical properties of amino acids; blue: acidic, red: small and hydrophobic, magenta: basic, green: hydroxyl, sulphhydryl, amine and glycine. Asterisks indicate bovine-specific amino acids



Table 3. Transmembrane domains of IFITM3 protein predicted by TMpred and SOSUI

Prediction methods	Species	Sequence	Position	Length	Type	Reference
TMpred	Human	LFNTLFMNPCCLGFI AFAY	62–80 (i→o)*	19	IMD1***	
		LILGILMTILLIVIPVLIF	111–129 (o→i)**	19	TMD2****	
	Sykes' monkey	LFNTLFMNPCCLGFI AFAY	62–80 (i→o)	19	IMD1	
		LILCIFMTILLIVIPVLIL	111–129 (o→i)	19	TMD2	
	Mouse	LFNTLFMNFCCLGFI AYAY	62–80 (i→o)	19	IMD1	
		VLSILMVVITIVSVIIVL	112–130 (o→i)	19	TMD2	
	Rat	LFNTLFMNFCCLGFI AYAY	62–80 (i→o)	19	IMD1	
		LVLVLSILMVITIVTVVIAL	111–130 (o→i)	20	TMD2	
	Duck	LCSTLYSNVCCLGFLALVFSV	51–71 (o→i)	21	IMD1	
		IFFIILIALIASGTITFV	104–122 (i→o)	19	TMD2	
	Chicken	LCTTLYANVCCLGFLALVFSV	50–70 (o→i)	21	IMD1	
		VFLIILIALVASGTIMVA	103–121 (i→o)	19	TMD2	
	Goose	LCSTLYANVCCLGFLALVFSV	51–71 (o→i)	21	IMD1	
		IFLVILIVALIASGTITIV	104–122 (i→o)	19	TMD2	
Cattle	LFNTIFMNVCCLG FVAFAYSV	62–82 (i→o)	21	IMD1	In this study	
	ICSLVGLILLTVVLIVLV	108–125 (i→o)	18	TMD2	In this study	
SOSUI	Human	WSLFNTLFMNPCCLGFI AFAYS	60–81	22	IMD1	
		NIWALILGILMTILLIVIPVLIF	107–129	23	TMD2	
	Sykes' monkey	WSLFNTLFMNPCCLGFI AFAYS	60–81	22	IMD1	
		NIWALILCIFMTILLIVIPVLIL	107–129	23	TMD2	
	Mouse	WSLFNTLFMNFCCLGFI AYAYS	60–81	22	IMD1	
		TLVLSILMVVITIVSVIIVLNA	110–132	23	TMD2	
	Rat	WSLFNTLFMNFCCLGFI AYAYS	60–81	22	IMD1	
		SLVLSILMVITIVTVVIALNA	110–132	23	TMD2	
	Duck	WSLCSTLYSNVCCLGFLALVFS	49–70	22	IMD1	
		ALLNIFFIILIALIASGTITF	99–121	23	TMD2	
	Chicken	WSLCTTLYANVCCLGFLALVFS	48–69	22	IMD1	
		HLINVFLLIILIALVASGTIMVA	99–121	23	TMD2	
	Goose	WSLCSTLYANVCCLGFLALVFS	49–70	22	IMD1	
		ALLNIFLVILIVALIASGTITI	99–121	23	TMD2	
Cattle	EVAVLGAPQSQAPLTTT VINIRS	28–50	23	IMD1	In this study	
	WSLFNTIFMNVCCLG FVAFAYS	60–81	22	IMD2	In this study	
	SLVGLILLTVVLIVLVSNGSLMI	110–132	23	TMD3	In this study	

* (i→o) indicates inside-to-outside helices.

** (o→i) indicates outside-to-inside helices.

***IMD: intramembrane domain.

****TMD: transmembrane domain.

Bold texts indicate distinct topology prediction of cattle.

DISCUSSION

Previous studies have reported that the IFITM3 protein is a vital antiviral effector present in a broad range of viruses in human, mouse, chicken and duck (Bailey et al., 2012; Everitt et al., 2012; Smith et al., 2015). However, to date, there is a shortage of studies on immune-related functions of the IFITM3 protein in cattle. Previous studies have reported that the endometrium in the preattachment period is related to type I interferon and interferon tau (IFNT) signalling in the bovine uterus (Pru et al., 2001; Klein et al., 2006). As a component of those signalling pathways, embryo-induced elevated expression of the bovine IFITM3 protein has been shown. In addition, elevated expression of the *IFITM3* gene was detected in nuclear transfer (NT) and *in vitro* produced (IVP) embryos (Smith et al., 2007). Furthermore, the *IFITM3* gene plays a pivotal role in feed efficiency in cattle (Kern et al., 2016). In a recent study in pigs, another host of

FMDV, the IFITM3 protein showed inhibition of FMDV infection *in vitro* and *in vivo*. In addition, pig IFITM3 plays a pivotal role in the inhibition of classical and African swine fever viruses (Munoz-Moreno et al., 2016; Li et al., 2019). Although the basic function of the IFITM3 protein is conserved among species, interspecific differences in the regulation, sequence diversity and topology of the IFITM3 protein have been identified in mammals and birds. Thus, investigation of the bovine IFITM3 protein represents an essential baseline study to reveal species-specific features.

In the present study, we compared nucleotide sequences between cattle and several other species. Interestingly, the lengths of the coding sequences were different, and nucleotide sequence homologies in cattle were very low relative to other species (Fig. 1, Table 2). These differences in nucleotide and amino acid sequences indicated that cattle were classified into an independent clade among several species, including primates, rodents and birds (Fig. 2). These distinct

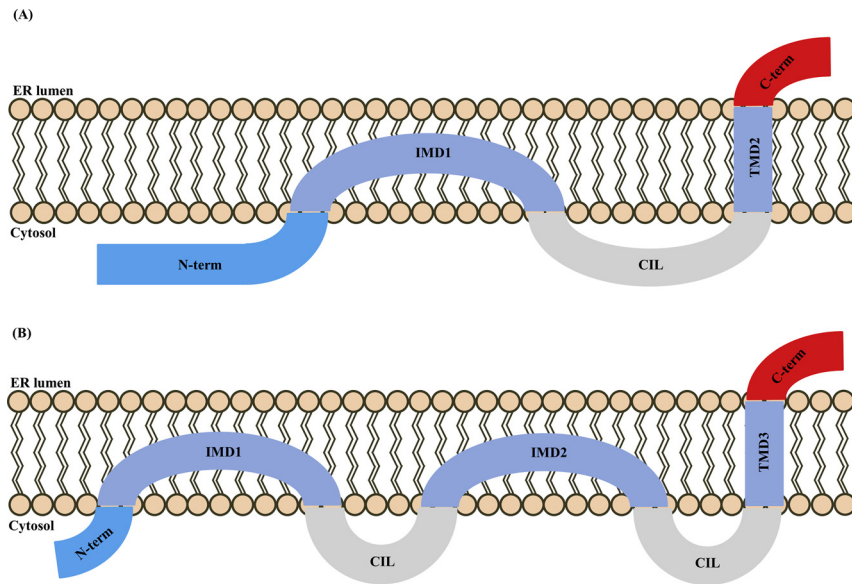


Fig. 4. Topology modelling of the bovine IFITM3 protein. (A): Topology of the IFITM3 protein was predicted by TMpred. (B): Topology of the IFITM3 protein was predicted by SOSUI. Abbreviations in boxes are as follows: NTD (N-terminal domain), IMD1 (intermembrane domain 1), IMD2 (intermembrane domain 2), CIL (conserved intracellular loop), TMD2 (transmembrane domain 2), TMD3 (transmembrane domain 3) and CTD (C-terminal domain)

features based on nucleotide sequences of the bovine *IFITM3* gene can also be caused by differences in the amino acid sequences of the IFITM3 protein. A total of 36 bovine-specific amino acids were identified by multiple sequence alignment of the IFITM3 protein (Fig. 3). Since a significant difference in amino acid sequences can induce unique topology of the bovine IFITM3 protein, we also analysed its topology. Notably, the IFITM3 protein of cattle showed a unique topology compared to that of primates, rodents and birds. In brief, the bovine IFITM3 protein showed inside-to-outside topology of IMD1 and inside-to-outside topology of TMD2. Furthermore, SOSUI predicts that the IFITM3 protein of cattle has an additional IMD compared to that of other species (Table 3, Fig. 4). A previous study has reported that the terminal domain of the IFITM3 protein physically prevents viral invasion from the host cell via constructing a chain-like structure between the IFITM3 protein monomers (Zani and Yount, 2018). Thus, the distinct feature of the N-terminal domain of the bovine IFITM3 protein may have an influence on the immunological capacity. Knowledge of these differences in nucleotide and amino acid sequences and topology of the IFITM3 protein is needed for further research in the future.

Intraspecies differences in nucleotide and amino acid sequences of the IFITM3 protein can be induced by genetic polymorphisms. Previous studies have reported that genetic polymorphisms of the *IFITM3* gene, including rs12252 SNP, rs34481144 SNP, rs3888188 SNP and rs6598045 SNP, showed association with susceptibility to the influenza A pandemic in 2009 and ulcerative colitis (Acutis et al., 2008; Seo et al., 2010; Prabhu et al., 2018; Kim et al., 2020, 2021a, 2021b; Kim and Jeong, 2020). In addition, non-synonymous SNPs of the chicken *IFITM3* gene result in topological

differences relative to those of mammals (Kim et al., 2019). Based on the nucleotide sequence differences of the bovine IFITM3 protein found in this study, genetic polymorphisms are expected to deepen the genetic differences of the bovine *IFITM3* gene compared to that of other species. Further study of the genetic polymorphisms of the bovine *IFITM3* gene is highly desirable in the future.

In conclusion, we compared nucleotide sequences of the *IFITM3* gene among species and found that cattle showed a significantly different length and sequence identity relative to primates, rodents and birds. In addition, we carried out phylogenetic analyses and confirmed that the bovine *IFITM3* gene and IFITM3 protein constituted an independent evolutionary clade. We also performed multiple sequence alignment of the IFITM3 protein and found 36 bovine-specific amino acids. Bovine-specific amino acids result in topological differences of the IFITM3 protein relative to other species. Strikingly, the bovine IFITM3 protein preferred inside-to-outside topology of IMD1 and inside-to-outside topology of TMD2 as determined by TMpred and three membrane embedding domains as determined by SOSUI.

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