African Journal of Agricultural Research Vol. 5(18), pp. 2551-2555, 18 September, 2010
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Distribution and characterization of the equine type I IFN family

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Accepted 10 May, 2010

The interferon (IFN) was described as a substance secreted by virally infected cells and endowed with the ability to render cells capable of interfering with a subsequent viral infection. In eutherian mammals, type I IFNs comprises seven major homologous subgroups including IFNA, IFNB, IFND, IFNE, IFNK, IFNW and IFNX. They are the key cytokines orchestrating host antiviral defense and other physiological processes. All type I IFNs are clustered together in specific regions in humans, mice and bovine genomes. Two mammalian IFN genes of ancient origin, IFNB and IFNE, define the outer limits of the locus, with all the other genes, except IFNK, distributed between these two markers. Twenty four type I IFN genes were identified in the genome of horse (*Equus caballus*). They included eight IFNWs (two are pseudogenes), six IFNAs, three IFNBs and IFNXs, two IFNDs, one IFNK and IFNE. Interestingly, IFND, which has been discovered only in pigs to date, was also found in equine genome. However, IFNT, which was identified in bovine genome, did not exist in equine genome. The specific distribution of type I IFN in equine genome suggested these IFNs were required in immune defense against particular pathogens affecting horse itself. The equine type I IFN locus also had two sub-loci (1 & 2) distributed in different chromosomes. The larger sub-loci encompassing 530 kb was located on chromosomes 23. Another smaller sub-loci encompassing 86 kb was located on chromosomes Un0111. It seemed that equine type I IFN locus showed its specific property different from those of mouse, human, pig and bovine genome, which were located on the same chromosomes.

Key words: Type I IFN, equine genome, viral infection, phylogenetic analyses.

INTRODUCTION

The interferon (IFN) was described by Isaacs and Lindenmann more than 50 years ago as a substance secreted by virally infected cells and endowed with the ability to render cells capable of interfering with a subsequent viral infection (Isaacs and Lindenmann, 1957). According to structural criteria and interaction with distinct cell surface receptors, IFN are subdivided into three types. Type II IFN is IFN-γ, whose most important function is to activate macrophages for microbicidal activity. Type III IFN are the IFN-λ, a three-member family (IFN-λ 1-3) also called IL-28A, IL-28B and IL-29 (Yang et al., 2010). The remaining IFNs belong to type I IFN, which include most members of IFNs in mammals. In eutherian mammals, type I IFNs comprise seven major homologous subgroups including IFNkappa (IFNK), IFN-beta (IFNB), IFN-epsilon (IFNE), IFNdelta(IFND), IFN-zeta (IFNZ), IFN-alpha (IFNA), IFNomega (IFNW), and IFN-tau (IFNT) (Krause and Pestka, 2005). They are the key cytokines orchestrating host antiviral defense and other physiological processes (Goodbourn et al., 2000). Recently, a new type I IFN family (IFNX) was identified which diverged from the IFNA lineage at least 83 million years ago (Walker and Roberts, 2009). Not all subgroups exist in all eutherian mammals, as with IFNT which is found in ruminants and IFND which has been discovered only in pigs to date (Lefevre and Boulay, 1993). With the exception of IFND, each of type I IFNs is encoded by multiple gene families, at least in some species (Woelk et al., 2007). For example, the IFNA subfamily contains 13 and 14 genes in humans and mice, respectively (Woelk et al., 2007). Moreover, there exists species-specific

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Table 1. Query sequences used for the genomic searches.

Species	Gene	Accession no.
Equine	IFNA	XP_001497752
Equine	IFNA	XP_001497640
Equine	IFNA	NP_001108009
Equine	IFNW	XP_001497165
Equine	IFNW	XP_001497504
Equine	IFNW	XP_001497775
Equine	IFNB	XP_001917608
Equine	IFNB	XP_001497044
Bovine	IFNT	AF196320
Bovine	IFNT	AF196322
Procine	IFND	Z22706
Procine	IFND	Z22707
Human	IFNK	NM_020124
Human	IFNE	NM_176891
Human	IFNL	AY184374
Murine	IFNZ	NM_197889

expansion and contraction of type I IFN families. For example, 24 potential IFNW and at least 8 pseudogenes were identified in cow genome (Walker and Roberts, 2009). However, a single functional IFNW and at least two pseudogenes are present in humans, and only a single pseudogene can be identified in mice (Woelk et al., 2007).

All type I IFN genes in human are clustered in an approximately 400 kb length region located on the short arm of chromosome 9 (9p21) in human genome (Hardy et al., 2004). Mouse type I IFN are clustered on the centromere-proximal region of chromosome 4 (4C4) in mouse genome (Hardy et al., 2004). Two mammalian IFN genes of ancient origin, IFNB and IFNE, define the outer limits of the locus, with all the other genes, except IFNK, distributed between these two markers. However, the bovine type I IFN locus is organized differently with that of human and mouse (Walker and Roberts, 2009). They have two type I IFN sub-loci (1 and 2) encompassing 701 kb and 441 kb, respectively, separated by a gap estimated to be approximately 11 megabases (Mb) in bovine genome (Walker and Roberts, 2009).

IFNXs were identified in bovine genome, but is absent in all other sequenced genomes with the possible exception of the horse, a non-ruminant herbivore (Walker and Roberts, 2009). It implied that there are similarities in the organization of the type I IFN locus of bovine and equine genome. We previously identified four IFN-λs in equine genome (Yang et al., 2010), but no IFN-λs in bovine genome. It implied that equine type I IFN locus may have some unique features different from other species including cow. The recent sequencing of the equine genome has provided the first opportunity for a detailed study of the type I IFN locus in this species. Here we provide a detailed description and full annotation of the equine type I IFN locus and found there existed two type I IFN locus distributed in different chromosome, significantly different with that of bovine genome.

METHODS

Identification of type I IFNs from equine genome

Type I IFNs genes were searched in the genome sequences of horse (*Equus caballus*) by the method described before (Yang et al., 2010; Walker and Roberts, 2009) using the IFNs genes listed in Table 1 as queries in Ensembl database (http://www.ensembl.org). The assemblies used in this study, was horse Equ Cab 2. The identified putative type I IFNs genes were blasted against the nr database of GenBank to confirm that the best hits were type I IFNs genes. The identified type I IFNs information such as exons, chromosome location, and transcript direction were also extracted from Ensembl database.

General naming rules for the identified gene

The identified type I IFN genes were named using the following naming rules: The first three letters were named as IFN, belongs to the type I family; the fourth letter was on behalf of the sub-family; the last numbers came from the last four numbers of type I IFN genes in Ensembl database.

Phylogenetic analyses of equine type I IFNs

The amino acid sequences of the identified type I IFNs were deduced from the identified type I IFNs genes and aligned using Clustal X 1.8 software (Thompson et al., 1997). The phylogenetic tree of type I IFNs was obtained by using ML (maximum likelihood) (PHYML v2.4.4) (Guindon et al., 2005) and NJ (neighbor-joining) (MEGA 3.0) (Kumar et al., 2004) methods, and the reliability of the tree was evaluated by the bootstrap method with 1,000 replications.

RESULTS AND DISCUSSION

The identification of type I IFN genes in equine genomes

Twenty four type I IFN genes were identified in the genome of horse (*E. caballus*). They included eight IFNWs (two are pseudogenes), six IFNAs, three IFNBs and IFNXs, two IFNDs, one IFNK and IFNE (Table 2; Figure 1). Former study identified 156 IFNA genes from 17 eutherian species and found that IFNA genes form species-specific clusters except for the primate section. Only six IFNAs were identified in horse genomes. They are smaller than those of human, mouse and cow genomes (Table 2). It implied the horse exhibited its specific IFNA clusters, different from those of other mammals. IFNW family is greatly expanded in equine genome compared to human and mouse genomes, but less than that of bovine genome, which includes 24 IFNWs and 8 pseudogene. The IFNB are present in

Table 2. Cross-species comparison of IFN subfamilies among human, mouse, cow and horse.

Superfamily	Gene number			
	Human	Mouse	Cow	Horse
IFNA	13	14	13	6
IFNB	1	1	6	3
IFND	0	0	0	2
IFNE	1	1	1	1
IFNK	1	1	1	1
IFNT	0	0	3	0
IFNX	0	0	3	3
IFNW	1	0	24	8
IFNZ	0	2	0	0
IFNL	3	3	0	0
Total	17	17	51	24

The number of predicted IFN genes in each subfamily based on genomic analysis of mouse, human, bovine and horse is as shown. The equine Type I IFN locus has an expansion of both the IFNB and IFNW subfamilies. Horse have also acquired a novel IFN subfamily termed IFND, discovered during this analysis, different from human, mouse, and cow.

multiple copies in equine genome, less than that of bovine genome, but more than that of human and mouse genome. Interestingly, IFND, which has been discovered only in pigs to date (Lefevre and Boulay, 1993), was also found in equine genome. The recently described IFNX family was also identified in equine genome, which is also reported to exist in cow genome and present as pseudogene in human, mouse, porcine, feline and canine genome. IFNK and IFNE are present in single copy in equine genome, consistent with that of humans, mice and bovine genomes.

However, IFNT, which was identified in bovine genome, did not exist in equine genome. Type I IFNs are predominantly produced by leukocytes in response to virus infection, the presence of double stranded RNA (dsRNA), or the recognition of pathogen associated molecular patterns by Toll-like receptors (Rubinstein et al., 1979; Goodbourn et al., 2000). IFNA and IFNB constituted the primary viral defense mechanism. IFNW has been implicated in protection against specific viruses, such as parvovirus, herpesvirus, calicivirus, coronavirus and rotavirus (Paltrinieri et al., 2007), while murine IFNZ suppress viruses targeting the bone marrow and spleen (Oritani et al., 2000). IFNK is predominately expressed in keratinocytes and acts through a unique cell-associated viral protection mechanism (Buontempo et al., 2006; LaFleur et al., 2001). IFNE is suggested to serve a specific role in reproductive tissues either in viral protection or early placental development (Matsumiya et al., 2007). The specific distribution of type I IFN in equine genome suggested these IFNs are required in immune defense against particular pathogens affecting horse itself.

Locus map of type I IFN gene in equine genomes

The type I IFN locus is organized similarly in mouse, human and pig genome. Two mammalian IFN genes of ancient origin, IFNB and IFNE, define the outer limits of the locus, with all the other genes, except IFNK, distributed between these two markers. Moreover, the genes in ty type I IFN locus are predominantly (but not exclusively) localized on one strand and transcribed in the same direction as the IFNB and IFNE. The bovine type I IFN locus have two sub-loci (1 and 2) encompassing 701 kb and 441 kb, respectively, separated by a gap estimated to be approximately 11 Mb (Walker and Roberts, 2009). The majority of the genes in both sub-loci are transcribed in the same direction as the distally placed IFNB, except one cluster of IFNW and IFNA and the solitary IFNE, which are transcribed in the opposite direction. The equine type I IFN locus also have two sub-loci (1 and 2) distributed in different chromosomes (Figure 2). The larger sub-loci encompassing 530 kb is located on chromosomes 23. Another smaller sub-loci encompassing 86 kb is located on chromosomes Un0111. It seemed that equine type I IFN locus showed its specific property different from those of mouse, human, pig and bovine genome, which are located on the same chromosomes.

Two mammalian IFN genes of ancient origin, IFNB and IFNE, define the outer limits of the type I IFN locus on chromosomes 23, with all the other genes, except IFNK, distributed between these two markers. IFNK is present in a single copy and separated (5.0 Mb) from IFNE. This organization of type I IFN locus on chromosomes 23 was similar to those in mouse, human, pig and bovine genome. However, many genes (7/18) in type I IFN locus on chromosomes 23 are transcribed in the reversed direction as the IFNB and IFNE, which is not observed in human, mouse, pig and bovine genomes. There are three clusters of IFNA/IFNW. The first IFNA/IFNW cluster (IFNW6599-IFNA6690-IFNW6768-IFNA6805-IFND6933) was transcribed in the same direction as the IFNB and IFNE. The second IFNA/IFNW cluster (IFNW0976-IFNA1107-IFNW1785-IFNA2305-IFND2978) is just the copy of the first IFNA/IFNW cluster, but transcribed in the reversed direction as the first IFNA/IFNW cluster. It seemed that the second IFNA/IFNW cluster may be the gene duplication of first IFNA/IFNW cluster. The third IFNA/IFNW cluster is IFNA3168-IFNA3771-IFNW4269, which is different from the first and second IFNA/IFNW clusters and transcribed in the reversed direction as the first IFNA/IFNW cluster. These three clusters of IFNA/IFNW were arranged one by one, without any other genes among them. There are also three clusters of IFNA/IFNW in cow genome, two of them are on sub-locus1, one at the proximal end, the second placed about half way along. Moreover, the gene set in the first IFNA/IFNW cluster 1 is a palindrome to one in the second cluster.

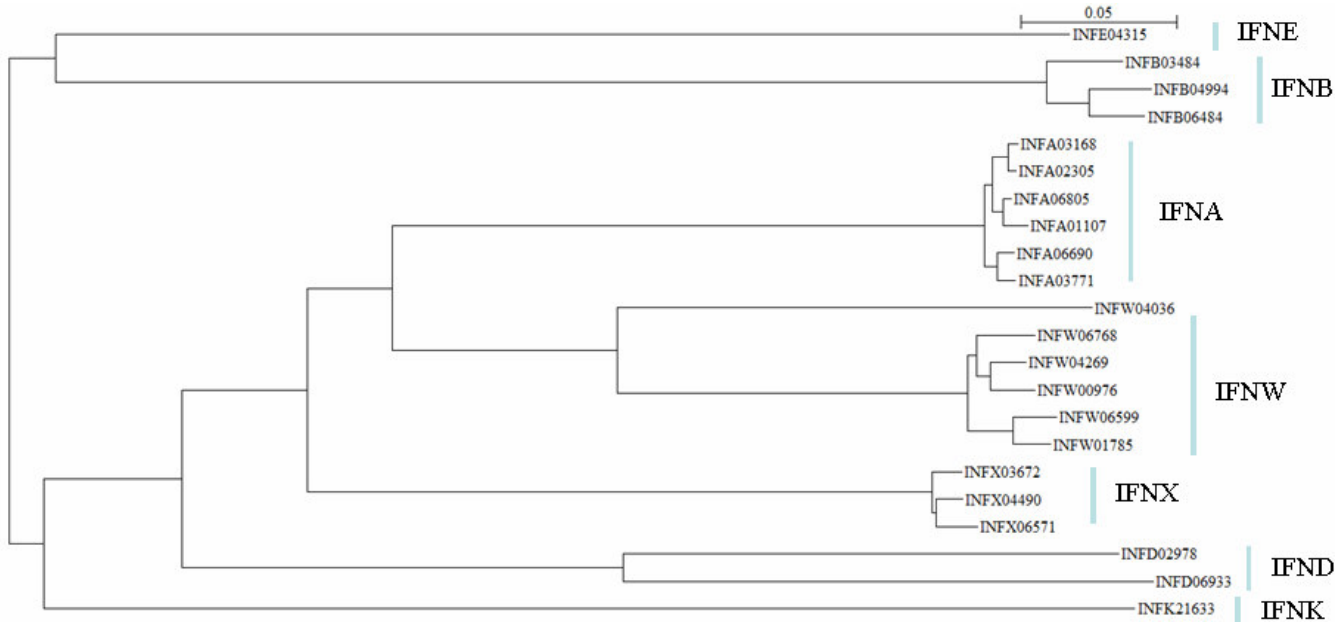

Figure 1. The equine type I IFN phylogenetic tree. The evolutionary history of equine type I IFN was inferred by using the Neighbor-Joining (NJ) method with bootstrap test (1000 replicates). The tree was rooted to IFNK and calculations were based on uniform rates of change for all sites. IFND emerged prior to IFNA in this analysis. IFND, IFNA and IFNW branched from a common ancestor in this analysis. Predicted pseudogenes based on frame shift mutations or stop codons within the first 100 aa of the coding sequence have been excluded from the table.

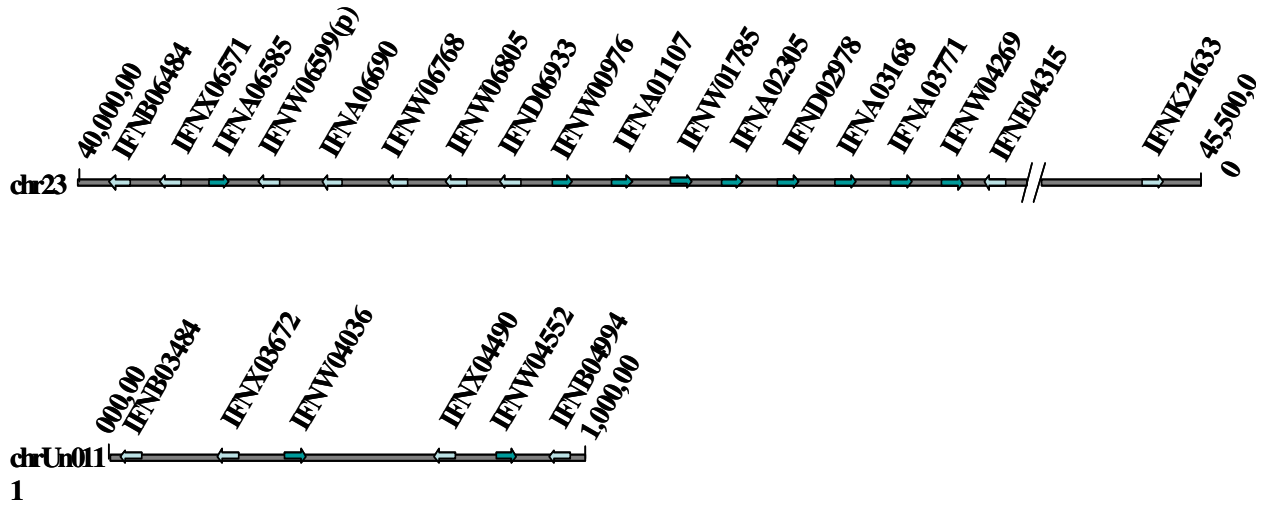

Figure 2. Genomic map of the bovine type I IFN locus. Genomic map of the equine type I IFN locus. Blast searches of the equine genome database revealed that all matches to known IFN genes, reside within two sub-loci, illustrated in the gene map shown. Both the position of each gene relative to the line and direction of the arrow on the map denote the direction of transcription. The subfamily for each gene is designated by the final letter of the abbreviated name. Pseudogenes are indicated by a box instead of an arrow and the letter "p" after the subfamily designation.

The third cluster of IFNA/IFNW is at the distal end of sub-locus 2, but lacks the duplicated group of four genes in IFNA/IFNW clusters 1 and 2. The multiple IFNA/IFNW clusters in cow and horse genomes suggested that the type I IFN locus has broadened as gene duplication.

Besides chromosomes 23, a smaller type I IFN locus in horse genome was identified on chromosomes Un0111. Two IFNB define the outer limits of this locus, with all the other genes, distributed between these two markers. We noticed that the first three type I IFN genes (IFNB3484-

IFNX3672-IFNW4036) were similar to the first three type I IFN genes (IFNB6484-IFNX6571-IFNW6585) on chromosomes 23. Moreover, the transcribed direction of IFNB3484-IFNX3672-IFNW4036 was just the same as those of IFNB6484-IFNX6571-IFNW6585 on chromosomes 23. Another three type I IFN genes (IFNX4490-IFNW4552-IFNB4994) on chromosomes Un0111 was just the copy of IFNB3484-IFNX3672-IFNW4036 and only the different IFNW and IFNX location. It suggested that type I IFN locus in chromosomes Un0111 was formed by the gene conversion and duplication of the partial type I IFN locus on chromosome 23.

REFERENCES

Buontempo PJ, Jubin RG, Buontempo CA, Wagner NE, Reyes GR, Baroudy BM (2006). Antiviral activity of transiently expressed IFNkappa is cell-associated. J. Interferon Cytokine Res., 26: 40-52.

Goodbourn S, Didcock L, Randall RE (2000) Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol., 81: 2341-2364.

Goodbourn S, Didcock L, Randall RE (2000). Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol., 81: 2341-2364.

Guindon S, Lethiec F, Duroux P, Gascuel O (2005). PHYML Online--a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res., 33: 557-559.

Hardy MP, Owczarek CM, Jermiin LS, Ejdeback M, Hertzog PJ (2004). Evolution of the interferon alpha gene family in eutherian mammals. Characterization of the type I interferon locus and identification of novel genes. Genomics, 84: 331-345.

Isaacs A, Lindenmann J (1957). Virus interference. I. The interferon. Proc. Soc. Lond. B, 147: 258-267.

Krause CD, Pestka S (2005). Evolution of the Class 2 cytokines and receptors, and discovery of new friends and relatives. Pharmacol. Ther., 106: 299-346.

Kumar S, Tamura K, Nei M (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform, 5: 150-163.

LaFleur DW, Nardelli B, Tsareva T, Mather D, Feng P, Semenuk M,. Taylor K, Buergin M, Chinchilla D, Roshke V, Chen GX, Ruben. SM, Pitha PM, Coleman TA, Moore PA (2001). Interferon-kappa, a novel type I interferon expressed in human keratinocytes. J. Biol. Chem., 276: 39765-39771.

Lefevre F, Boulay V (1993). A novel and atypical type one interferon gene expressed by trophoblast during early pregnancy. J. Biol. Chem., 268: 19760-19768.

Matsumiya T, Prescott SM, Stafforini DM (2007). IFN-epsilon mediates TNF-alpha-induced STAT1 phosphorylation and induction of retinoic acid-inducible gene-I in human cervical cancer cells. J. Immunol., 179: 4542-4549.

Oritani K, Medina KL, Tomiyama Y, Ishikawa J, Okajima Y, Ogawa M, Yokota T, Aoyama K, Takahashi I, Kincade PW (2000). Limitin: An interferon-like cytokine that preferentially influences B-lymphocyte precursors. Nat. Med., 6: 659-666.

Paltrinieri S, Crippa A, Comerio T, Angioletti A, Roccabianca P (2007). Evaluation of inflammation and immunity in cats with spontaneous parvovirus infection: consequences of recombinant feline interferon-omega administration. Vet. Immunol. Immunopathol., 118: 68-74.

Rubinstein M, Rubinstein S, Familletti PC, Miller RS, Waldman AA, Pestka S (1979). Human leukocyte interferon: production, purification to homogeneity, and initial characterization. Proc. Natl. Acad. Sci., USA, 76: 640-644.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic. Acids Res., 15: 4876-4882.

Walker AM, Roberts RM (2009). Characterization of the bovine type I IFN locus: rearrangements, expansions, and novel subfamilies. BMC Genomics, 10: 187.

Woelk CH, Frost SD, Richman DD, Higley PE, Kosakovsky Pond SL (2007). Evolution of the interferon alpha gene family in eutherian mammals. Gene, 397: 38-50.

Yang L, Wei J, He S (2010). Integrative genomic analyses on interferon-lambdas and their roles in cancer prediction. Int. J. Mol. Med., 25: 299-304.