

## RESEARCH ARTICLE

# Distinct evolution process among type I interferon in mammals

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## ABSTRACT

Interferon (IFN) is thought to play an important role in the vertebrate immune system, but systemic knowledge of IFN evolution has yet to be elucidated. To evaluate the phylogenetic distribution and evolutionary history of type I IFNs, 13 genomes were searched using BLASTn program, and a phylogenetic tree of vertebrate type I IFNs was constructed. In the present study, an IFN $\delta$ -like gene in the human genome was identified, refuting the concept that humans have no IFN $\delta$  genes, and other mammalian IFN genes were also identified. In the phylogenetic tree, the mammalian IFN $\beta$ , IFN $\epsilon$ , and IFN $\kappa$  formed a clade separate from the other mammalian type I IFNs, while piscine and avian IFNs formed distinct clades. Based on this phylogenetic analysis and the various characteristics of type I IFNs, the evolutionary history of type I IFNs was further evaluated. Our data indicate that an ancestral IFN $\alpha$ -like gene forms a core from which new IFNs divided during vertebrate evolution. In addition, the data suggest how the other type I IFNs evolved from IFN $\alpha$  and shaped the complex type I IFN system. The promoters of type I IFNs were conserved among different mammals, as well as their genic regions. However, the intergenic regions of type I IFN clusters were not conserved among different mammals, demonstrating a high selection pressure upon type I IFNs during their evolution.

**KEYWORDS** type I IFN, evolutionary history, vertebrate, gene cluster

## INTRODUCTION

Interferon was first recognized half a century ago for its antiviral

activities (Isaacs and Lindenmann, 1957), and its anti-proliferation and immune-regulatory activities were then subsequently discovered (Stark et al., 1998). According to the receptors that they bind, IFNs can be divided into three types: type I, type II, and type III IFNs (Sheppard et al., 2003). There are many different kinds of type I IFNs, such as IFN $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ,  $\kappa$ ,  $\tau$ , and  $\omega$ , but type II and type III IFNs are only by a single kind each, IFN $\gamma$  and IFN $\lambda$ , respectively (Pestka et al., 2004).

Type I IFNs predominantly function through the typical Jak-Stat pathway. When type I IFNs bind to their high-affinity receptors, a heterotrimer named interferon-stimulated gene factor 3 (ISGF3) is formed to activate the expression of interferon-stimulated gene (ISGs). Aside from the canonical Jak-Stat pathway, IFNs can also activate the MAPK pathway (Stancato et al., 1997; David, 2002). However, due to its complex activities in the immune system, new IFN pathways and roles remain to be discovered.

Type I IFNs have been identified in zebrafish, Atlantic salmon, grass carp, and Fugurubripes, which several of them display similar activities to their mammal homologs (Altmann et al., 2003; Robertsen et al., 2003). IFN $\beta$  is hypothesized to have arisen from birds, and the other type I IFNs are present only in mammals (Sick et al., 1996). IFN $\zeta$ , also known as limittin, was discovered for its ability to arrest the growth of or kill lympho-hematopoietic cells, and is thought to only exist in mice (Oritani et al., 2000). IFN $\delta$  has been described in sheep, pigs, and horses and is expressed at 15 days of gestation in conceptus (Lefèvre and Boulay, 1993; Cochet et al., 2009). IFN $\tau$  is considered to only exist in ruminants but is present in the human genome as a pseudogene (Whaley et al., 1994). Amphibian type I IFN genes were inferred as intron-containing and 4-cysteine-containing IFN genes, similar to zebrafish IFN $\beta$  and IFN $\kappa$ , while reptile type I IFNs were inferred from genomic se-

quence as intronless forms (Sun et al., 2009; Qi et al., 2010).

Robert has hypothesized a good model for type I IFN evolution, which suggests that IFN $\omega$  diverged from IFN $\alpha$  and that IFN $\tau$  arose from IFN $\omega$  approximately 36 million years ago (Roberts et al., 1998). The evolution of IFN $\alpha$  is well described, and both gene conversion and duplication played important roles in the formation of the IFN $\alpha$  gene family (Woelk et al., 2007). However, the detailed phylogenetic distribution and evolutionary history of type I IFNs in vertebrates remains unclear (Qi et al., 2010). Towards a better understanding of type I IFN evolution, we report several novel IFNs and illustrated the evolution history of type I IFNs in vertebrates. The gene synteny analysis demonstrates a high selection pressure upon type I IFNs during their evolution.

RESULTS

Type I IFN gene clusters in genomes

Different types IFN genes are located on different chromosomes. In humans, type I IFN genes represent a gene cluster on chromosome 9 that includes 14 IFN $\alpha$  genes, one IFN $\omega$  gene, one putative IFN $\delta$  gene, one IFN $\tau$  pseudogene, one IFN $\beta$  gene and one IFN $\epsilon$  gene. The IFN $\kappa$  gene is not adjacent to this gene cluster, and a similar situation was found in other mammals. In mouse, type I IFNs form a gene cluster on chromosome 4, which includes 16 IFN $\alpha$  genes, one IFN $\omega$  pseudogene, 14 limitingenes, and one IFN $\epsilon$  gene. In most species, the IFN $\epsilon$  and IFN $\beta$  genes are located on the two extremities of type I IFN clusters, while the other type I IFN genes were randomly located relative to one another, except limitin, which forms a small cluster within the type I IFN cluster.

As indicated by the BLAST results, IFN $\delta$  and IFN $\omega$  genes may not exist in the mouse and dog genomes and this result is not due to lack of sequence coverage as the mouse and dog genomes have been fully characterized (Fig. 1), which is consistent with previous studies (Hardy et al., 2004). Limitins only exist in mice. Indeed, even in the rat genome, the limitin homolog is a pseudogene with an early stop codon and considerably degraded sequence (date not shown).

A putative IFN $\delta$  gene, containing a pseudogene named IFN $\alpha$ 12p, was identified in the human genome (Fig. 2). This gene is located in the type I IFN gene cluster and represents a single gene that is quite different from the other IFN $\delta$  genes in sheep and pigs. Sharing 62.3% similarity with horse IFN $\delta$ 1 and <50% similarity with the other human IFNs, the putative human IFN $\delta$  gene is likely a new subtype of human type I IFN (Table 1). Located immediately beside the IFN $\delta$  gene lies the IFN $\alpha$ 8 gene and IFN $\alpha$ 11p, which is unexpressed in humans (Henco et al., 1985). In addition, the homolog of human pseudogene in other primates is similar with itself, represents as a single gene with an early stop codon (date not shown).

Four IFN $\epsilon$  genes were identified in the cow, horse, cat, and dog genomes (Fig. 2A). All of these genes display >75% similarity with human IFN $\epsilon$  and <50% similarity with the other IFNs previously identified in these genomes. Similar to human IFN $\epsilon$ ,

these four IFN $\epsilon$  genes are located on the extremities of the type I IFN clusters, *i.e.*, there is high conservation of the IFN $\epsilon$  genomic location. In addition, three IFN $\kappa$  genes were identified in the horse, cat, and dog genomes (Fig. 2B). Both the phylogenetic tree and sequence similarity indicate that these genes are true IFN $\kappa$  genes. All of these genes are located outside of the type I IFN gene cluster, just like the human IFN $\kappa$  gene.

The complex relationship among type I IFNs in vertebrates

Within the type I IFN grouping, avian type I IFNs forms a separate clade from piscine and mammalian type I IFNs (Fig. 3). In our analysis, piscine type I IFNs are the outgroup of avian and mammalian type I IFNs, consistent with the course of vertebrate evolution. Unique to piscine IFNs, the divergence pattern of type I IFNs does not match the speciation of fish, and this

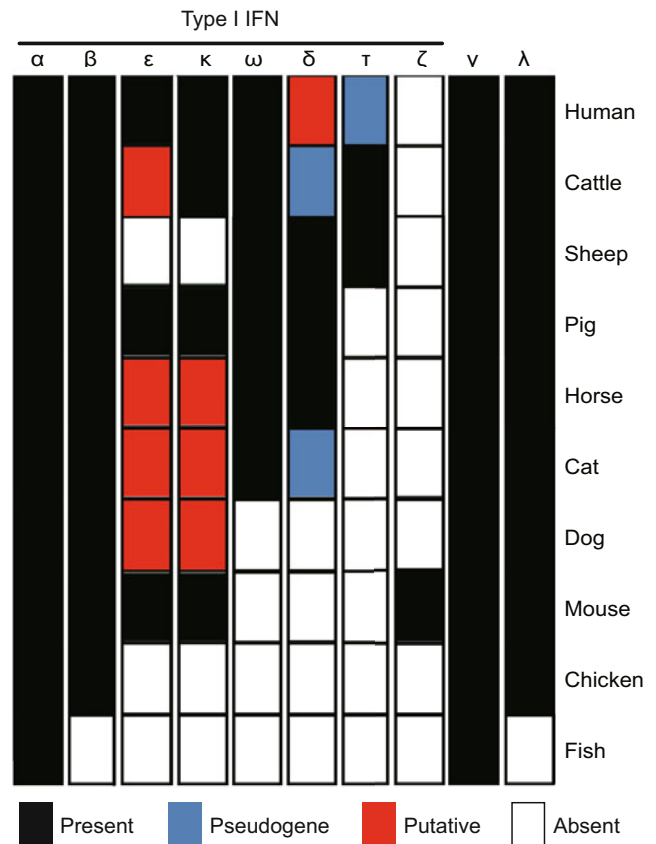
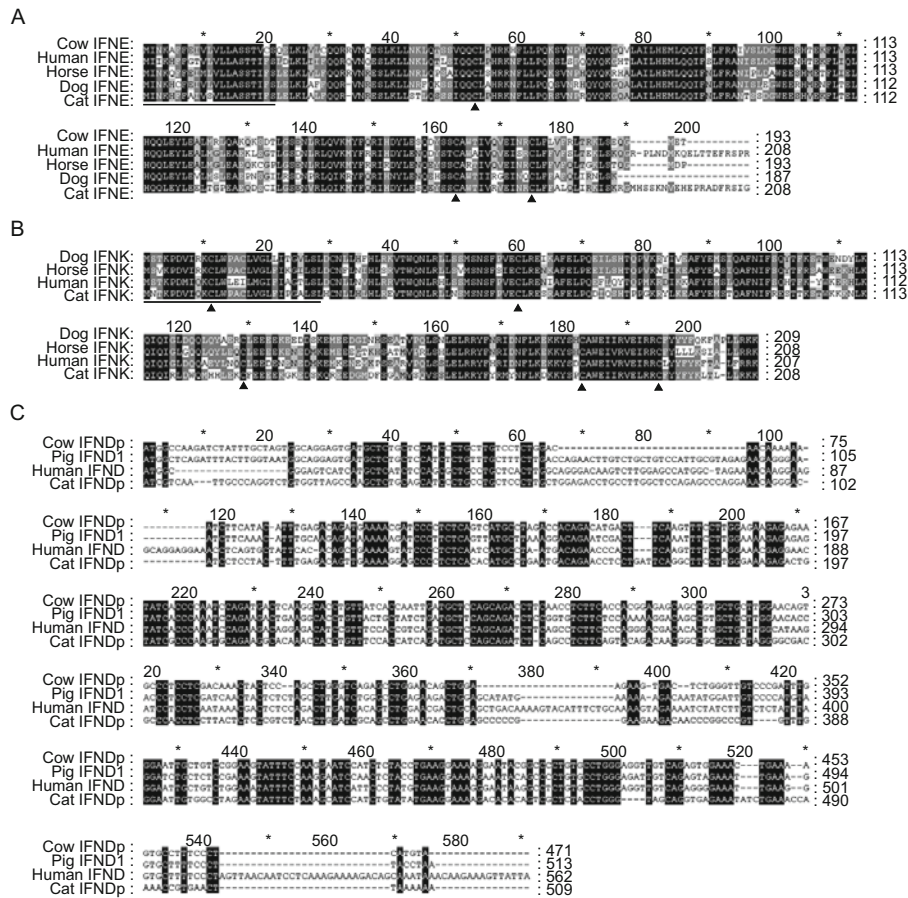


Figure 1. Absence/presence plots in a subset of 10 vertebrate genomes. Type II IFN and IFN $\alpha$  exist in all vertebrates, while type III IFN is absent in fish genomes. IFN $\beta$  can be found in both avian and mammalian genomes, and the other type I IFN only exist in mammal. Mouse and dog lost their IFN $\omega$  genes and IFN $\delta$  genes during species evolution, but limitins arise in mouse genome. 4 IFN $\epsilon$  genes and IFN $\kappa$  genes was inferred, suggesting that IFN $\epsilon$  and IFN $\kappa$  exist in all mammals. IFN $\tau$  only exist in ruminants.



**Figure 2. Multiple alignment and gene synteny analysis of novel identified IFNs from human, cat, horse, cow and pig.** The alignment was generated by CLUSTALW and then edited by GENEDOC. (A and B) Multiple alignment of novel identified IFNε and IFNκs. The putative signal peptide sequences predicted by SignalP4.0 are underlined and conserved cysteines are marked with black trilateral. Conserved regions are shadowed. (C) Multiple alignment of novel identified IFNδ pseudogene.

disparity was not resolved by restricting the list of piscine type I IFN species in the phylogenetic analyses. Thus, the reason for this discrepancy is the divergence of 2C-containing IFNs and 4C-containing IFNs in fish.

The avian and mammalian IFNs form distinct species-specific clades in the phylogenetic tree (as the piscine IFNs do). Even though IFNs have been purified from reptiles and have similar biophysical properties as mammalian IFNs, the failure to clone these IFNs makes it difficult to further study reptile type I IFNs.

The major subgroups of IFNs identified thus far form subgroups within the mammalian type I IFN family. Phylogenetically, the mammalian type I IFN subtypes form clades consistent with mammalian speciation in the phylogenetic tree. The first diverging group of IFNs within the mammalian type I IFN clade is that of the unduplicated IFNs. IFNκ forms an outgroup in this subclade, which indicates a different evolutionary route compared to IFNβ and IFNε. The next subgroup to diverge from the remaining mammalian type I IFNs is that of IFNε and IFNβ. These two subgroups may in fact be more related to

each other than to other mammalian type I IFN subtypes.

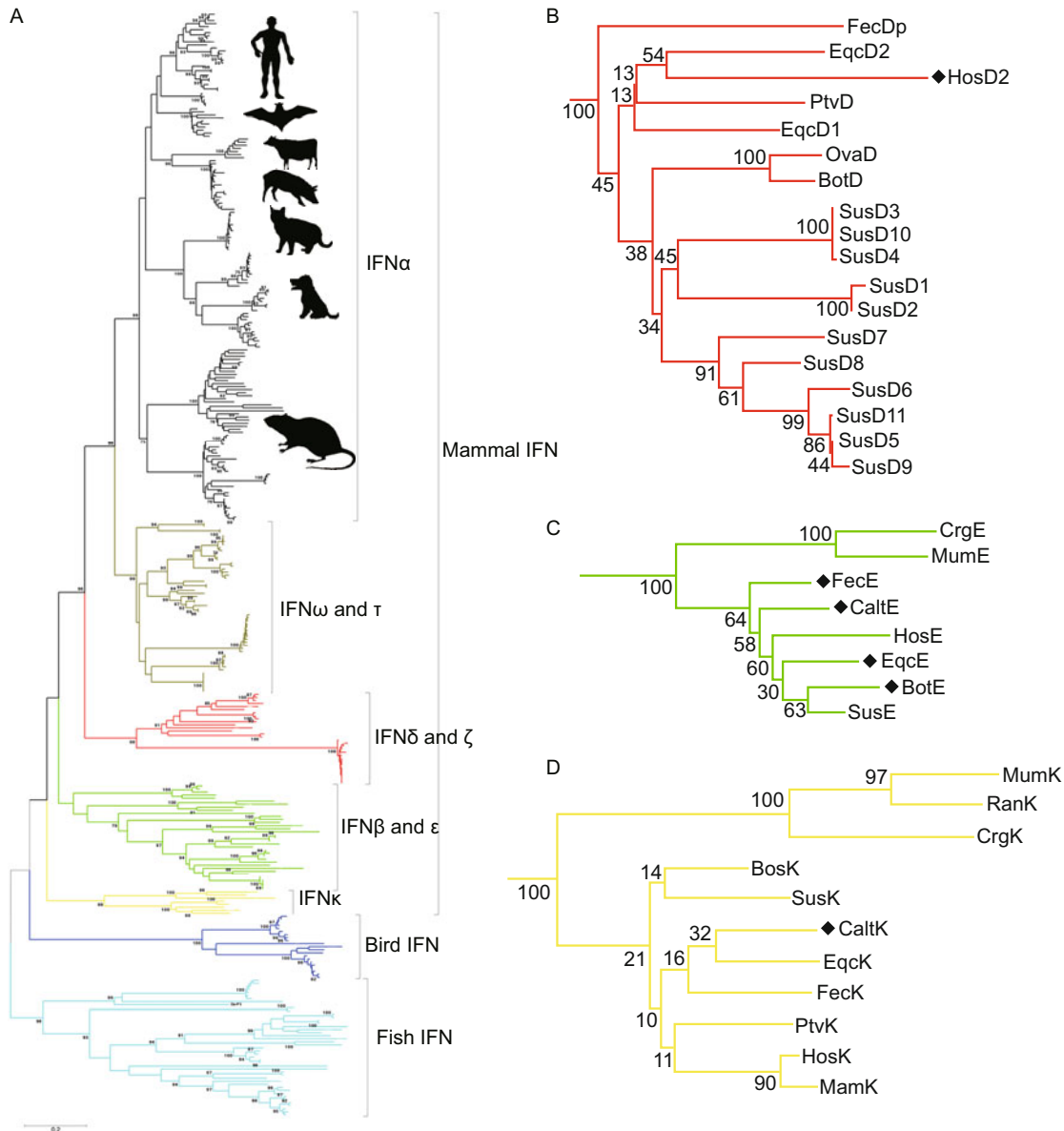
Both IFNδ and IFNζ form an outgroup from the remaining mammalian IFNs. Like the piscine IFNs, the divergence pattern of porcine IFNδ genes does not form a single clade in the phylogenetic tree, suggesting a close evolutionary distance among IFNδ genes from different species. The failure to find limitin in the pig and horse genomes, which have several IFNδ genes, and the failure to find IFNδ genes in mice and rats, suggests a preferential evolutionary relationship between IFNδ and IFNζ.

IFNω and IFNτ form a subgroup in the phylogenetic tree, and porcine and bovine IFNω, together with ovine and bovine IFNτ, form a core clade in this subgroup. Though IFNω and IFNτ display intimate relationships, their functions are quite different: IFNω is an antiviral and immuno-regulator, just like IFNα, while the preferential function of IFNτ is to ensure the pregnancy continues through preventing the corpus luteum from degradation. Aside from their differences in function, the species distribution of IFNω is more extensive than that of IFNτ, which is only found in sheep and cattle. According to the phylogenetic tree and the characteristics of IFNω and IFNτ,

Table 1. Sequence identities and divergences among type I IFNs

	CalfA1	EqcA1	FecA1	HosA1	BotB	CalfB	FecB	EqcB	HosB	EqcD1	HosE
CalfA1	-	69.5	73.2	65.2	32.3	35.8	39	38.7	32.8	44	36.2
EqcA1	30.3	-	73.5	79.8	34.2	37.3	39.1	40.7	39.6	51.8	40
FecA1	25.4	22.9	-	69.8	35.1	36.2	37.8	38.5	34.4	46.6	36.8
HosA1	36.4	19.6	27.3	-	36.4	36.4	39.2	40.5	36.5	52.6	42.5
BotB	93.6	88.1	87.8	91.1	-	65.8	67	70.1	65.4	33.7	39.6
CalfB	85.6	85	88.4	88.6	38.6	-	82	74.2	71.5	36.7	41
FecB	79.2	77.9	79.4	81.5	35.4	17.5	-	75.4	71.7	34.5	41.2
EqcB	77.8	73.5	76.2	77.9	31.8	28.4	25.2	-	73.8	37.3	40.5
HosB	87.3	80.6	90	86.5	36.8	30.2	28.7	27.6	-	34.7	43.1
EqcD1	65.8	56.1	62.6	55.7	92.1	86.7	94.1	84.5	86.1	-	38.3
HosE	90.5	81	88	73.8	87.3	81.1	82.8	76.1	77.3	90.1	-
EqcW1	51.4	41.6	47.5	41.8	83.7	78.4	74.3	68.4	69.1	58.1	75.2
FecW1	53.1	47.7	51.6	50.3	91.3	89.3	83.4	79.6	87.6	65.6	93.2
HosW	51.2	39.1	42.3	38.9	79.3	74	72.2	64.4	73.7	52.4	77.8
HosT	45.8	36.9	39.4	37.5	83.4	77.3	75.6	66.6	76.6	59	75
FecDp	86	71.1	83.2	71.9	87.8	82	91.1	85	98.4	30.6	82.9
HosD	79.7	71.3	75.5	63.2	89.5	86.9	96.4	90	94.4	28.9	96.1
CalfE	91.3	76.4	89.2	77.5	92.2	88.1	85.1	83.6	80.7	88.6	19.6
CalfK	126.8	111.3	122.1	101.8	144.3	124.2	127.9	136.8	123.1	129.8	108.1
EqcE	96.7	82.8	91.1	79.3	84.3	79.1	81.4	75.2	78.3	91	16.7
EqcK	122.6	110.1	117.6	105.3	122	116.5	112.1	119.8	114.4	132.7	107.2
FecE	92.5	81.2	91.2	81.1	91.2	83.3	81.9	80.2	77.4	89.7	22.1
	EqcW1	FecW1	HosW	HosT	FecDp	HosD	CalfE	CalfK	EqcE	EqcK	FecE
CalfA1	54.1	51.2	53.7	58	34.6	32.4	35.3	35.3	34.6	35.5	36.5
EqcA1	62.3	56.9	63.2	64.3	41.6	40	43.2	41.8	40.9	43.1	43.4
FecA1	54.9	51.9	59.3	60	34.3	35.4	37.2	36.8	37	35.4	39.3
HosA1	61.8	54.9	63.5	65.3	38.6	45	42.9	37.9	42.6	39.5	44.2
BotB	37.3	31.6	38.5	39.2	31.6	31.9	35.5	28.9	39.2	32.6	36.5
CalfB	38.7	35.5	40.8	39.6	33.1	33.3	38.9	33.3	39.9	35.7	39.8
FecB	41.5	37.8	41.7	41.4	30.7	31	38.9	36.5	40.3	38	40.8
EqcB	39.9	38.3	43.5	43.1	28.9	32.8	36.9	36.5	40.6	37.1	38.3
HosB	43.3	33.7	40.1	41	30.4	28.8	39.9	36.9	41.5	39.9	40.2
EqcD1	50.2	44.8	51.6	48.2	62.3	64.3	39.3	34.1	39.1	34.1	40.1
HosE	41.2	37.1	40.3	40.6	36.7	36.1	79.4	36.2	83.5	34.6	76.9
EqcW1	-	61.7	69	69.6	41	41.3	41.8	36.6	41.4	37.6	40.8
FecW1	40.1	-	66.7	70.4	36.7	35.8	38.7	33.3	37.8	31.6	37.7
HosW	33.6	34.5	-	73.8	44	43.8	41.5	39.8	40.2	39.6	40
HosT	31.7	30.1	27.3	-	44.6	38.4	43.3	37.9	41.8	38.4	44.4
FecDp	66	73.3	63.7	63	-	49.7	38.3	28	36.4	28.3	38.3
HosD	70.1	80.2	60.9	72.6	36.3	-	31.5	31.7	31.9	28.1	32.9
CalfE	78.8	96.1	85.3	77.9	84.2	103.9	-	39	82.6	36.7	88.3
CalfK	128.1	121.3	103	121.3	135.1	142.4	99.4	-	37.5	82.1	32.2
EqcE	74.7	93.3	82.2	77.8	84	98.6	15.4	100	-	37.1	80.9
EqcK	136.6	130	103.2	117.4	127	145.3	98.9	16.8	99.5	-	32.9
FecE	81.6	96.7	92.5	76.4	80.8	108.6	11.8	108.5	17.3	101.9	-

Percent similarity in upper triangle and percent divergence in lower triangle



**Figure 3. Phylogenetic tree analysis of all type I IFNs including the novel identified IFNs.** (A) Overview for the phylogenetic tree of type I IFNs. Symbols inside the IFN $\alpha$  clade are human, bat, cow, pig, cat, dog, mouse, respectively. (B) Details for IFN $\delta$  clades. (C) Details for the IFN $\epsilon$  clades. (D) Details for the IFN $\kappa$  clades. Bootstrap values over 75% are shown.

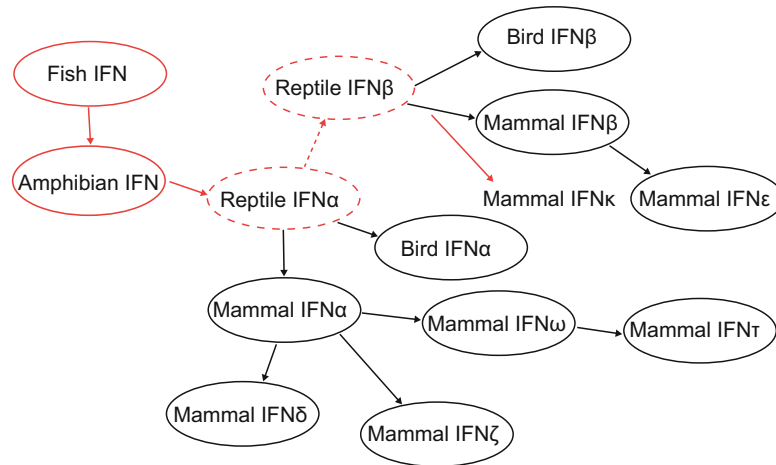
IFN $\tau$  maybe the last mammalian type I IFN to evolve.

IFN $\alpha$  generally forms species-specific clades in the IFN $\alpha$  subgroup unless the two species in question are closely related. This renders direct homologs of IFN $\alpha$  between different species difficult to find, suggesting a high selection pressure on IFN $\alpha$  during evolution.

**Overview of type I IFN evolution**

On the basis of the phylogenetic analysis above and the known characteristics of type I IFNs, we propose a hypothesis for type I IFN evolution: The ancestral type I IFN gene likely

contained introns, similar to piscine type I IFNs, and was liable to duplicate which seems to be the ancestor of IFN $\alpha$  genes instead of IFN $\beta$  genes. Synchronously with species evolution, type I IFN genes retain original characteristics in amphibian genomes. IFN $\beta$  evolved from the ancestral IFN $\alpha$ -like genes soon after reptiles arose (Fig. 4). This ancient IFN $\beta$  gene may be encoded by several exons, as no obviously homologs of avian IFN $\beta$  genes can be found in the green lizard genome, and this gene represents the ancestor of avian and mammalian IFN $\beta$  genes, as avian type I IFNs are homolog of mammalian type I IFNs (Sick et al., 1998). Consistent with species evolution from



**Figure 4. Evolution history of type I IFNs.** The type I IFN genes of fish and Amphibian contain 4–5 introns. No reptile IFN genes were cloned as recent studies, but it is no doubt that the IFN $\alpha$  genes do exist as the completeness of the evolution routes. The red circle denotes a gene with introns, and dotted line represents a gene or a route which is inferred.

reptiles to birds and mammals, a retrotransposition occurred in the type I IFNs other than IFN $\kappa$ , as evident by the fact that most bird and mammal type I IFNs are intronless. IFN $\kappa$  genes may have arisen from a putative reptile IFN $\beta$  gene before this retrotransposition occurred, and avian type I IFNs remain in their ancestral state. In contrast, mammals encountered more evolutionary pressure to survive in tougher environments, and their type I IFNs divided into many subtypes.

IFN $\beta$  genes duplicated, and one of them represented the ancestor of mammalian IFN $\epsilon$ . This subtype of type I IFNs likely arose at the very beginning of mammalian evolution as practically every mammal possess IFN $\beta$  and IFN $\epsilon$ . Afterward, IFN $\beta$ , together with IFN $\epsilon$  and IFN $\kappa$ , remained untouched as duplications of these genes seem to be difficult (only platypus and cow have two IFN $\beta$ -like genes) for unknown reasons.

As avian IFN $\alpha$  and mammalian IFN $\alpha$  genes are strict homologues of the ancient IFN $\alpha$ -like genes and IFN $\alpha$  genes show broader distribution than the other multigene Type I IFNs, the complex mammalian Type I IFN system should be shaped by ancient mammalian IFN $\alpha$  genes. In addition, mammalian IFN $\alpha$  exhibits common activities compared with the other mammalian IFNs. In contrast to IFN $\beta$ , it is much easier to duplicate IFN $\alpha$  as approximately 10 IFN $\alpha$  genes are present in most vertebrates. With selective pressure, some of these genes gained specific functions. One of these genes became the forerunner of IFN $\omega$  and duplicated into a cluster of genes. Afterward, a few of the IFN $\omega$  genes in the ruminant ancestor diverged into IFN $\tau$  to help form placentas. One of the ancient IFN $\alpha$  genes evolved into the forerunner of the IFN $\delta$  and IFN $\zeta$  genes. The divergence of IFN $\delta$  and IFN $\zeta$  is likely different results of the same selection pressure on different species. During this selection, IFN $\delta$  gained a reproductive response element in the porcine and ovine genomes, while it failed in the mouse genome.

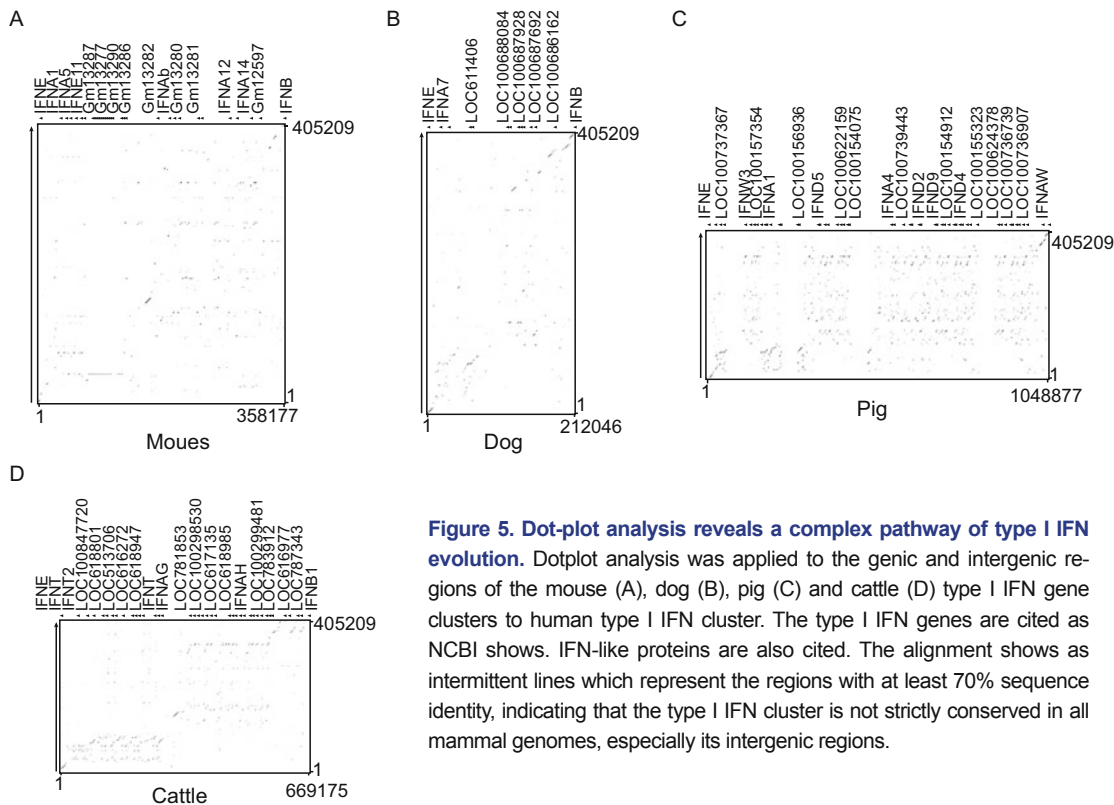
#### Synteny alignment of the mouse, dog, pig, and cow type I IFN clusters against the human type I IFN cluster

The type I IFN cluster is not strictly conserved in all mammal genomes, especially interspersed regions. Part of the limitin genes displays limited similarity with human IFN $\alpha$ , and all of the limitin genes are located together in the type I IFN cluster (Fig. 5). This indicates that the master force for limitin gene family conformation was duplication instead of conversion, and this kind of duplication occurred after the speciation of mice. All mouse IFN $\alpha$  genes have homologous regions in the human genome, and most of the upstream region of these genes is conserved, ensuring that IFN $\alpha$  can be induced by viral infection and pathological processes. The corresponding region of human IFN $\omega$  in the mouse genome seems to be mouse IFN $\alpha$ 14, which is evidence for the hypothesis that IFN $\omega$  evolved from IFN $\alpha$ .

IFN $\tau$  cannot be identified in the human type I IFN cluster, and only a previously described pseudogene located outside of the gene cluster on the IFN $\beta$  side. However, a discontinuous region of the human genome displays limited conservation with IFN $\tau$  genes, and further analysis revealed that these regions are microRNAs, such as the MIR31 host gene.

#### DISCUSSION

In the present study, an IFN $\delta$ -like gene in the human genome was identified, refuting the concept that humans have no IFN $\delta$  genes (Pestka et al., 2004). This gene is located on chromosome 9, with a pseudogene named IFNA12p inside of it. Both phylogenetic and sequence similarity analyses demonstrated that this gene is an IFN $\delta$  gene. In the phylogenetic tree, the mammalian IFN $\beta$ , IFN $\epsilon$ , and IFN $\kappa$  genes formed a clade separate from the other mammalian type I IFNs, while piscine and avian IFNs formed distinct clades. Based on our phylogenetic



**Figure 5. Dot-plot analysis reveals a complex pathway of type I IFN evolution.** Dotplot analysis was applied to the genic and intergenic regions of the mouse (A), dog (B), pig (C) and cattle (D) type I IFN gene clusters to human type I IFN cluster. The type I IFN genes are cited as NCBI shows. IFN-like proteins are also cited. The alignment shows as intermittent lines which represent the regions with at least 70% sequence identity, indicating that the type I IFN cluster is not strictly conserved in all mammal genomes, especially its intergenic regions.

analysis and the known characteristics of the various type I IFNs, we hypothesized the evolutionary history of type I IFNs. In this evolution route, IFN $\alpha$  formed a core from which new IFNs divided during vertebrate evolution. One of the IFN $\alpha$  genes evolved away from the other genes and became the ancestor of IFN $\beta$  in reptiles, and most of these genes lost introns through retrotranspositions when birds and mammals diverged from reptiles. The ancestor IFN $\alpha$ -like genes duplicated more frequently than the ancestor IFN $\beta$ -like gene and divided into several subtypes (including IFN $\delta$ , IFN $\tau$ , and IFN $\zeta$ ) during the evolution of some animals, while IFN $\beta$  evolved into IFN $\epsilon$  and IFN $\kappa$  at the very beginning of the emergence of mammals.

The doubts and suspicions in this evolutionary model focus on the time when the type I IFN introns disappeared and whether reptiles genomes encode IFN $\beta$  genes. Type I IFNs exist in all kinds of vertebrates, but only the fish and amphibian type I IFN genes contain four introns as recent research (Qi et al., 2010). In the fish genome, IFNa, IFNb, IFNc and IFNd are considered to be different kinds of type I IFNs that only exists in fish and not strictly orthology of higher vertebrates IFN genes. Both IFNa, IFNb, IFNc and IFNd displays a considerable distance to the other type I IFNs in our phylogenetic tree, but IFNa and IFNc show similar antiviral activities and ability to induce antiviral genes, like mammalian IFN $\alpha$  do, while IFNb and IFNd show little activities (Sun et al., 2009; Svingerud et al., 2012).

When the introns of these type I IFN genes disappeared remains unknown, but the IFN genes in amphibians may have

introns (Qi et al., 2010). It is very likely that reptile genomes encode IFNs for the sake of the completeness of evolution routes, even though no reptiles IFN gene has yet been cloned, only been inferred. The failure to detect reptile IFNs when using chicken IFN $\alpha$  genes with BLAST against the green lizard genome suggests that IFN genes in reptiles are much similar to fish IFN genes than those in warm-blooded animals. However, the IFN $\kappa$  genes of mammals have one intron each, suggesting that the existence of intron-containing reptile IFN $\beta$  genes exist.

Amphibian IFNs represent as intron-containing IFNs with five different molecules as inferred by present research, however, functional study on frog type I IFNs is still needed (Qi et al., 2010). A retrotransposition event likely occurred to type I IFNs during reptile evolution as avian and mammalian type I IFNs are intronless genes. Type I IFN genes in lizards may contain introns as no obvious homologs of avian and mammalian type I IFN genes can be found in the green lizard genome. The situation in snakes and turtles remains unknown, and perhaps intron-containing and intronless type I IFN genes coexist in their genomes. IFN $\beta$  genes likely exist in reptile genomes because these genes appear to exist in all avian and mammalian genomes. The IFN $\beta$  gene may have duplicated during reptile evolution into mammals, and retrotransposition occurred simultaneously. Further, the IFN $\kappa$  genes in mammals each contain an intron, and IFN $\epsilon$  genes are intronless, suggesting the possibility that duplicated IFN $\beta$  genes or the ancestor of

IFN $\kappa$  genes exist in reptilian genomes.

Even though all type I IFNs bind to the same receptors, different IFN display different bioactivities. The functions of type I IFN maintain good relation with their evolutions. The antiviral activity and anti-proliferation activity of IFN $\beta$  is lower than most of the IFN $\alpha$ s, and the antiviral activity of IFN $\alpha$ -evolved IFNs is stronger than that of IFN $\kappa$  and IFN $\epsilon$ s (Sang et al., 2010; La-voie et al., 2011). The ISGs that IFN $\beta$  induces tend to regulate host immunoreactions, while IFN $\alpha$  tends to induce antiviral proteins, such as 2'-5' OAS and PKR (Qu et al., 2013). This may be caused by the different ways that they interact with IFN receptor subunits. Despite their differences in sequence, IFN $\omega$  demonstrates little difference with IFN $\alpha$  in their expression, function, ternary complex structures with receptors, and abilities to induce ISGs (Thomas et al., 2011). Expressed by trophoblasts before they attach to the placenta, IFN $\tau$  is important to prevent the corpus luteum from degradation and, hence, ensuring the pregnancy continues, while IFN $\delta$  is expressed in conceptus and shows a potent ability to regulate pregnancy (Chelmońskasoyta, 2002; Roberts, 2007). It has been hypothesized that IFN $\tau$  is not virally inducible and that its function has no relationship to pathogenesis, which is quite different from the other multigene Type I IFNs, but recent research shows that bovine IFN $\tau$  is an antiviral protein capable of inducing 2'-5' OAS with less toxicity to the cells, which suggests that IFN $\tau$  could be a better drug than IFN $\alpha$  for patients suffering from viruses (Johnson et al., 2001). The IFN $\beta$ -evolved IFNs, IFN $\epsilon$  and IFN $\kappa$ , tends to induce effectors which can regulate host immunoreactions. Over expression of IFN $\kappa$  in pancreatic islets can induce diabetes in mouse, and recent research has shown that the expression of IFN $\kappa$  in skin has a close association with systemic lupus erythematosus and inflammation (Vassileva et al., 2003; Harley et al., 2010). IFN $\epsilon$  can enhance the lymphocyte recruitment to lung alveoli with reduced inflammation, promote migration of antigen-specific CD8<sup>+</sup> T cells to the gut, which suggests IFN $\epsilon$  an important role in mucosal immunity (Xi et al., 2012). This relationship between evolution and function of type I IFN may provide a new view for type I IFN function analysis.

## MATERIALS AND METHODS

### Genome selection

A total of 496 IFN sequences from 99 species were acquired from NCBI (<http://www.ncbi.nlm.nih.gov/>). Allelic genes and pseudogenes were omitted for the sake of phylogenetic analyses. The IFN genes from human, mouse, and pig have already been characterized in detail before, and some of the features of IFN $\tau$  genes from ruminants and IFN $\delta$  genes from pigs have also already been described (Bazer et al., 1997). To find undetected IFN genes, BLASTn (Buhler et al., 2007) was used to search 13 genomes (human, mouse, dog, cat, sheep, pig, cow, horse, chicken, zebrafish, green anole, Nile tilapia, and western clawed frog). Human IFN $\alpha$ 2b (AY255838.1), IFN $\omega$  (X58822.1), IFN $\beta$  (M28622.1), IFN $\epsilon$  (AY190045.1), and IFN $\kappa$  (AF315688.1), mouse IFN $\zeta$ 1 (NM\_197889.2), pig IFN $\delta$ 1 (GQ415074.1), and cow

IFN $\tau$ 1 (AF238611.1), chicken IFN $\alpha$ 1 (AB021153.1) and chicken IFN $\beta$  (AY974089.1) were chosen as initial queries. All of the default alignment/search parameters were used. Sequences displaying >10% similarity were collected for further identification. Short homologous sequences were extended to 600 bp to calculate the similarity with given sequences.

### Phylogenetic analyses

All of the IFN sequences were aligned with ClustalW (EBI, [www.ebi.ac.uk/clustalw/](http://www.ebi.ac.uk/clustalw/)) (Thompson et al., 2002). After manual correction of the sequences, a neighbor-joining tree was constructed using MAGE 5.0 with the following parameters: method = NJ, substitution model = Poisson correction method, and 1000 bootstrap replicates (Tamura et al., 2011). We also constructed a maximum likelihood tree with the following parameters to evaluate the reproducibility of the grouping of the IFN genes: method JTT and 1000 bootstrap replicates.

### Gene synteny analysis

MultiPipmaker was used to align both the genic and intergenic regions of the mouse, dog, pig, and cow type I IFN gene clusters to the human type I IFN cluster (Schwartz et al., 2003). Default parameters were used except that the "search one strand" and "single coverage" options were chosen. Genes were cited as shown by NCBI.

Competing Interests: The authors have declared that no competing interests exist.

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## ABBREVIATIONS

Bot, *Bostaurus* Cow; Crg, *Cricetulusgriseus* Hamster; Calf, *Canis lupus familiaris* Dog; Eqc, *Equuscaballus* Horse; Fec, *Feliscatus* Cat; Hos, *Homo sapiens* Human; ISG, interferon-stimulated gene; ISGF, interferon-stimulated gene factor; Mum, *Musmusculus* Mouse; Ova, *Ovisaries* Sheep; Ptv, *Pteropus vampyrus* Bat; Ran, *Rattusnorvegicus* Rat; Sus, *Susscrofa* Pig

## COMPLIANCE WITH ETHICS GUIDELINES

Xu Lei, Yang Limin, and Liu Wenjun declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

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