Genome-Wide Identification and Evolutionary Analysis of the Animal Specific ETS Transcription Factor Family

Zhipeng Wang and Qin Zhang

State Key Laboratory for Agrobiotechnology, Key Laboratory of Animal Genetics and Breeding of the Ministry of Agriculture, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China. Email: qzhang@cau.edu.cn

Abstract: The ETS proteins are a family of transcription factors (TFs) that regulate a variety of biological processes. We made genome-wide analyses to explore the classification of the ETS gene family. We identified 207 ETS genes which encode 321 ETS TFs from ten animal species. Of the 321 ETS TFs, 155 contain only an ETS domain, about 50% contain a ETS_PEA3_N or a SAM_PNT domain in addition to an ETS domain, the rest (only four) contain a second ETS domain or a second ETS_PEA3_N domain or another domain (AT_hook or DNA_pol_B). A Neighbor-Joining phylogenetic tree was constructed using the amino acid sequences of the ETS domain of the ETS TFs. The results revealed that the ETS genes of the ten species can be divided into two distinct groups. Group I contains one nematode ETS gene and 18 vertebrate animal ETS genes. Group II contains the majority of the ETS TFs and can be further divided into eleven subgroups. The sequence motifs outside the DNA-binding domain and the conservation of the exon-intron structural patterns of the ETS TFs in human, cattle, and chicken further support the phylogenetic classification among these ETS TFs. Extensive duplication of the ETS genes was found in the genome of each species. The duplicated ETS genes account for ~69% of the total of ETS genes. Furthermore, we also found there are ETS gene clusters in all of the ten animal species. Statistical analysis of the Gene Ontology annotations of the ETS genes showed that the ETS proteins tend to be related to RNA biosynthetic process, biopolymer metabolic process and macromolecule metabolic process expected from the common GO categories of transcriptional factors. We also discussed the functional conservation and diversification of ETS TFs.

Keywords: ETS transcription factors, metazoan animal, genome-wide, phylogenetic analysis
Introduction

Transcription factors (TFs) are the key regulators of gene expression at the transcriptional levels. They play crucial roles in the life cycle or biological processes of all living organisms, such as development, growth, and responses to environmental stimulus. TFs are usually classified into different families and subfamilies based on the sequence of DNA-binding domains they contain, which are highly conserved among species.\(^1,2\) Some of these families are common to most eukaryotic organisms, and some are specific to a given taxonomic group.

The ETS TF family is one of the largest families of TFs. All members of this family share a highly conserved DNA-binding domain of 85 amino acid residues named the ETS domain. The ETS family is further sub-classified into a number of subfamilies\(^3,4\) based on the sequence similarities of the ETS domain and the presence of additional conserved domains. The ETS TFs are present throughout the body and are involved in a wide variety of functions including the regulation of cellular differentiation, cell cycle control, cell migration, cell proliferation, apoptosis (programmed cell death), and angiogenesis.\(^4\) Many ETS TFs have been found to be associated with cancer through gene fusion. For example, the fusion of TEL to the JAK2 protein results in early pre-B acute lymphoid leukaemia.\(^4\) Some ETS TFs appear to regulate positively or negatively other transcription factor activities.\(^3\) This allows combinatorial control of gene expression and enhances the action specificity of the ETS-domain proteins. For example, Ets-1 interacts with bHLH proteins,\(^5\) which activates transcription regulation and enhances DNA binding. Since the ETS TFs are important factors in the network of protein-protein interactions that govern transcription regulation, identifying the extent of ETS TFs and the subfamilies they belong to at the genome-wide level is an important step to understand the gene regulatory network.

With genome sequence data for more and more species becoming available, it is now possible to compare the ETS genes among different animal species at the genome-wide level. Determining the phylogenetic relationships of the ETS genes is an important step for elucidating the evolutionary and functional divergence of this gene family. Phylogenetic analyses have been conducted for many other TF families including the bHLH family,\(^7,8\) the homeobox family,\(^9,10\) the nuclear receptor family,\(^11\) the WRKY family,\(^12\) the MADS family,\(^13\) the GATA family,\(^14,15\) the AP2 family,\(^16\) the DOF family,\(^17\) the SBP-box family,\(^18\) the heat shock family,\(^19\) the ERF family,\(^20\) the NF-Y family,\(^21\) the basic leucine zipper family,\(^22\) the Sox family,\(^23\) and the CCCH zinc finger family.\(^24\) As for the ETS family, Laudet\(^25\) studied the evolutionary relationship between the ETS genes using sequences of all known ETS family members extracted from the EMBL data library, Genbank, NBRF, and the Infobiogen network (www.infobiogen.fr). They showed that the genes of the ETS family can be divided into 13 groups namely ETS, ER71, GABP, PEA3, ERG, ERF, ELK, DETS4, ELF, ESE, TEL, YAN, and SPI.

In this study, we first collected all putative ETS TFs from 10 species and classified them into subfamilies based on their ETS domains. Then, we performed phylogenetic analysis to explore the evolutionary relationship of these ETS genes. The features of the gene structures, the patterns of the conserved motifs, and the function divergence were discussed as well.

Materials and Methods

Identification of ETS TFs and ETS genes

From the DBD database,\(^31\) we identified all putative TFs at the genome-wide level of ten species of the animal kingdom, one or two from each genus, for which the full genome sequences are available. The ten species are human (\textit{Homo Sapiens}), mouse (\textit{Mus musculus}), rat (\textit{Rattus norvegicus}), cattle (\textit{Bos Taurus}), chicken (\textit{Gallus gallus}), sea squirt (\textit{Ciona intestinalis}), frog (\textit{Xenopus tropicalis}), zebrafish (\textit{Danio rerio}), fruit fly (\textit{Drosophila melanogaster}), and nematode (\textit{Caenorhabditis elegans}).

Then, we collected the proteome sequences of all these TFs from the Flybase database (for fruit fly) (http://flybase.org/),\(^32\) and the ENSEMBL database release 47 (for all other species) (ftp://ftp.ensembl.org/pub/release-47/fasta/).\(^33,34\) We performed the HMMER (http://hmmer.wustl.edu/)\(^35\) search for the ETS domain in the sequences using the profile PF00178 of the Pfam database (http://pfam.sanger.ac.uk/).\(^36\) and refined the results manually to obtain the ETS TFs. The genes encoding these ETS TFs were identified according to their annotation information. In addition, we collected the genome sequences of all identified ETS genes from the two databases.
Phylogenetic analysis
For phylogenetic analysis, we considered only the amino acid sequences of the DNA-binding domains, i.e. the ETS domain, in the ETS TFs which exist in all or most species. For a gene with more than one splicing-isoforms, we retained only the longest sequence encoded by it. We used ClustalX (v1.81) for multiple sequence alignment with default settings and manually refined the alignment by removing the common gaps of some sequences. We used PhyML (v3.0) to construct the maximum likelihood (ML) phylogenetic trees with 1000 replicate bootstrap tests and set a cut-off bootstrap value of 65 to define clades in the ML trees. Representations of the calculated trees were constructed using MEGA (v4.0).

Exon-intron structure and motif analysis
The diagrams of the exon-intron structures of the ETS genes were obtained from the ENSEMBL database (http://www.ensembl.org/). The sequence logos were generated using the online platform Weblogo (http://weblogo.berkeley.edu/). We got the conserved motifs of the ETS proteins using the online platform MEME (http://meme.sdsc.edu/meme).

GO enrichment analysis
The gene ontology (GO) hierarchy annotations were downloaded from the Gene Ontology database (http://www.geneontology.org/). The enrichment of the GO categories was analyzed using the tool DAVID (http://david.abcc.ncifcrf.gov/home.jsp). DAVID calculates the functional enrichment score of the same gene set based on the GO categories including biological process, molecular function and cellular component. In addition to a p value, it also provides a FDR (false positive rate) value for each enrichment score. A FDR value of 0.05 was used as the significance threshold for defining a GO term.

Results
ETS subfamilies and distributions of ETS TFs in different subfamilies and in the ten species
We identified a total of 321 ETS TFs from the ten species, which are encoded by 207 genes. Of the 321 ETS TFs 155 contain only an ETS domain, about 50% contain a ETS_PEA3_N or a SAM_PNT domain in addition to an ETS domain, the rest (only four) contain a second ETS domain or a second ETS_PEA3_N domain or an another domain (AT_hook or DNA_pol_B). We classified the ETS TFs into seven subfamilies according to the domain combinations they contain. The distributions of the ETS TFs in different subfamilies and in the ten species are shown in Table 1. Subfamilies ETS and ETS&SAM_PNT exist in all of the ten species. Subfamily ETS&ETS_PEA3_N exists in 7 species. The other subfamilies exist only in one species. The number of ETS TF proteins (genes) varies in different species, from 11 (10) in nematode to 71 (29) in human. However, the proportions of the number of ETS TF proteins (genes) to the total number of TF proteins (genes) are very similar in all species, ranging from 2% ~3%, except in sea squirt (about 4%) and in nematode (about 1%).

Phylogenetic relationship of ETS genes in the ten species
Three ETS subfamilies, ETS, ETS&ETS_PEA3_N, and ETS&SAM_PNT, which distribute in all or most of the ten species and correspond to 203 ETS genes (Table 1), were used to construct the phylogenetic tree. We designate these genes as “aabbnn”, where “aa” is the abbreviation of the species (hs for human, bt for cattle, mm for mouse, etc); “bbb” refers to the subfamily (ets for subfamily ETS, pea for subfamily ETS&ETS_PEA3_N, sam for subfamily ETS&SAM_PNT), “n” refers to the sequence number of the ETS gene in this subfamily. For example, hsets1 refers to the first gene of subfamily ETS in human. The detailed information of these genes is given in Supplemental Table S1.

To resolve the phylogenetic relationship between the ETS family members, we constructed an unrooted Maximum likelihood (ML) phylogenetic tree (Fig. 1, Supplemental Fig. S3) for the 203 EST TFs from the 10 species based on the amino acid sequences of their ETS-domain. Of the 203 EST TFs, 197 were classified into two groups in the ML tree. The other 7, drets7, xtets11, ggets4, hsets14, bttets8, rnets2, and mmetts2, which were not able to be classified into these two groups, were independent from each other and hence removed from the ML tree. In the ML tree, Group I contains one nematode ETS TF and 11 vertebrate animal ETS TFs which can be divided into two clades. Group II contains 184 ETS TFs which are
Table 1. ETS TF subfamilies and distribution of ETS TFs in different subfamilies and in the ten species of animal kingdom. First row: numbers of proteins, second row: numbers of genes encoding these proteins.

| Subfamily* | ETS TFs/TFs (%) | Total No ETs TFs | Total No TFs | ETS TFs/TFs (%) |
|------------|-----------------|-----------------|--------------|-----------------|
|            |                 |                 |              |                 |
| Species    | ETS             | ETS SAM_PNT     | ETS PEA3_N   | ETS PEA3_N+2    | ETS DNA_pol_B | ETS_AT_hook & SAM_PNT |
|------------|-----------------|-----------------|--------------|-----------------|
| Human      | 30              | 34              | 0            | 0               | 0              | 0                        | 71 | 2740 | 2.6 |
| Mouse      | 15              | 20              | 0            | 0               | 0              | 0                        | 71 | 2930 | 2.0 |
| Rat        | 24              | 13              | 1            | 1               | 0              | 0                        | 28 | 1336 | 2.1 |
| Cattle     | 19              | 10              | 3            | 3               | 0              | 0                        | 28 | 1336 | 2.1 |
| Chicken    | 14              | 10              | 0            | 0               | 0              | 0                        | 0  | 176   | 0.9 |
| Zebrafish  | 14              | 10              | 0            | 0               | 0              | 0                        | 0  | 176   | 0.9 |
| Sea squirt | 11              | 8               | 1            | 1               | 0              | 0                        | 11 | 579   | 1.9 |
| Fruit fly  | 11              | 8               | 0            | 0               | 0              | 0                        | 11 | 579   | 1.9 |
| Nematode   | 9               | 12              | 0            | 0               | 0              | 0                        | 0  | 176   | 0.9 |
| Total      | 155             | 131             | 31           | 1               | 1              | 1                        | 1  | 104   | 1.3 |

ETS TFs are classified into different subfamilies according to the DNA-binding domains they contain. For example, ETS TFs in subfamily ETS contain only an ETS domain, those in subfamily ETS SAM_PNT contain an ETS domain and a SAM, PNT domain, those in subfamily ETS + 2 contain two ETS domains, etc.
further divided into 12 subgroups. This classification is in general consistent with that of Laudet et al.\textsuperscript{30} with minor difference (see Discussion). Following their nomenclature, we named group I SPI, and the 11 subgroups of group II, which are in common with their classification, ESE, TEL, ELF, DETS4, PEA3, ELK, ETS, ER71, GABP, ERF, and ERG. The other subgroup containing three nematode genes, cieets1, cieets4, and cieets8, which were not included in Laudet et al.\textsuperscript{30} was named CEETS. All members in group SPI contain only an ETS domain. Members in subgroups ELF, ELK, ER71, and ERF contain only the ETS-domain except cisam8 in ELF which contain the ETS and SAM-PNT domains. About 1/5 members in ERG contain only the ETS-domain and the rest contain the ETS and SAM_PNT domains. Members in PEA3 contain the ETS and ETS_PEA3 N domains except cieets2 and dmets4 which contain only the ETS domain. Members in ESE, TEL, DETS4, ETS, and GABP contain the ETS and SAM_PNT domain except dmets5, drets9, and xtets10 in ETS which contain only the ETS domain. In addition, using the ME (minimum evolution) and NJ (Neighbor-Joining) methods, we obtained trees with similar topology (data not shown). But in the ME tree, the subgroups PEA3, ELK, ETS, ER71, GABP, ERF, and ERG in the NJ and ML trees were merged into one group.

Based on the topology structure of the phylogenetic tree, we classified the ETS genes into four categories in the same way of Xiong et al.\textsuperscript{43} i.e. one-to-one category in which a gene in one species and its corresponding gene in the other species have a common ancestor, many-to-many category in which gene duplication occurred in one or some lineages, lineage-specific expansion category which includes clades that have two or more genes in one lineage and no gene in other lineages, and other. We then constructed the phylogenetic trees for three pairs of species, i.e. cattle and chicken, mouse and rat, and human and nematode, respectively. Based on these phylogenetic trees, we estimated the number of ancestral genes for each pair of species. For example, for the phylogenetic tree of cattle and chicken, there are 17 ancestral genes. From the phylogenetic tree for mouse and rat, we found all ETS genes in the two species are derived from 27 ancestral genes except genes rnpea1, mmpea1, and mmpea3.

Sequence logos
The ETS domain in the ETS TFs is necessary for the specific recognition of a purine-rich core sequence GGAA/T flanked by more variable but not random 5' and 3' sequences.\textsuperscript{44,45} The most conserved part of the ETS domain is the sequence MNY(DE)KLSR(GA)LRYYY (Fig. 2). However, considerable variation in this sequence was observed among different ETS subgroups in the ML tree (Table 2). Such variation may have relation to the subgroup-specific functions of the ETS proteins. Indeed, the alteration of a single amino acid at the carboxy-terminal end of the DNA-recognition helix in the ETS domain can markedly alter its DNA-binding specificity and its interactions with other transcription factors.\textsuperscript{46,47}

Genomic distribution and duplication of ETS genes
To determine the distribution and duplication of the ETS genes in the genome, we searched the DNA
sequence of each ETS genes in the genome database of each species and determined the chromosomal location of each ETS gene. Since the genome sequence of frog has not been assigned to individual chromosomes, we were not able to determine the chromosomal distribution of the ETS genes in frog. For all other species, the distributions of the ETS genes seem to be uneven among chromosomes, as having been observed for other gene families. Figure 3 illustrates the distribution of ETS genes in human genome (for other eight species, see Supplemental Fig. S4). Their chromosomal distribution patterns reveal that certain chromosomes and chromosomal regions have a relatively high density of ETS genes. For instance, in the human genome, four ETS genes are located on chromosome 1, whereas 12 chromosomes have no ETS gene at all.

There are some ETS genes that reside tandem next to one another. In this study, two or more ETS genes that occurred within a 200 kb genomic region were considered an ETS gene cluster. In Figure 3, these genes are marked with a red line. All species (except frog) considered in this study have one or more ETS gene clusters, and the larger the genome, the more such clusters. For example, the numbers of ETS gene clusters in human and nematode genome are five and one, respectively, which account for about 30% and 20% of the total ETS genes, respectively.

To detect the possible relationship between the ETS genes and the potential duplications of ETS genes in the genome, we constructed the phylogenetic tree for each species (data not shown). Genes at the terminal branches on the phylogenetic tree may represent recently duplicated genes. As shown in Figure 3, we identified 21 duplicated ETS genes (at nine terminal branches) in the human genome. Most of the duplicated genes are located on different chromosomes, a few of them are within a cluster defined as above. The numbers of duplicated ETS genes in other eight species are 17 in cattle, 20 in mouse, 20 in rat, 17 in chicken, 15 in zebrafish, 15 in frog, 8 in fruit fly, 4 in nematode, and 4 in sea squirt (see Supplemental Fig. S4).

**Structure analyses of the human, cattle, and chicken ETS genes**

We construct a ML phylogenetic tree (Fig. 4a) based on the ETS domain amino acid sequences of 29 human, 27 cattle, and 20 chicken ETS TFs. The topology of this tree is similar to that constructed using all 203 ETS TFs from the 10 species (Fig. 1). Furthermore, we analyzed the exon-intron structure of the human, cattle, and chicken ETS genes. Figure 4b

**Table 2. Core conserved sequence of the ETS domain in different (sub)groups in the ML tree.**

| Group | Sequence logo | Group | Sequence logo | Group | Sequence logo |
|-------|---------------|-------|---------------|-------|---------------|
| SPI   | MTKYKMARALRNYG | ESE   | MTEKLSRALRYYY | TEL   | MTKYKMSRALRHYY |
| ELF   | MNYETMGRALRYYY | DETS4 | MNYDCLKSRLQYY | PEA3  | MNYDCLKSRLRYY |
| ELK   | MNYDCLKSRLRYYY | ETS   | MNYEKLKRRLYYY | GABP  | MNYEKLKRRLYY |
| ERF   | MNYDCLKSRLRYYY | ERG   | MNYDCLKSRLRYYY | CEETS | MNYDMSRGRFLFY |

Figure 2. The whole sequence logo of the ETS domain and its core conserved sequence MAY(DE) KLSR(GA)LRYYY. The overall height of each stack indicated the sequence conservation at that position, whereas the height of symbols within each stack reflects the relative frequency of the corresponding amino acid.
shows the basic gene structural patterns of these group/subgroups. The genes in the same subgroup have similar structural pattern with minor exceptions in some subgroups. Exceptions occur in group SPI (gene hsets15), subgroups TEL (gene hsets9), ETS (gene hssam8), and ERF (gene btets6). The results also reveal that all ETS genes have two or more exons which encode an ETS domain except those in group SPI and subgroup ERG. The details of the structures of all ETS genes of the three species are given in Supplemental Figure S1.

Conserved motifs in ETS genes out off the conserved domains

We made MEME search of the conserved protein motifs flanking the ETS domain and other domains and uncovered 13 conserved motifs in the ETS TFs. As shown in Figure 4c, the ETS TFs in the same group (subgroup) share similar number and pattern of conserved motifs. The details of all conserved motifs of each ETS gene are given in Supplemental Figure S2. Each conserved motif appears only in one group (subgroup) except motif 3 and motif 5 that are found in two subgroups (ERG and ELK) and three subgroups (ELF, GABP, and ERF), respectively. The ETS TFs in group SPI, subgroups ESE, TEL, DETS4, ETS, and ER71 contain no such conserved motifs, but those in subgroup ELF contain eight motifs, three of which exist in the N-terminal of all ETS TFs in this subgroup, and the rest in the C-terminal of some proteins. For example, proteins hsets10, mmets6, rnets12, and btets4 contain motif 6 in their C-terminal, but the other ETS TFs do not.

Function analysis of the ETS genes

The ETS TFs have been proved to be related to many biological processes. To understand the genome-wide functions of the ETS family, we used the online software DAVID to interpret its functions using gene ontology hierarchies. We uploaded the human, mouse, rat, chicken, fruit fly and sea squirt ETS gene list, and compared it with the existing reference gene list. The significant GO terms (FDR < 0.05) are shown in Table 3. The detailed p value and FDR
value for each significant GO term in each species are given in Supplement Table S2. In the molecular function category, the significant GO terms include sequence specific DNA binding, nucleic acid binding, DNA binding, and transcription factor activity. In the biological process category, in addition to those which are in the common GO categories of transcriptional factors such as transcription, regulation of transcription and metabolic process, the ETS genes in the categories of RNA biosynthetic process, biopolymer metabolic process, macromolecule metabolic process also have highly significant enrichment annotation. Furthermore, positive regulation of transcription and cellular metabolic process also have highly significant enrichment annotation in the mouse ETS genes.

**Discussion**

**Features of the ETS TFs**

In this study, several features of the animal kingdom ETS TFs were revealed. First, the ETS TFs exist in all of the ten species studied. When searching for the ETS TFs in the yeast proteome, we did not find any homologues of ETS proteins. Several studies show that ETS TFs exist neither in plants, such as rice, Arabidopsis, and poplar, nor in bacteria and archaea. So, it seems that the ETS family is unique to metazoan animals, as suggested by Degnan and Laudet. Second, the number of ETS genes is proportional to the genome size. Although the numbers of ETS genes are different significantly in different lineages, the proportions of number of ETS genes to the total number of TF genes in lower organisms are similar to that in the higher organisms. The same phenomenon was also observed for non-ETS genes. Third, about 50% ETS TFs have either a SAM_PNT domain or a ETS_PEA3_N domain besides the ETS domain. The combination of DNA binding domains would be a source of generation of novel TFs, as suggested by Riechmann et al.
Table 3. The significant gene ontology (GO) terms of the ETS gene (FDR < 0.05).

| Category               | GO term   | GO definition                                                      | Species                                      |
|------------------------|-----------|--------------------------------------------------------------------|----------------------------------------------|
| **Molecular Function** | 0043565   | sequence-specific DNA binding                                      | human, mouse, rat, chicken, sea squirt, fruit fly |
|                       | 0003676   | nucleic acid binding                                               | human, mouse, rat, chicken, fruit fly        |
|                       | 0003677   | DNA binding                                                        | human, mouse, rat, chicken, fruit fly        |
|                       | 0003700   | transcription factor activity                                       | human, mouse, rat, chicken, fruit fly        |
|                       | 0030528   | transcription regulator activity                                    | human, mouse, rat, chicken, fruit fly        |
|                       | 0016563   | transcription activator activity                                    | human, mouse                                |
| **Cellular Component** | 0005634   | nucleus                                                            | human, mouse, rat, chicken                   |
|                       | 0043231   | intracellular membrane-bound organelle                             | human, mouse, chicken                        |
|                       | 0043227   | membrane-bound organelle                                           | human, mouse, chicken                        |
|                       | 0043229   | intracellular organelle                                            | human, mouse                                |
|                       | 0043226   | organelle                                                          | human, mouse                                |
|                       | 0044424   | intracellular part                                                 | human, mouse                                |
|                       | 0005622   | Intracellular                                                      | mouse                                        |
| **Biological Process** | 0006350   | Transcription                                                      | human, mouse, rat, chicken, fruit fly        |
|                       | 0010467   | gene expression                                                    | human, mouse, rat, chicken, fruit fly        |
|                       | 0010468   | regulation of gene expression                                       | human, mouse, rat, chicken, fruit fly        |
|                       | 0019219   | regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | human, mouse, rat, chicken, fruit fly        |
|                       | 0019222   | regulation of metabolic process                                     | human, mouse, rat, chicken, fruit fly        |
|                       | 0050789   | regulation of biological process                                    | human, mouse, rat, chicken, fruit fly        |
|                       | 0050794   | regulation of cellular process                                      | human, mouse, rat, chicken, fruit fly        |
|                       | 0031323   | regulation of cellular metabolic process                            | human, mouse, rat, chicken, fruit fly        |
|                       | 0006139   | nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | human, mouse, rat, chicken                   |
|                       | 0006351   | Transcription, DNA-dependent                                        | human, mouse, rat, chicken                   |
|                       | 0006355   | regulation of transcription, DNA-dependent                          | human, mouse, rat, chicken                   |
|                       | 0016070   | RNA metabolic process                                               | human, mouse, rat, chicken                   |
|                       | 0032774   | RNA biosynthetic process                                            | human, mouse, rat, chicken                   |
|                       | 0045449   | regulation of transcription                                         | human, mouse, rat, fruit fly                 |
|                       | 0065007   | biological regulation                                               | human, mouse, chicken                        |
|                       | 0043283   | biopolymer metabolic process                                        | human, mouse, chicken                        |
|                       | 0044237   | cellular metabolic process                                          | human, mouse                                |

(Continued)
Duplications of the ETS genes

Duplication at both gene and genome levels is a pervasive process and contributes to the origin of biological novelty in evolution. Duplications on genome level are thought to have occurred throughout the process of animal and plant evolution. Xiong analyzed TFs of the rice genome, and found twelve pairs of large duplicated segments which account for ~45% of the rice genome. About 62% (991) of the 1611 TF genes identified in rice reside in the duplicated segments, of which 592 are retained as duplicated pairs. From the phylogenetic tree for each of the ten animal species, we found that the duplicated ETS genes account for 69% of the total ETS genes, ranging from 36.4% in Sea squirt to 85% in chicken. High proportions of duplicated genes were also reported in other TF families, e.g. ~60% in GATA family in Arabidopsis.

In addition, we also observed an interesting phenomenon. In all studied endotherm animals (human, cattle, mouse, rat, and chicken), an ETS gene cluster located on one chromosome is duplicated on another chromosome. For example, in human a cluster (containing genes hssam2 and hssam10) residing on chromosome 11 at position q23.24 is duplicated on chromosome 21 at position q22 (containing genes hssam8 and hssam1). This kind of duplication can be used as a support for the vertebrate specific block duplication event, leading to increase of various paralogous copies of genes.

Functional divergence of ETS genes

The difference in gene structure and amino acid sequence among different subgroups provide us with some hints that ETS genes may have a variety of physiological functions. The variety of subgroups within group II reflects a big spectrum of structural and functional diversity of this group. It has been found through cell culture that some ETS proteins, e.g. those which are classified into group SPI and subgroups ELF, ETS, and ERG preferentially expressed in cells which are developed from mesoderm, such as hematopoietic, vascular endothelial, kidney, intestine, and liver cells. Sharrocks proved that the ETS TFs were involved in various processes during embryonic development in several organisms, such as fruit flies, worms, fishes, frogs and mice. Multiple ETS factors have been found to be associated with cancer. For example, the ERG ETS TF is fused to the EWS gene. Many ETS TFs are known to represent nuclear targets of signaling pathways. Some ETS-domain subfamily play key role in immune system. Gene function prediction of the mouse and human ETS TF family was performed in this study. Besides the common GO categories of TFs, many ETS TFs in mouse have significant enrichment annotation in

---

Table 3 (Continued)

| Category | GO term | GO definition | Species |
|----------|---------|---------------|---------|
| 0044238  | primary metabolic process | human, mouse |
| 0043170  | macromolecule metabolic process | human, mouse |
| 0006357  | regulation of transcription from RNA polymerase II promoter | human, mouse |
| 0008152  | metabolic process | human, mouse |
| 0006366  | transcription from RNA polymerase II promoter | human |
| 0045935  | positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | mouse |
| 0045941  | positive regulation of transcription | mouse |
| 0031325  | positive regulation of cellular metabolic process | mouse |
| 0009893  | positive regulation of metabolic process | mouse |
categories of cell cycle, organ development, and cell differentiation. However, the categories of immune system process and immune response do not have significant enrichment annotation.

The overall conservation of protein sequences often implies the similar molecular and biological functions of them. The ETS TFs of the same group or subgroup usually have equivalent or similar biological functions. For example, the members in group SPI, including three human, three mouse, three rat, two cattle, two chicken, two zebrafish, three frog, and one nemolade ETS genes, were reported to be involved in immune system such as B-cell function and myeloid and lymphoid differentiation. However, some ETS TFs of the same group play different biological roles. For example, genes drpea1, hspea1, and mmpea4 (named pea3 in NCBI) and genes ggpea1, hspea2, and mmpea3 (named er81 in NCBI) in subgroup PEA3 are involved in muscle differentiation and directing sensory-motor neuron connections, respectively; genes mmmsam7, mmsam10, and hssam10 (named ETS1 in NCBI) and genes ggsam3, hssam8, mmsam4 (named ETS2 in NCBI) in subgroup ETS are related to T-cell survival and hair development, respectively; genes hsets5 and mmnets10 (named Sap1 in NCBI) and gene ceets5 named lin1 in NCBI in subgroup ELK participate in T-cell differentiation and vulval development, respectively. Moreover, some particular ETS genes possess multiple biological functions, for example, genes ggsam3, hssam8, mmsam4 (named ETS2 in NCBI) plays roles in extraembryonic tissue generation, extracellular matrix remodeling, and hair development; genes ggsam5, hssam7, mmsam10 (named Tel in NCBI) plays roles in yolk-sac angiogenesis and adult haematopoiesis.

Evolution of the ETS genes

We constructed the molecular phylogenetic tree of the ETS TF family for ten species of animal kingdom. The overall divergence pattern of the ETS genes appears similar to that of other gene families such as the homeobox family and the nuclear receptor genes. Laudet et al. constructed a phylogenetic tree of the ETS gene family using 61 known ETS genes and showed the ETS TF family members can be classified into 13 groups, which could be further clustered into five subfamilies. Our classification is in general consistent with theirs. The ETS genes which were included in both studies were all classified into same groups except that the gene Drosophila YAN (named dmsam5 in our study) was classified into group YAN which contains only this gene, while in our study it was classified into group TEL. In addition, the three nematode genes, ceets1, ceets4 and ceets8, which were not included in Laudet et al., were classified into one subgroup named CEETS. So, the total number of subgroups in group II is the same in both studied. Furthermore, in our phylogenetic tree, the 12 subgroups in group II are all in relatively independent branches, while in their phylogenetic tree, groups ETS, ER71, GABP, PEA3, ERG, ERF and ELK are merged into a large branch and thus are classified into one subfamily (named ETS), similarly, groups ELF and ESE are classified into another subfamily (named ELF), and the other groups are in different individual subfamilies.

The ETS TFs within each subgroup generally contain the same domain combination. With only minor exceptions, the ETS TFs in subgroups ELF, ER71, ELK, CEETS and ERF contain only one ETS domain, those in subgroup PEA3 contain both ETS and ETS_PEA3_N domains, and those in subgroups ESE, TEL, DETS4, and ETS contain both ETS and SAM_PNT domains. So, we infer that the ancestor gene of group II might have duplicated into three copies. The first copy might evolve to subgroups ELF, ER71, ELK, CEETS and ERF contain only one ETS domain, those in subgroup PEA3 contain both ETS and ETS_PEA3_N domains, and those in subgroups ESE, TEL, DETS4, and ETS contain both ETS and SAM_PNT domains). So, we infer that the ancestor gene of group II might have duplicated into three copies. The first copy might evolve to subgroups ELF, ER71, ELK, CEETS and ERF contain only one ETS domain, those in subgroup PEA3 contain both ETS and ETS_PEA3_N domains, and those in subgroups ESE, TEL, DETS4, and ETS contain both ETS and SAM_PNT domains. A confused situation is that in subgroup ERG about 2/3 ETS TFs contain both ETS and SAM_PNT domains and the rest contain only one ETS domain.

Our results show the ETS genes of mammalian animals exist in both groups I and all subgroups of group II. So, we infer that the diversification of these genes predates the divergence of mammalian animals. Moreover, Degnan and Laudet suggested that the diversification of the ETS TF family was already achieved before the separation of the major phylum of metazoans. We deem that the question of the origin of the ETS genes remains open, and that it would be interesting to investigate the ETS genes in other lower metazoan animals.

Acknowledgements

This study was supported by the National Basic Research Program of China (Grant NO. 2006CB102104) and the Key project of the National Natural Science Foundation of China (Grant No. 30430500).
Disclosures
The authors report no conflicts of interest.

References
1. Luscombe NM, Austin SE, Berman HM, Thornton JM. An overview of the structures of protein—DNA complexes. *Genome Biol.* 2000;1(1):1–37.
2. Riechmann JL, Heard J, Martin G, Reuber L, et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science.* 2000;290:2105–10.
3. Gutierrez-Hartman A, Duval DL, Bradford AP. ETS transcription factors in endocrine systems. *Trends Endocrinol Metab.* 2007;18(4):150–8.
4. Sharrocks AD. The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol.* 2001;2(11):827–37.
5. Butticè AG, Dutereque-Coquillaud M, Basuyaux JP, Carrere S, Kurkinen M, SteA helin D, Erg, an Ets-family member, differentially regulates human collagenase1 (MMP1) and stromelysin1 (MMP3) gene expression by physically interacting with the Fox/Jun complex. *Oncogene.* 1996;13:2297–306.
6. Sieweke MH, Tekotte H, Jarosch U, Graf T. Cooperative interaction of ets-1 with USF-1 required for HIV-1 enhancer activity in T cells. *EMBOJ.* 1998;17:1728–39.
7. Li J, Liu Q, Qiu MS, Pan YC, Li YX, Shi TL. Identification and analysis of the mouse basic/Helix-Loop-Helix transcription factor family. *Biochemical and Biophysical Research Communications.* 2006a;350:648–56.
8. Wang Y, Chen K, Yao Q, Wang W, Zhi Z. The basic helix-loop-helix transcription factor family in Bombby mori. *Dev Genes Evol.* 2007;217:715–23.
9. Toledo-Ortiz G, Huq E, Quail PH. The Arabidopsis Basic/Helix-Loop-Helix Transcription Factor Family. *The Plant Cell.* 2003;15:1749–70.
10. Marc AH, Marc J, Martin W, Cathie M, Bernd W, Paul CB. The Basic Helix-Loop-Helix Transcription Factor Family in Plants: A Genome-Wide Study of Protein Structure and Functional Diversity. *Mol Biol Evol.* 2003;20(5):735–47.
11. Li X, Duan X, Jiang H, et al. Genome-Wide Analysis of Basic/ Helix-Loop-Helix Transcription Factor Family in Rice and Arabidopsis, *Plant Physiol.* 2008b;141:1167–84.
12. Holland PW, Booth HA, Bruford E. Classification and nomenclature of all human homeobox genes. *BMC Biology.* 2007;5:47–73.
13. Lau, Q. Evolution of the nuclear receptor superfamly: early diversification from an ancestral orphan receptor. *J Mol Endocrinol.* 1997;19:207–26.
14. Wu KL, Guo ZJ, Wang HH, Li J. The WRKY family of transcription factors in rice and Arabidopsis and their origins. *DNA Res.* 2005;12:9–26.
15. Zhang Y, Wang L. The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. *Mol Biol Evol.* 2005;5:1–12.
16. Zahm LM, Kong H, Leebens-Mack JH, et al. The evolution of the SEPALATA subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history. *Genetics.* 2005;169:2209–23.
17. Immin NG, Ferrario S, Busscher-Lange J, Kooiker M, Busscher M, Angenent GC. Analysis of the petunia MADS-box transcription factor family. *Mol Gen Genomics.* 2003;268:598–606.
18. Reyes JC, Muro-Pastor M, Florencio FJ. The GATA family of transcription factors in Arabidopsis and rice. *Plant Physiol.* 2004;134:1718–32.
19. Shigyo M, Hasebe M, Ito M. Molecular evolution of the AP2 subfamily. *Genes.* 2006;366:383–402.
20. Moreno-Risueno MA, Martinez M, Vicente-Carbajosa J, Carbonero P. The family of DOF transcription factors: from green unicellular algae to vascular plants. *Mol Genet Genomics.* 2007;277:379–90.
21. Shigyo M, Tabei N, Yoneyama T, Yanagisawa S. Evolutionary processes during the formation of the plant-specific DOF transcription factor family. *Plant Cell Physiol.* 2007;48:179–85.
22. Guo A, Zhu QH, Gu XC, Ge S, Yang J, Luo JC. Genome-wide identification and evolutionary analysis of the plant specific SBP-box transcription factor family. *Gene.* 2008a;418:1–8.
23. Cardon G, Hohmann S, Klein J, Nettesheim K, Saedler H, Hülsler P. Molecular characterisation of the Arabidopsis SBP-box genes. *Gene.* 1999;237:91–104.
24. Guo JK, Wu J, Ji Q, et al. Genome-wide analysis of heat shock transcription factor families in rice and Arabidopsis, *J Genet Genomics.* 2008b;35:105–18.
25. Toshtsu N, Kaoru S, Tatsuhiro F, Hideaki S. Genome-Wide Analysis of the ERF Gene Family in Arabidopsis and Rice. *Plant Physiology.* 2006;140:411–32.
26. Stephenson TJ, McIntyre CL, Coller C, Xue GP. Genome-wide identification and expression analysis of the NF-Y family of transcription factors in *Tricharium asarium,* *Plant Mol Biol.* 2007;65:77–92.
27. Nijhawan A, Jian M, Tyagi AK, Khurana JP. Genome-Scale Survey and Gene Expression Analysis of the Basic Leucine Zipper Transcription Factor Family in Rice. *Plant Physiology.* 2008;146:333–50.
28. Koopman P, Schepers G, Brenner S, Venkatesh B. Origin and diversity of the Sox transcription factor gene family-genome-wide analysis in Fugu rubripes. *Gene.* 2004;328:177–86.
29. Wang D, Guo YH, Wu CG, Yang GD, Li Y, Zheng CC. Genome-wide analysis of CCCH zinc ﬁnger family in Arabidopsis and rice. *BMC Genomics.* 2008;9:44–54.
30. Lauvet V, Hainni C, Stelhelin D, Dutereque-Coquillaud M. Molecular phylogeny of the ETS gene family. *Oncogene.* 1999;11;18(6):1351–9.
31. Wilson D, Charomassawan V, Kummerfeld SK, Teichmann SA. BDIX taxonomically broad transcription factor predictions: new content and functionality. *Nucleic Acids Res.* 2008 Jan;36:D88–92.
32. Wilson RJ, Goodman JL, Steblet VB, FlyBase Consortium. FlyBase: integration and improvements to query tools. *Nucleic Acids Res.* 2008 Jan;36:D588–93.
33. Sterk P, Kulikova T, Kersey P, Apweiler R. The EMBL Nucleotide Sequence and Genome Reviews Databases. *Methods Mol Biol.* 2007;406:1–22.
34. Kulikova T, Akhter R, Aldebert P, Althorpe N, EMBL. Nucleotide Sequence Database in 2006. *Nucleic Acids Res.* 2007 Jan35:D16–20.
35. Eddy SR. Profile hidden Markov models. *Bioinformatics.* 1998;14:755–63.
36. Finn RD, Mistry J, Schuster-Bockler B, Griffiths-Jones S, Hollich V, Pfnam: clans, web tools and services. *Nucleic Acids Res.* 2006;34(Database issue):D247–51.
37. Higgins DG, Thompson JD, Gibson TJ. Using CLUSTAL for multiple sequence alignments. *Methods Enzymol.* 1996;266:383–402.
38. Guindon S, Gascuel D. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology.* 2003;52(5):696–704.
39. Kumar S, Dudley J, Nei M, Tamura K. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics.* 2008;9:299–306.
40. Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: A sequence logo generator, *Genome Research.* 2004;14:1188–90.
41. Bailey TL, Williams N, Misleh C, Li WW. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Research.* 2006;34:W369–73.
42. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* 2009;4(1):44–57.
43. Xiong Y, Liu T, Tian C, Sun S, Li J, Chen M. Transcription factors in rice: a genome-wide comparative analysis between monocots and eudicots. *Plant Molecular Biology.* 2005;59:191–203.
44. Karam FD, Urenas LD, Thummel CS, et al. The Ets-domain: a new DNA-binding motif that recognizes a purin-rich core DNA sequence. *Genes Dev.* 1990;4:1451–3.
45. Wang CY, Petryniak B, Ho IC, Thompson CB, Leiden JM. Evolutionarily conserved Ets family members display distinct DNA binding specificities. *J Exp Med.* 1992;175:1391–9.
46. Shore P, Whitmarsh AJ, Bhaskaran R, Davis RJ, Waltho JP, Sharrocks AD. Determinants of DNA-binding specificity of ETS-domain transcription factors. *Mol Cell Biol.* 1996;16:3338–49.
47. Fitzsimmons D, Hodson W, Wheat W, Maira SM, Wiselyk B, Hagman J. Pax-5 (BSAP) recruits Ets-prote oncogene family proteins to from functional ternary complexes on a B-cell-specific promoter. *Genes Dev.* 1996;10:2198–211.
48. Oliver B, Misteli T. A non-random walk through the genome. *Genome Biol.* 2005;6(4):214–29.
49. Gao G, Zhong Y, Guo A, Zhu Q, Tang W. DRTF: a database of rice transcription factors. Bioinformatics. 2006;22:1286–7.

50. Guo A, He K, Liu D, et al. DATF: a database of Arabidopsis transcription factors. Bioinformatics. 2005;21:2568–9.

51. Zhu QH, Guo AY, Gao G, et al. DPTF: a database of poplar transcription factors. Bioinformatics. 2007;23:1307–8.

52. Minezaki Y, Homma K, Nishikawa K. Genome-Wide Survey of Transcription Factors in Prokaryotes Reveals Many Bacteria-Specific Families Not Found in Archaea. DNA Research. 2005;12:269–80.

53. Degnan BM, Degnan SM, Nagagnuma T Morse DE. The ets multigene family is conserved throughout the Metazoa. Nucl Ac Res. 1993;21:3479–84.

54. Lauder V, Niel C, Duterque-Coquillaud M, Leprince D, Stehelin D. Evolution of the ets gene family. Biochem Biophys Res Com. 1993;190:8–14.

55. Shiu SH, Shih MC, Li WH. Transcription Factor Families Have Much Higher Expansion Rates in Plants than in Animals. Plant Physiology. 2005;139:18–26.

56. Adams KL, Wendel JF. Polyploidy and genome evolution in plants. Curr Opin Plant Biol. 2005;8:135–41.

57. Kent WJ, Baertsch R, Hinrichs A, Miller W, Haussler D. Evolution’s cauldron: duplication, deletion, and rearrangement in the mouse and human genomes. Proc Natl Acad Sci U S A. 2003;100:1148–9.

58. Cannon SB, Mitra A, Baumgarten A, Young ND, May G. The roles of segmental and tandem gene duplication in the evolution of large gene families in Arabidopsis thaliana. BMC Plant Biol. 2004;4:10–6.

59. Mehan MR, Freimer NB, Ophoff RA. A genome-wide survey of segmental duplications that mediate common human genetic variation of chromosomal architecture. Hum Genomics. 2004;1:335–44.

60. Katsanis N, Fitzgibbon J, Fisher EMC. Paralogy mapping: identification of a region in the human MHC triplicated onto human chromosomes 1 and 9 allows the prediction and isolation of novel PBX and NOTCH loci. Genomics. 1996;35:101–8.

61. Kasahara M, Hayashi M, Tanaka K, et al. Chromosomal localization of the proteasome Z subunit gene reveals an ancient chromosomal duplication involving the major histocompatibility complex. Proc Natl Acad Sci U S A. 1996;93:9096–101.

62. Tsuneyuki Oiikawa, Tosiyuki Yamada. molecular biology of the ETS family of transcription factors. Gene. 2003;303:11–34.

63. Wasylyk B, Hagman J, Gutierrez-Hartmann A. A Ets transcription factors: nuclear effectors of the Ras-MAP-kinase signalling pathway. Trends Biochem Sci. 1998;23:213–6.

---

**Publish with Libertas Academica and every scientist working in your field can read your article**

“I would like to say that this is the most author-friendly editing process I have experienced in over 150 publications. Thank you most sincerely.”

“The communication between your staff and me has been terrific. Whenever progress is made with the manuscript, I receive notice. Quite honestly, I’ve never had such complete communication with a journal.”

“LA is different, and hopefully represents a kind of scientific publication machinery that removes the hurdles from free flow of scientific thought.”

**Your paper will be:**
- Available to your entire community free of charge
- Fairly and quickly peer reviewed
- Yours! You retain copyright

[http://www.la-press.com](http://www.la-press.com)