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Whole genome-wide analysis of DEP family members in sheep (*Ovis aries*) reveals their potential roles in regulating lactation

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Abstract

Background: Sheep milk is an attractive nutritional and functional food source. Some Dishevelled, Egl-10 and pleckstrin (DEP) domain-containing proteins can regulate lactation performance in mammals. However, systematic study of the role of sheep DEP family members in regulating lactation is currently lacking. This study aimed to reveal the roles of sheep DEP family members in lactation by exploring their genetic characteristics and functional features at the whole genome-wide level.

Results: Twenty DEP family members were identified in the sheep genome, and they can be divided into four major groups. Ka/Ks calculations suggest that the purifying selection is the main pressure acting on DEP genes. In the late pregnancy and lactation periods, the expression levels of eight DEP genes exhibited significant differences in the mammary gland. In addition, nutritional conditions have a great influence on the expression of DEP family members, and the DEP gene family underwent more expansion than the average gene family in the early stages of biological evolution. The Mirrortree assays indicated that the DEP family members coevolved in biological evolution.

Conclusions: Our research provides a better understanding of the characters of the DEP domain-containing protein family and their potential roles in regulating lactation in sheep. Moreover, these results of our study may contribute to the genetic improvement of milk performance in dairy sheep breed.

Keywords: Lactation, DEP, Gene family, Expression, Evolution

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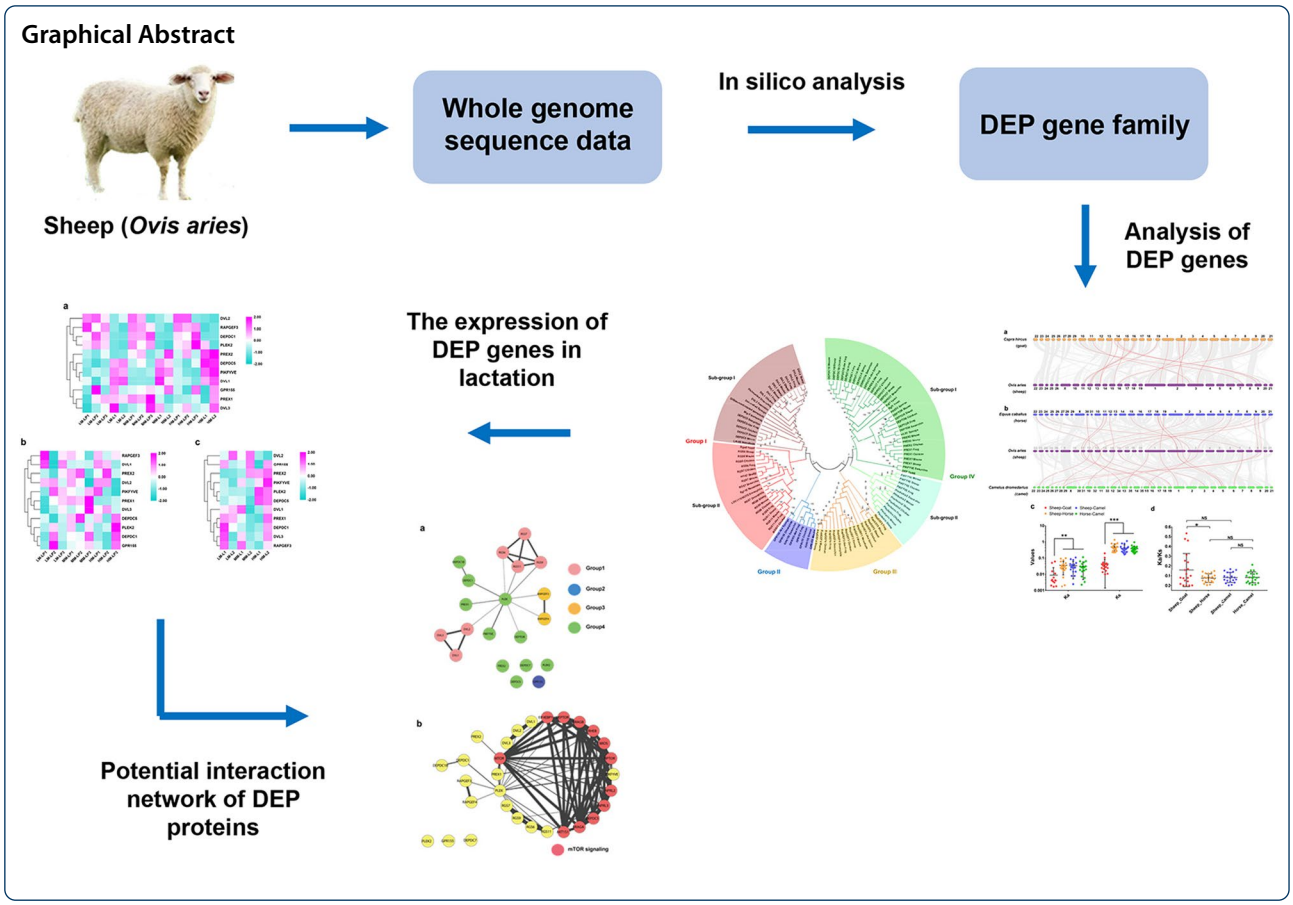

Background

Ovis aries (sheep) is an important domestic animal worldwide. Sheep milk contains more proteins (~5.5 g/100 g milk) than cow milk (3.4 g /100 g milk), and it can serve as a perfect nutrition source for humans [1, 2]. Many hormones are involved in the initiation and maintenance of lactation in sheep, including prolactin (PRL), adrenocorticotropic hormone (ACTH), somatotropin, thyroid stimulating hormone (TSH), oxytocin and progesterone [3–7]. After parturition, the secretion of various pregnant hormones, like estrogen and progesterone, is greatly inhibited [8]. All these hormones regulate protein and lipid synthesis and milk secretion through different mechanisms.

Hormones are known to regulate lactation mainly through some cell signaling pathways [9–11]. PRL is one of the most important lactation-regulated hormones, and it functions mainly through the JAK–STAT pathway [11]. PRL interacts with its membrane receptor (the PRL-receptor) and induce the dimerization of it, then, the dimerized PRL receptor further phosphorylates JAK and initiates JAK–STAT signaling to activate the transcription of milk proteins [11]. Moreover, the dimerization of PRL-R consequently contributes to the activation of PI3K and AKT, inhibits the GTPase activating protein (GAP) activity of the TSC1/TSC2/TBC1D7 protein complex and further activates mTOR to promote intracellular protein anabolism [12, 13]. Similarly, insulin binds to its membrane receptor (receptor protein tyrosine kinase, RPTK), and RPTK phosphorylates insulin receptor substrate (IRS) and further activates PI3K/AKT/mTOR signaling [14]. In addition, mTOR signaling also participates in regulating milk lipid synthesis, which is mainly achieved by changing the transcriptional activity of SREBP1/2 [10, 15].

Acting as a dominator of intracellular metabolism, mTOR signaling plays essential roles in regulating the synthesis of milk components, such as milk proteins and milk lipids [15, 16]. mTOR signaling contains many functional elements that precisely regulate the bioactivity of mTOR in different ways. DEP domain is a globular domain that consists of approximately 80 residues, and most DEP domain-containing proteins exert important functions in mTOR signaling [17]. DEPTOR is a negative regulatory subunit of mTORC1; it binds and inhibits the kinase activity of mTORC1 [18]. DEPDC5 is a subunit of

the GATOR1 complex, which acts as a negative regulator of mTORC1 [19]. In addition, previous studies also suggest that some other DEP domain proteins may be involved in mTOR signaling [20–22].

Previous reports suggest that some DEP proteins can regulate lactation [23–26]. However, systematic studies of the roles of sheep DEP family members in regulating lactation are currently lacking. Genome-wide analysis can provide a comprehensive landscape of a specific gene family, including the molecular evolution, genetic characteristics, member classification and expansion of a gene family [27, 28]. The aim of this study was to reveal the roles of DEP gene family members in regulating sheep lactation by exploring their genetic characteristics and functional roles at the whole genome-wide level. These results are expected to increase our understanding of the biological and genetic background of sheep lactation, and to provide the gene targets for the genetic improvement of milk performance in dairy sheep breed.

Methods

Genome-wide identification of DEP genes

First, the protein sequence of sheep (*Ovis aries*) was downloaded from the NCBI, and obtained the HMM profile of the DEP domain (accession number: PF00610) from the Pfam database. Then, the hmmsearch program was used to identify the DEP proteins from the protein sequence dataset (*E*-value $\leq 1 \times 10^{-5}$). In addition, all these identified proteins were further verified in the InterPro database. Similarly, the protein sequences of 21 other species (seven vertebrates: *Homo sapiens*, *Mus musculus*, *Columba livia*, *Gallus*, *Xenopus laevis*, *Crocodylus porosus*, and *Danio rerio*; eight invertebrates: *Octopus bimaculoides*, *Strongylocentrotus purpuratus*, *Tribolium castaneum*, *Penaeus vannamei*, *Drosophila hydei*, *Limus polyphemus*, *Aedes aegypti*, and *Caenorhabditis elegans*; six fungi: *Candida albicans*, *Coprinellus micaceus*, *Schizosaccharomyces pombe*, *Aspergillus fumigatus*, *Dictyostelium discoideum*, and *Cryptococcus neoformans*) were downloaded, and the same method was employed to identify DEP proteins in these organisms.

Analysis of domain organization and physiochemical characteristics of DEP proteins

The molecular weights and isoelectric points of DEP proteins were calculated in the ExPASy server, and both the Mw and pI values were compared through the one-way ANOVA method. The conserved protein domains in sheep DEP proteins were identified in the SMART database and the domain distributions were visualized in Evolview [29].

Phylogenetic analysis

The sequences of DEP proteins from ten organisms (sheep, mouse, chicken, frog, Danio, sea urchin, Drosophila, nematode, sponge and yeast) were aligned through the *ClustalW* method in MEGA software. Then, phylogenetic trees were constructed using the neighbor-joining (N-J) method with 1000 bootstrap replicates.

Genome-wide collinearity and Ka/Ks analysis

Analyses of homologous gene pairs of DEPs among *Ovis aries*, *Capra hircus*, *Equus caballus*, and *Camelus dromedarius* at the genome level were carried out. Whole-genome FASTA sequences and GTF (GTF gene transfer format) files of each species were obtained from the ENSEMBL database. Protein sequences were aligned through the blastp method, and collinearity information was queried and visualized in the jcvi (MCScan) package in Python [30]. The values of Ka and Ks were calculated for the DEP family members between *Ovis aries*, *Capra hircus*, *Equus Caballus* and *Camelus dromedarius*. All DEP protein pairs were aligned using the MUSCLE method, then the KaKs_Calculator (version 2.0) program was used to calculate the Ka/Ks values from the aligned pairs [31].

Ortholog analyses

Orthologous analyses of sheep DEP genes were carried out as previously described [32]. Orthologs of sheep genes in zebrafish (*D. rerio*), Drosophila (*D. melanogaster*), arabidopsis (*A. thaliana*), and yeast (*S. cerevisiae*) were identified in the InParanoid8 online server. The DEP genes were classified into five different phylogenetic patterns according to the existence of orthologs. The status of orthologs was identified "none of the other four species" (represented by the symbols $----$), "only in zebrafish" ($+---$), "only in zebrafish and drosophila" ($++--$), "only in zebrafish, drosophila and arabidopsis" ($+++-$), and "both in zebrafish, Drosophila, arabidopsis and yeast" ("$++++$"). The different phylogenetic patterns indicated the different evolutionary stages.

Gene expression analysis

To analyze the expression patterns of sheep DEP genes in the mammary gland during the late pregnancy and lactation periods, we downloaded the gene expression data from the NCBI GEO database (accession number: GSE71424). This dataset titled "Maternal Nutritional Programming in the Ovine Mammary Gland" contained 15 samples, nine from late pregnancy and six from the lactation period. In addition, all 15 samples can also be divided into three groups based on their nutritional conditions: HM means high maintenance, MM means moderate maintenance, and LM means

low maintenance. Five samples obtained from each nutrition group: three were in late pregnancy, and two were in the lactation period. The expressional heatmaps of sheep DEP genes were drawn by the pheatmap program in the R suite.

Molecular coevolution analyses

The coevolution of different DEP proteins was detected by the Mirrortree method [33]. The full-length amino acid sequences of 20 sheep DEP proteins were obtained from NCBI, and then the coevolution coefficients of 180 different DEP–DEP sequence pairs were computed separately.

Three-dimensional structural analysis

The 3D structural alignments were performed as described previously [28]. Briefly, the 3D structures of three DEP domains from bovine (PDB: 6n9p), mouse (PDB: 3ml6), and human (PDB: 5suy) were downloaded from the PDB database. Structural alignments were conducted by PyMOL software. The ConSurf online server was used to exhibit the molecular surface conservation of DEP domains.

Protein–protein interaction (PPI) network study

The PPI data of sheep DEP proteins were retrieved from the STRING database (version 11.0). The minimum interaction score was set to high confidence (0.7). The interaction network maps were drawn by Cytoscape software (version 3.7.2) and the size of edges between different nodes was drawn according to their combined scores.

Results

Identification and sequence analysis of sheep DEP family members

A total of 20 nonredundant DEP domain-containing proteins were identified in sheep (Table 1), and they were given the same name as their orthologues in humans. The lengths of these 20 DEP genes ranged from 806,166 (*RGS6*) to 7600 bp (*RGS11*), their ORFs ranging from 6333 (*PIKFYVE*) to 1053 bp (*PLEK*). The isoelectric points (pIs) of sheep DEP proteins varied from 5.93 (DVL2) to 10.15 (RGS9). In addition to sheep, we further identified the DEP genes in 21 other organisms, including eight vertebrates, eight invertebrates and six fungi. Generally, the quantities of DEP genes increased with the evolutionary process (Fig. 1a). There are more DEP genes (~ 20) in vertebrates, and with only 5–10 in invertebrates and less than 5 in fungi. In addition, we analyzed the pI and Mw values of all these identified DEP proteins. No significant difference was found between the overall pI values of the different organisms (Fig. 1b). However, the

Table 1 Characterization of identified DEP genes in sheep

Gene name	Chromosome ID	Exon count	Gene length (bp)	ORF (bp)	Mw (kDa)	pI
DEPTOR	9	11	127,694	1368	50.96	10.06
PREX1	13	40	180,451	4962	188.50	6.42
PREX2	9	30	285,114	4988	144.56	8.15
RAPGEF4	2	33	328,691	2982	115.45	6.84
PIKFYVE	2	43	80,608	6333	238.31	6.51
RGS7	12	18	523,486	1434	55.62	8.18
DVL3	1	16	157,775	3877	78.29	6.78
DEPDC7	15	9	23,037	1536	58.69	8.43
DEPDC1	1	12	21,711	1587	61.77	9.24
DVL2	11	15	7830	2211	78.84	5.93
RGS6	7	20	806,166	1464	60.58	7.22
RAPGEF3	3	30	21,658	2676	99.34	8.16
PLEK2	7	9	20,085	1524	39.81	10.00
PLEK	3	10	28,079	1053	40.04	8.62
DVL1	12	14	11,832	2190	78.33	7.63
DEPDC1B	16	11	100,539	1590	61.70	8.79
GPR155	2	18	38,415	2607	96.60	7.06
RGS11	24	17	7600	1398	52.99	9.20
RGS9	11	19	52,639	1950	73.99	10.15
DEPDC5	17	43	73,423	4809	180.41	6.67

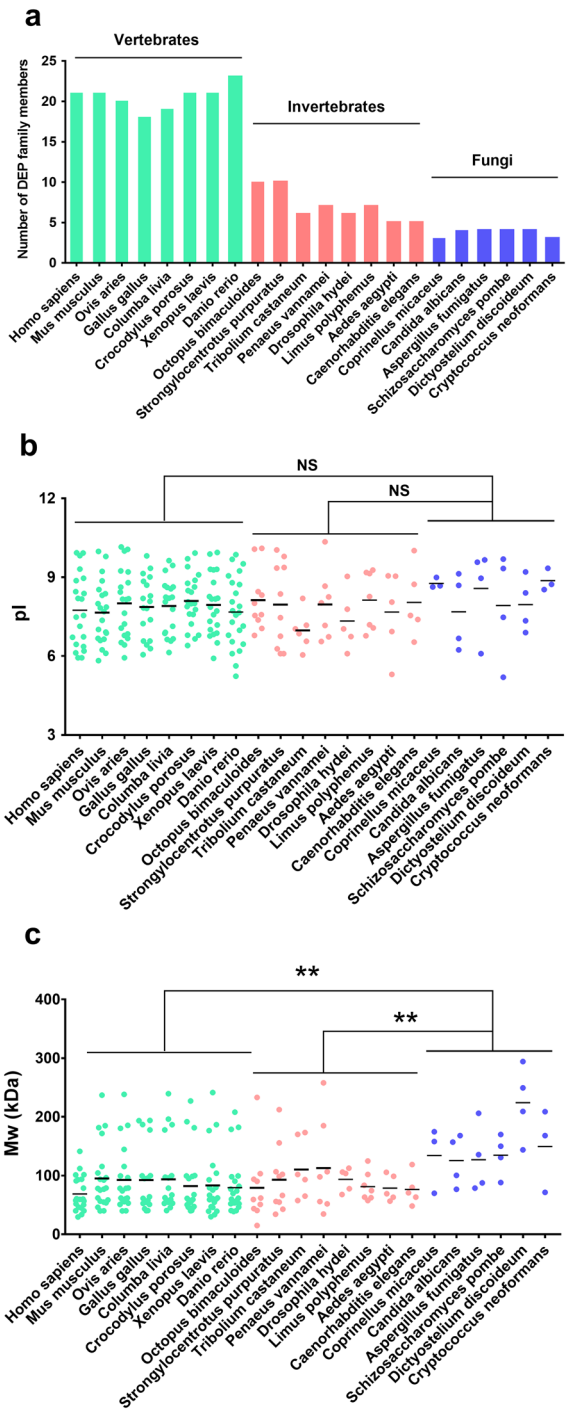

Fig. 1 The identification and sequence characteristics of DEPs in different organisms. **a** The quantities of DEP genes from eight vertebrates, eight invertebrates and six fungi. **b** Isoelectric points of 111 DEP proteins from 22 organisms. **c** Molecular weights of sheep DEP proteins from 22 organisms. ** Indicates that the *P* value of the one-way ANOVA test was less than 0.01. NS indicates a *P* value larger than 0.05

Mw values of DEP proteins from the six fungi were significantly larger than those from the eight vertebrates ($P < 0.01$) and eight invertebrates ($P < 0.01$) studied here (Fig. 1c).

Phylogenetic analysis of DEP members

The 111 DEP protein sequences from sheep and nine other nine organisms (mouse, chicken, frog, Danio, sea urchin, Drosophila, nematode, yeast and sponge) were aligned through *ClustalW* method, then the N-J phylogenetic trees were built (Fig. 2). This phylogenetic tree showed that all 111 DEP proteins could be categorized into four groups, named Groups I to IV. Moreover, Group I and Group IV can be divided into two subgroups according to the topology of the phylogenetic trees. Group IV was the largest group, including 46 DEPs. Group II was the smallest group, containing eight DEPs. All 20 sheep DEPs were divided into four groups, Group I contained eight DEPs, Group II contained one DEP, Group II contained three DEPs, and the other eight DEPs belonged to Group IV.

Collinearity analysis of DEP genes from different mammals

We surveyed the collinear relationship among the orthologous DEP genes from *Ovis aries, Capra hircus* (goat), *Equus caballus* (horse) and *Camelus dromedaries* (camel) to investigate the potential clues of evolutionary events. The sheep genome consists of 26 autosomes, and the goat genome contains 29 autosomes. Collinearity results showed that large-scale rearrangements and duplications between the genomes of sheep and goat. In addition, the collinearity results of sheep–horse and sheep–camel showed that large-scale chromosomes arrangements also existed in the genomes of horse and camel. The DEP genes of sheep, goat, horse and camel exhibited a collinear relationship (Fig. 3a, b).

Furthermore, the selected types of orthologous DEP gene pairs were determined according to the nonsynonymous substitutions (Ka) and synonymous substitutions (Ks). We obtained the Ks, Ka, Ka/Ks values and the selection types of 20 orthologous gene pairs (Table 2 and Additional file 1: Tables S1–S3). Both the Ka and Ks values of the sheep–goat pair were significantly lower than those of the other pairs, indicating fewer substitutions between the DEP genes from sheep and goat. The Ka/Ks ratios of all the sheep–goat orthologous pairs were < 1, indicating that purifying selection drove the nucleotide substitutions of DEP genes. Among the sheep–goat DEP gene pairs, the Ka/Ks values of DEPDC1B (0.5334), RGS6 (0.4768) and DEPDC7 (0.4597) were significantly larger

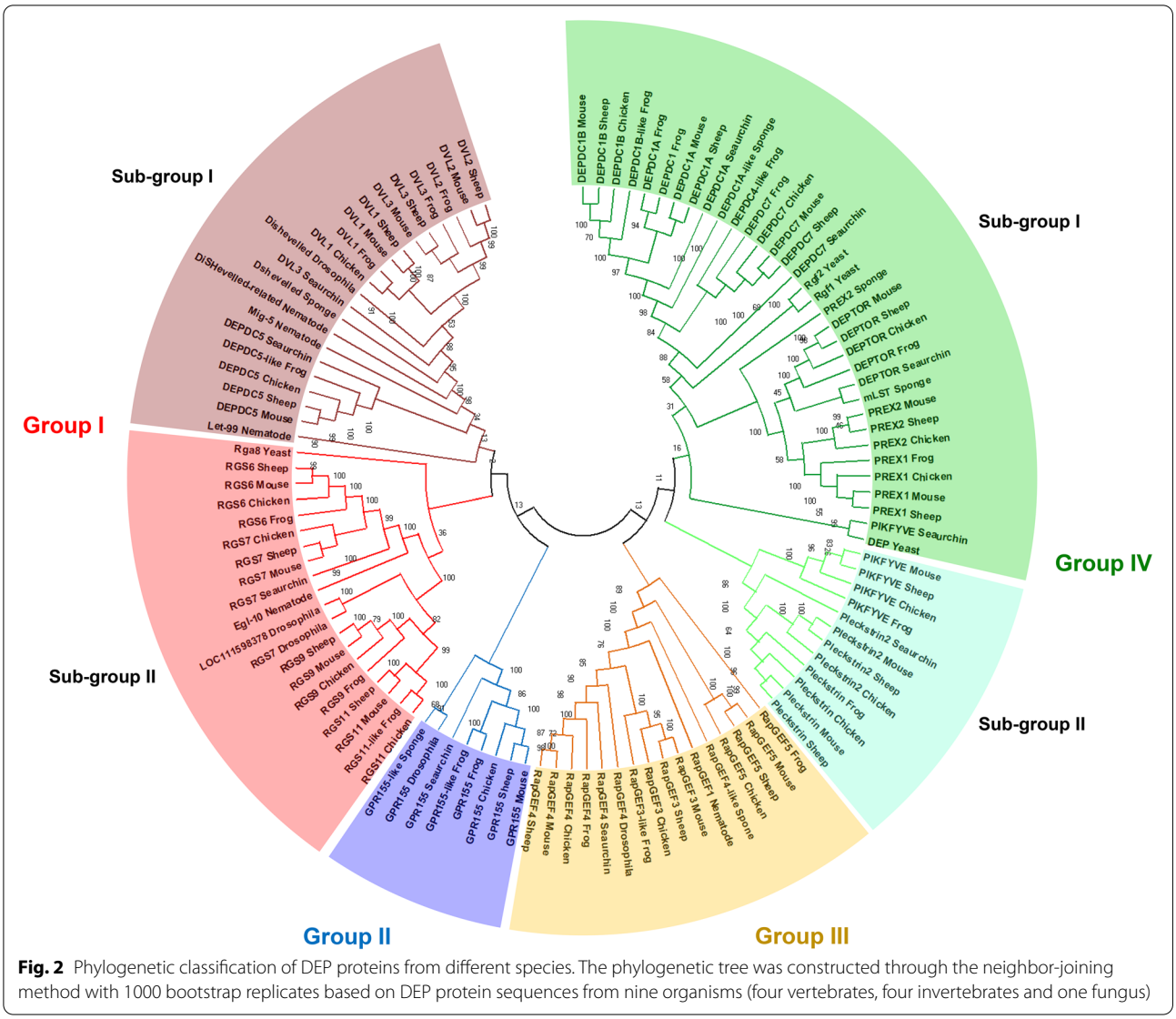

Fig. 2 Phylogenetic classification of DEP proteins from different species. The phylogenetic tree was constructed through the neighbor-joining method with 1000 bootstrap replicates based on DEP protein sequences from nine organisms (four vertebrates, four invertebrates and one fungus)

than that of the mean value (0.1509) of all DEP genes, which indicated that these three genes underwent less purifying selection than average. Moreover, statistical analysis showed that the Ka/Ks values of the sheep–goat DEP gene pairs were significantly larger than those of the sheep–horse DEP gene pairs, but there was no significant difference for the sheep–camel gene pairs.

DEP proteins were coevolved in the evolution of species

In the present study, we measured the Pearson correlation coefficients of the coevolution relationship between different sheep DEP proteins at the molecular level using Mirrortree method. The results showed that the coevolution between DEP proteins was highly positively correlated, and the mean value of Pearson correlation coefficients from all DEP–DEP pairs was 0.732 (Fig. 4a). Notably, the Pearson correlation coefficients of the PLEK-DEPs (mean value: 0.363) and PREX1-DEPs (mean value: 0.400) were significantly lower than those of the other DEP pairs. Moreover, the mean value of the coevolution correlation coefficients between DEPDC5 and the other 19 DEP proteins was the highest among all DEP proteins (mean value: 0.915, Fig. 4b). Overall, these results suggest that the DEP proteins were coevolved in the evolution of species.

The DEP family undergoes more expansion in the early evolutionary stage

In this study, we performed a phylogenetic analysis of DEP orthologs in yeast (single-celled eukaryote), Arabidopsis (plant), sea urchin (invertebrate) and zebrafish (vertebrate). The origin of these species indicates key time points in biological evolution (Fig. 5a). The sheep DEPs with orthologs in both of the other organisms

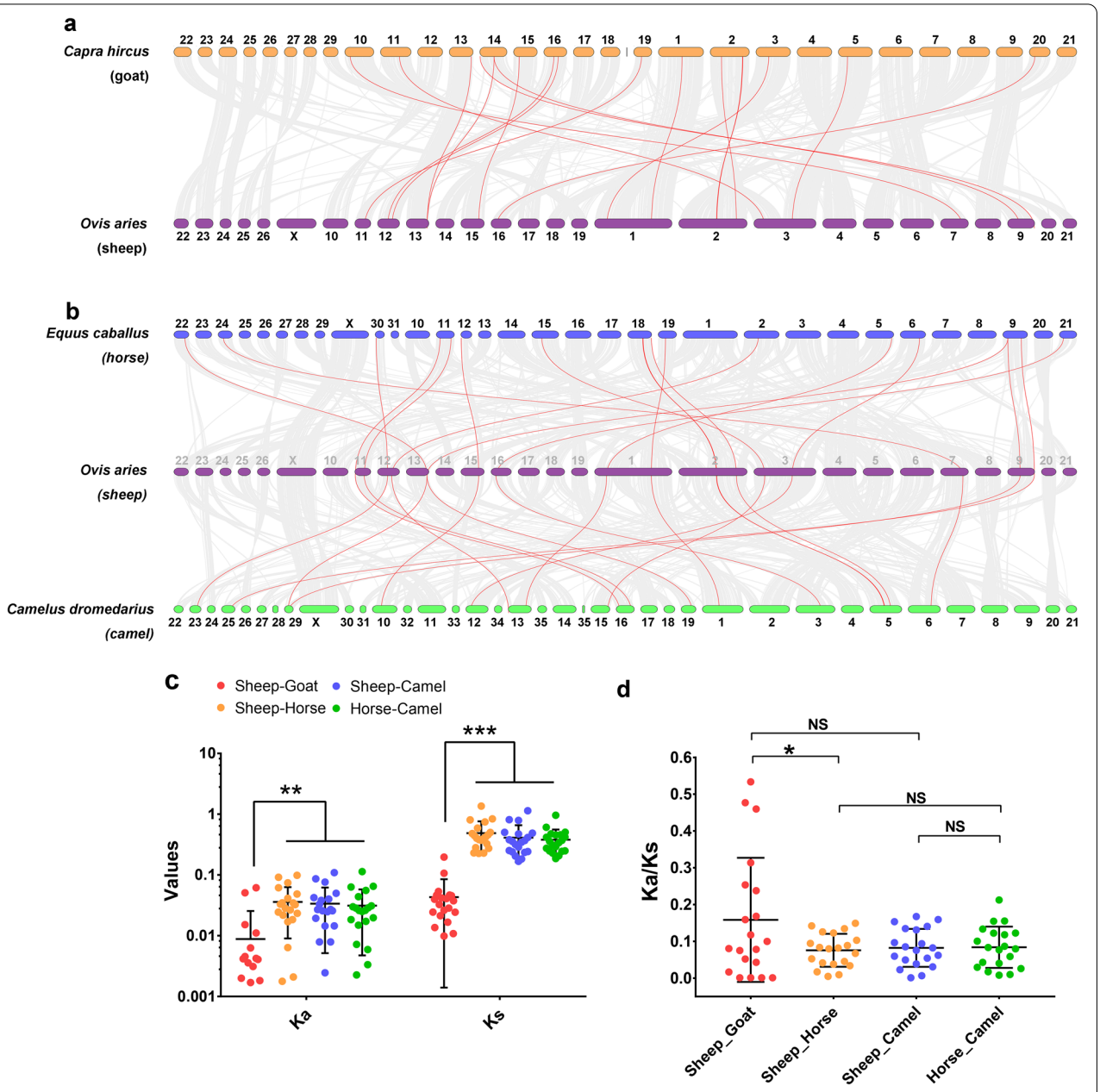

Fig. 3 Collinearity relations of DEP genes from different mammals. **a** The collinearity relations of DEP genes between goat and sheep. **b** The collinearity relations of DEP genes between horse, sheep and camel. Light gray lines link these collinear genes between different species. The red lines indicate homologous DEP gene pairs. **c** Ka and Ks values of different DEP gene pairs. **d** Ka/Ks ratios of different DEP gene pairs. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. NS means $P>0.05$

were labeled '++++', and eight (40%) DEP proteins belonged to this group, which indicates that they originated with the appearance of eukaryotes. Two (10%) DEP proteins in sheep have orthologs in Arabidopsis, Drosophila and zebrafish, and this group was labeled '+++−'. These results indicate that half of the DEP family members emerged before the separation of plants and animals. Eight (40.0%) DEP proteins had orthologs only in Drosophila (++−−), suggesting that they appeared before the appearance of vertebrates. Two DEPs had orthologs only in zebrafish (+−−−), suggested that it appeared before the separation between terrestrial and aquatic vertebrates. No DEP family members emerged with the terrestrial vertebrates' lineage expansion (−−−−) (Fig. 5b). In the mammal genome, most gene families expand in the late evolutionary stage.

Table 2 Ka/Ks calculation and divergent time of the orthologous gene pairs between sheep and goat

Orthologous gene pairs	Ka	Ks	Ka/Ks	*P*-value (Fisher)	Purify selection
sDEPDC1B-gDEPDC1B	0.0152	0.028492	0.53349	0.083627	Yes
sDEPDC1-gDEPDC1	0.00423	0.016691	0.25342	0.02419	Yes
sDEPDC5-gDEPDC5	0.003111	0.039759	0.078244	1.51E−17	Yes
sDEPDC7-gDEPDC7	0.004535	0.009866	0.45967	0.174907	Yes
sDEPTOR-gDEPTOR	0.061567	0.196262	0.313699	1.69E−07	Yes
sDVL1-gDVL1	0.001828	0.024372	0.074995	1.24E−05	Yes
sDVL2-gDVL2	0.000697	0.041884	0.016651	6.24E−13	Yes
sDVL3-gDVL3	4.56E−05	0.045613	0.001	0	Yes
sGPR155-gGPR155	0.003582	0.021299	0.168178	0.000139	Yes
sPIKFYVE-gPIKFYVE	0.000872	0.010846	0.080406	1.52E−07	Yes
sPLEK2-gPLEK2	3.75E−05	0.03755	0.001	0	Yes
sPLEK-gPLEK	0.004166	0.026212	0.158933	0.003478	Yes
sPREX1-gPREX1	0.001696	0.032617	0.052005	1.32E−17	Yes
sPREX2-gPREX2	0.011105	0.046718	0.237698	1.68E−09	Yes
sRAPGEF3-gRAPGEF3	0.001997	0.046463	0.042971	1.05E−14	Yes
sRAPGEF4-gRAPGEF4	1.36E−05	0.013616	0.001	0	Yes
sRGS11-gRGS11	0.004079	0.040875	0.099789	1.05E−05	Yes
sRGS6-gRGS6	0.050801	0.10654	0.476828	0.000921	Yes
sRGS7-gRGS7	2.45E−05	0.024452	0.001	0	Yes
sRGS9-gRGS9	0.006291	0.053467	0.117655	1.45E−08	Yes

In contrast, the DEP family underwent expansion in the early evolutionary stages, which suggests its crucial role in life activities.

Domain organization analysis of sheep DEP proteins

An N-J phylogenetic tree was built according to the sequence alignment of all 20 sheep DEP proteins (Fig. 6). Most sheep DEPs contained one DEP domain, except PREX1/2 and DEPTOR, both of which contained two DEP domains. DEPDC7 and GPR155 only contained DEP domains; no other known functional domains were identified in their primary structure. The PDZ domain was the most common functional domain after the DEP domain; it was found in five DEPs: DVL1/2/3, DEPTOR and PREX2. Moreover, four DEPs, RGS6/7/9/11, contained the RGS domain, which was the second common functional domain following the DEP domain. Three DEPs contained the DIX domain, DVL1/2/3. In addition, some other functional domains, such as RhoGAP, RasGEF, DH, PH, PIPK and IML1, were identified in one or two DEPs. In general, the domain organization patterns of all sheep DEP proteins suggested that their biological functions were diverse.

GO analyses of sheep DEP proteins

GO analysis was performed to systematically reveal the biological functions of 20 sheep DEP proteins (Fig. 7). In the biological process section, the most significantly enriched term was "regulation of GTPase activity". In the molecular function section, the dominant term was "GTPase activator activity". The enriched cellular component ontology terms suggested that DEP proteins were mainly distributed in the "neuron-to-neuron synapse" cellular component. The results of all three sections indicated that these sheep DEPs are focused on the regulation of small GTPase activity.

Expression patterns of DEP family members in the mammary tissue of sheep in the late pregnancy and lactation periods

The switch from pregnancy to lactation involves a complicated hormone-signaling system. To investigate the functional roles of DEP family genes in this physiological switch, the relative expression levels of DEP genes were analyzed in sheep mammary tissues. Here, we analyzed the RNA-sequencing data (GSE71424) of samples from the mammary glands of 15 different sheep (Fig. 8a). These sheep could be divided into three groups based on their nutrition level during the pregnancy period: the low maintenance (LM) group, middle maintenance group (MM) and high maintenance group (HM). Moreover, according to the physiological state of sheep, they could also be divided into the late pregnancy group (LP)

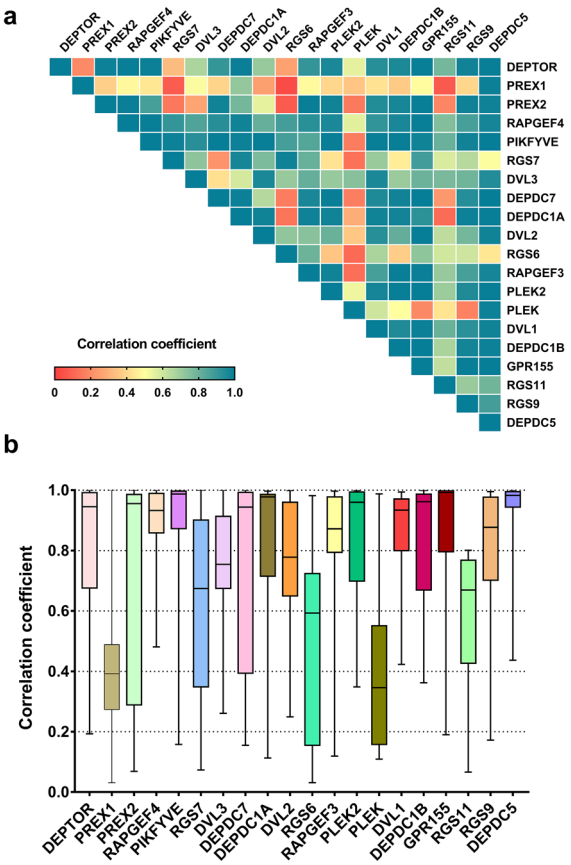

Fig. 4 DEP proteins were coevolved in the evolution of species. **a** The coevolution correlation coefficient matrix between 20 DEPs. **b** The box-plots exhibit the coevolution correlation coefficients of one DEP protein with the other 19 DEP proteins

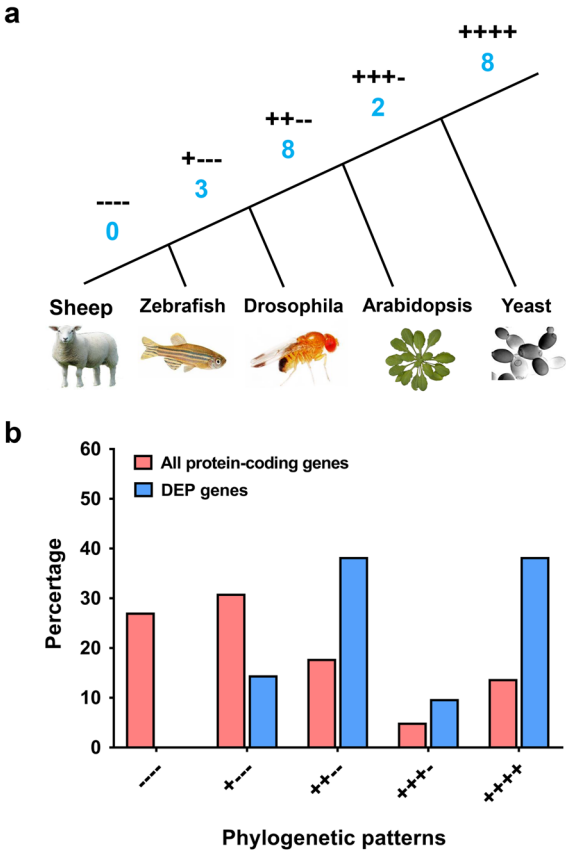

Fig. 5 The DEP family undergoes more expansion in the early evolutionary stage. **a** Phylogenetic patterns of 20 sheep DEP genes were analyzed. The symbols "− − − −", "+ − − −", "+ + − −", "+ + + −", and "+ + + +" represent different phylogenetic status. The numbers labeled below each symbol indicate the DEP gene numbers accordingly. **b** The ratios of sheep DEP genes to all sheep protein-coding genes

and lactation (L) group. In the late pregnancy stage, there was no obvious difference in the DEP genes of sheep under different nutrition conditions. In the lactation period, PREX2, PIKFYVE, PLEK2 and DEPDC5 were significantly upregulated in the sheep of the high nutrition maintenance group (Fig. 8b). Generally, RAPGEF3, PLEK2 and DEPDC1 were highly expressed in the late pregnancy state, and this high expression was unrelated to nutrition conditions. In contrast with these DEP genes, DEPDC5, PIKFYVE and DVL1 were downregulated in the lactation stage, and their downregulation was independent of nutrition conditions (Fig. 8c).

The 3D structure of the DEP domain is highly conserved in mammals

To compare the structural characteristics of the DEP domains of different organisms, we downloaded the 3D structures of DEP domains (*H. sapiens*: 5suy; *M. musculus*: 3ml6 and *B. taurus*: 6n9g) from the PDB database. All three DEP domains contain two helixes and two β-strands (Fig. 9a). Structural alignments showed that these DEP domains had similar spatial conformations, suggesting that the DEP domain was structurally conserved in biological evolution (Fig. 9c). Amino acid sequence alignment showed that the primary structures of these DEP domains were also conserved (Fig. 9b). Moreover, the surface conservation showed that the residues of C-terminal β strands made up a highly conservative region. This region is the potential binding surface of the interaction partner of DEP proteins.

The PPI networks of sheep DEP proteins

Cellular signaling transduction is achieved through protein–protein interactions (PPIs). To furtherly understand the functional mechanisms of sheep DEPs, the PPI networks of sheep DEPs were constructed using the interactome data of sheep in the STRING database. First, we generated a PPI network of 20 sheep DEPs (Fig. 10). This

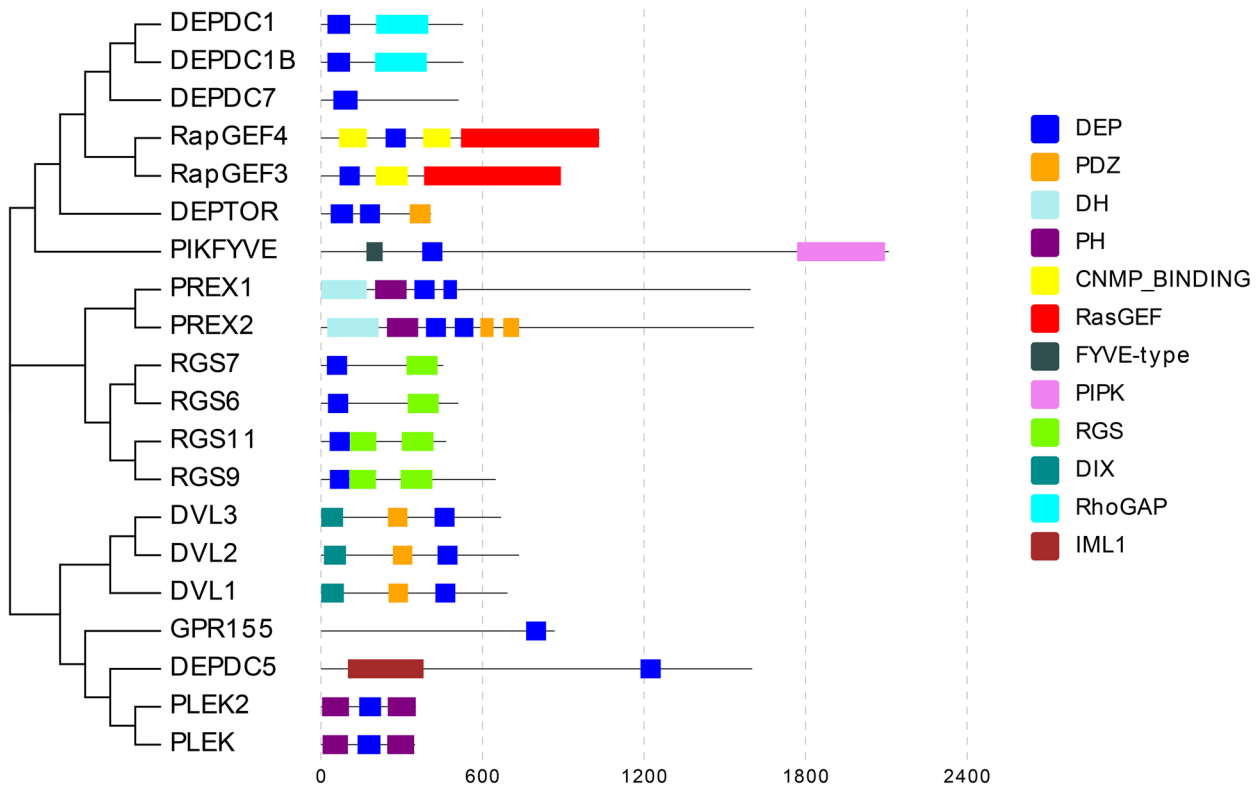

Fig. 6 Schematic representations of the domain organization patterns of sheep DEP proteins. The phylogenetic tree was generated using MEGA through the N-J method with 1000 bootstrap replicates

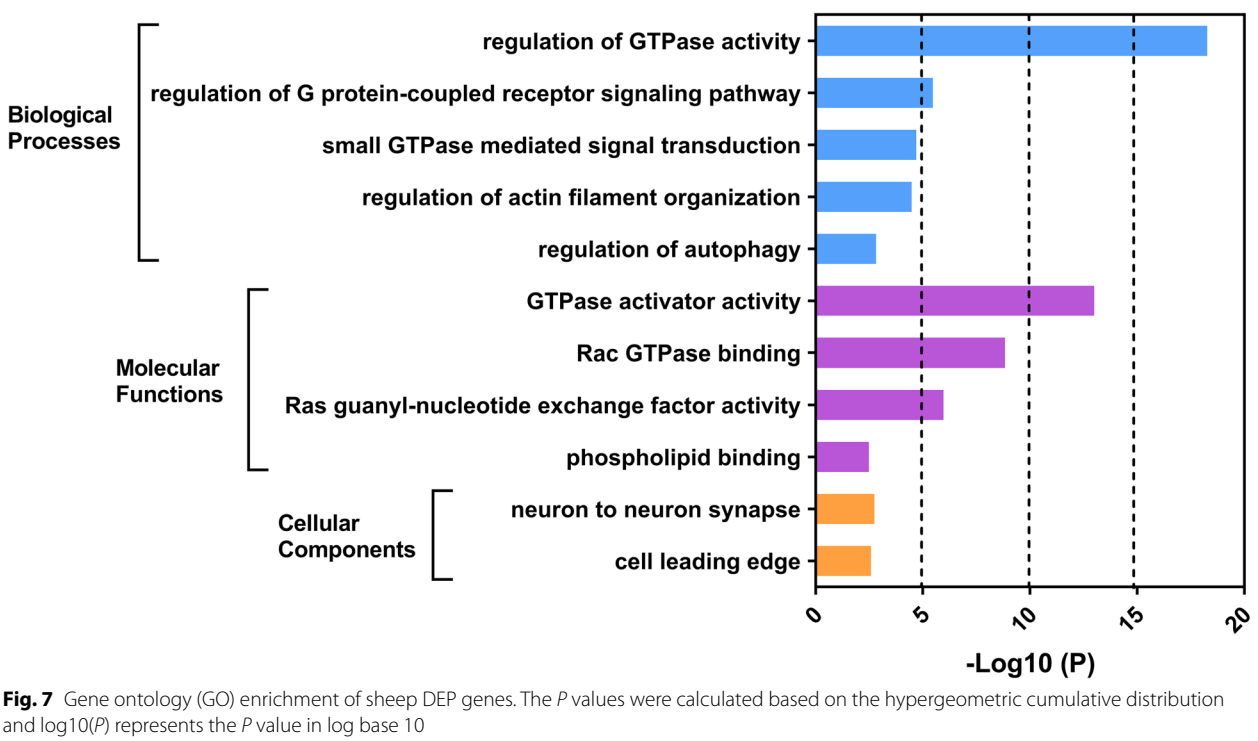

Fig. 7 Gene ontology (GO) enrichment of sheep DEP genes. The *P* values were calculated based on the hypergeometric cumulative distribution and log10(*P*) represents the *P* value in log base 10

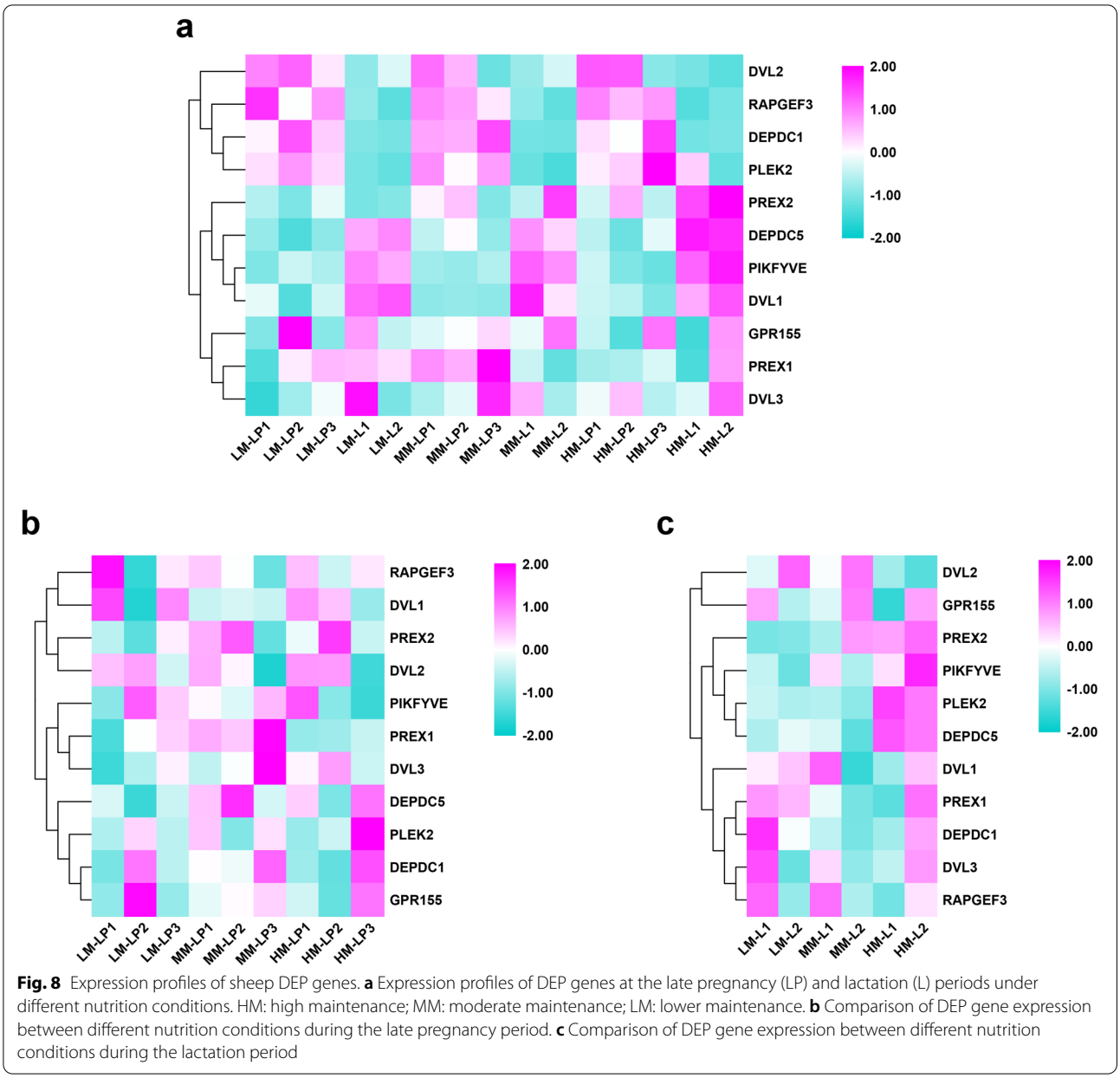

Fig. 8 Expression profiles of sheep DEP genes. **a** Expression profiles of DEP genes at the late pregnancy (LP) and lactation (L) periods under different nutrition conditions. HM: high maintenance; MM: moderate maintenance; LM: lower maintenance. **b** Comparison of DEP gene expression between different nutrition conditions during the late pregnancy period. **c** Comparison of DEP gene expression between different nutrition conditions during the lactation period

network showed that PLEK acted as a central mediator in the internal interaction of sheep DEP proteins. GPR155, DEPDC5, DEPDC7, PREX2 and PLEK2 did not participate in the interaction network of DEPs. Furthermore, when we expanded the nodes of this DEP interaction network, the results showed that many components of mTOR signaling participated in the network (Fig. 10). DEPTOR and DEPDC5 were core components of mTOR signaling; the other 15 sheep DEPs, except for DEPDC7, GPR155 and PLEK2 were directly or indirectly involved in regulating mTOR signaling.

Discussion

Lactation is a highly conserved process among mammals; it is controlled by various complicated regulatory networks [34]. Many important intracellular signaling networks are involved in regulating the initiation, maintenance and termination of the lactation process. mTOR signaling is one of the most important pathways that affects lactation by controlling the anabolism of milk proteins and lipids [10, 35]. Most DEP proteins participate in the regulation of various signaling pathways, including mTOR signaling [21, 36–38]. In eukaryotes, the quantity of DEP genes is increasing with evolution. Single-celled

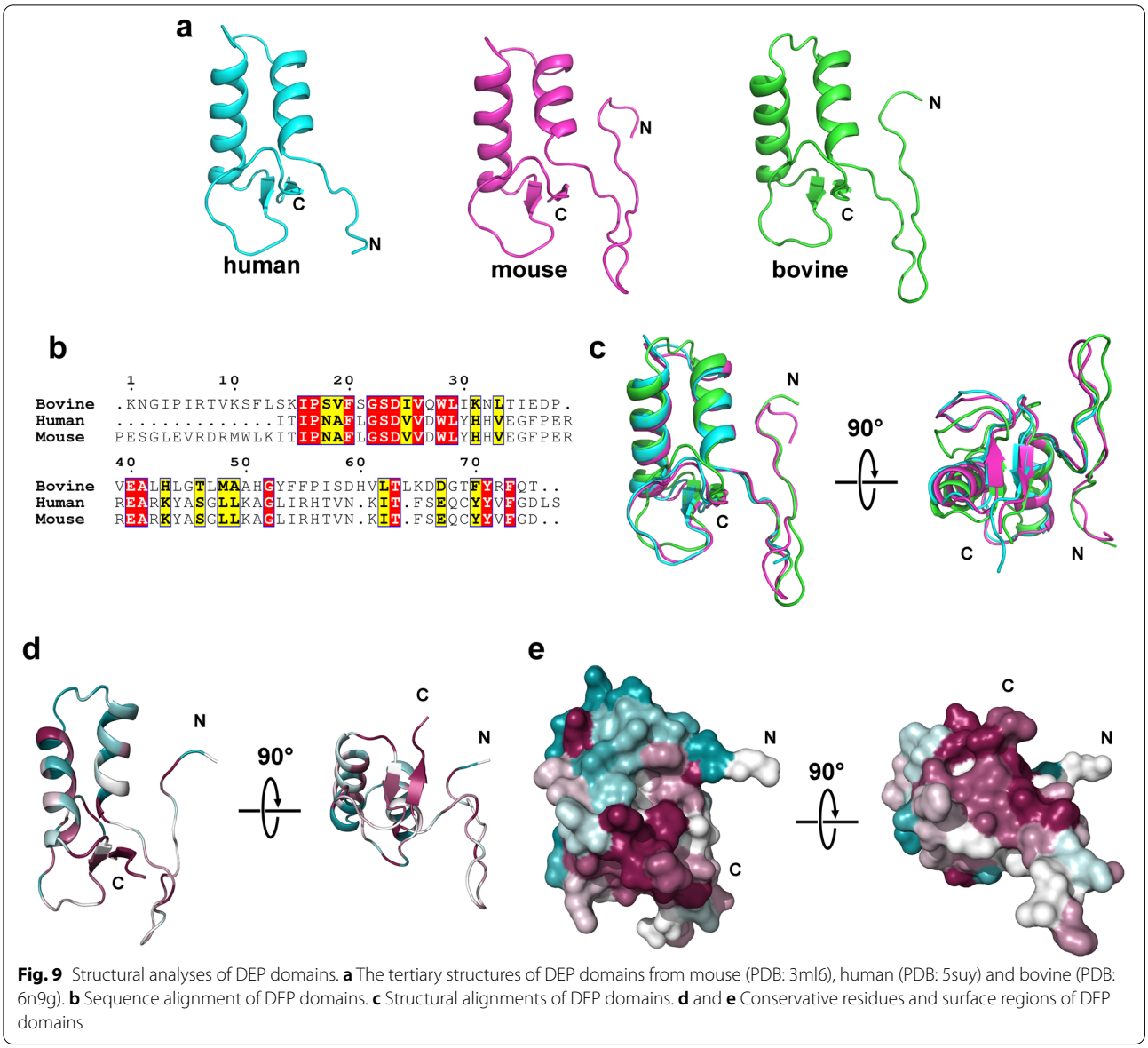

Fig. 9 Structural analyses of DEP domains. **a** The tertiary structures of DEP domains from mouse (PDB: 3ml6), human (PDB: 5suy) and bovine (PDB: 6n9g). **b** Sequence alignment of DEP domains. **c** Structural alignments of DEP domains. **d** and **e** Conservative residues and surface regions of DEP domains

eukaryotic organisms, such as yeast, contain three or four DEP genes in the genome. There were 5 to 10 DEP genes in invertebrates and approximately 20 DEPs in vertebrates. More DEP genes contribute to the normal operation of the complicated cellular signaling networks. The molecular weights of DEP proteins from fungi are significantly larger than those from both invertebrates and vertebrates. One reasonable explanation is that larger proteins usually consist of multiple functional domains, which contribute to various functions in life activities.

The Ka/Ks ratio can be used to determine the evolutionary selective pressure acting on a specific protein-coding gene [39]. A Ka/Ks ratio of less than one indicates that the main evolutionary press acting on themselves is purifying selection [40]. A Ka/Ks ratio of a specific gene greater than one indicates positive selective pressure. Usually, genes with a Ka/Ks ratio larger than 1 are undergoing fast evolution, and genes with a Ka/Ks ratio of less than 1 are conserved in evolution [41]. Here, we found that the Ka/Ks ratios were less than 1 for all DEP gene pairs compared between sheep, horse and camel, suggesting that purifying selection is the main pressure acting on DEP genes. Moreover, this result also indicates the conservative molecular function of DEP genes in different species.

Various studies have suggested that the hormones and relative regulatory pathways are different between the pregnancy and lactation periods [42, 43]. Moreover, the

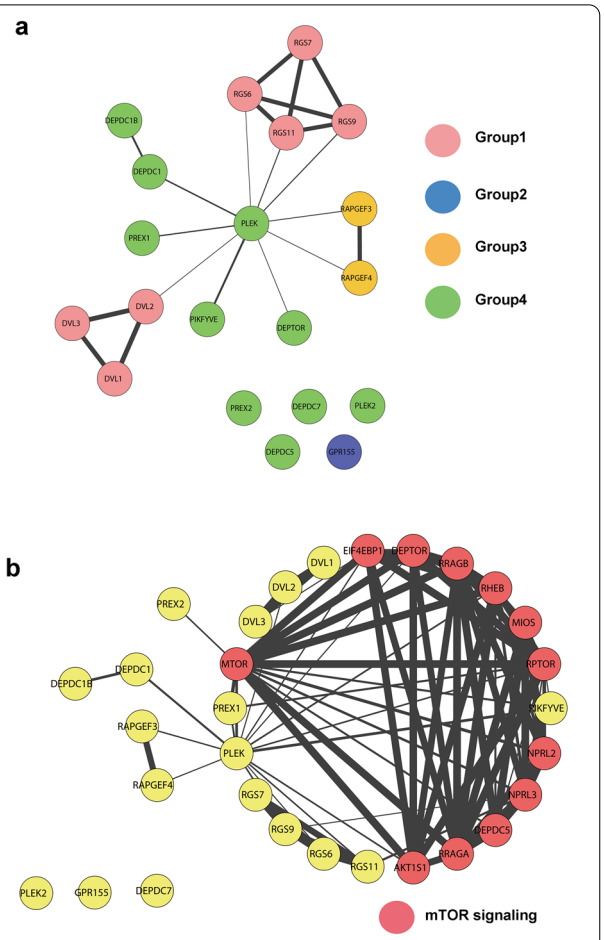

Fig. 10 The PPI networks of sheep DEP proteins. **a** The interaction network of all 20 sheep DEP proteins. **b** The interactions of sheep DEP proteins with other closely interacting partners. The size of edges between different nodes was drawn according to their combined scores

hormone secretions and cell physiological states in the pregnancy and lactation periods can also be affected by nutrition conditions [44, 45]. To address the potential roles of DEP genes in the late pregnancy period and lactation period under different nutrition conditions, we analyzed the public RNA expression data of the sheep mammary gland [46]. DVL2, RAPGEF3, DEPDC1 and PLEK2 were all downregulated in the lactation period when compared to the late pregnancy period in three different nutrition conditions. In contrast, DEPDC5, PIKFYVE and DVL1 were all upregulated in the lactation period. RAPGEF3 and DEPDC1 positively regulate cell proliferation, and their upregulation leads to the rapid development of the mammary gland. In lactation, the development of the mammary gland slows down, and both RAPGEF3 and DEPDC1 are downregulated [47, 48]. PLEK2 and DVL2 participate in cell skeleton organizations, and alterations in their expression contribute to the development of mammary gland cells, which are the main locations of milk synthesis and secretion [36, 49]. PIKFYVE is a crucial regulator of vesicular transport, its upregulation in lactation facilitates the synthesis and secretion of milk lipids and proteins [20, 37]. DEPDC5 is a component of mTOR signaling, which is highly active in the lactation period [50]. The upregulation of DEPDC5 can promote mTOR signaling and intracellular anabolism [19, 51]. Previous reports indicated that DEP proteins mainly function in various spatial and temporal control events [17]. In late pregnancy, there was no significant expressional difference between sheep under different nutrition conditions. However, PREX2, PIKFYVE, PLEK2 and DEPDC5 were highly expressed under high nutrition conditions. As mentioned above, both PIKFYVE and PLEK2 are involved in cell growth and cell skeleton organization [36, 37]. Moreover, PREX2 can interact with and inhibit PTEN to activate PI3K signaling, and the activation of PI3K further initiates downstream biosynthesis of lipids and proteins [52]. This result also implies that these DEP genes exert important roles in the lactation period.

Previous studies suggested that many gene families of terrestrial mammals experienced large-scale expansion in late evolutionary stages, such as the UDP-glucuronosyl transferase, glutathione S transferase and cytochrome P450 families [53]. Instead, our results suggested that the DEP family experienced more expansion during early evolutionary stages. Most DEP family members participate in the spatial and temporal control of diverse signaling pathways, such as mTOR signaling, and Wnt signaling [54]. Both of these intracellular signaling pathways play essential roles in cell metabolism and organ development. The early origin and high evolutionary stability of DEP genes contribute to their functional conservation in biological evolution. Moreover, the results of the coevolution analyses further suggest that close relationships between DEP family members have been maintained throughout evolution.

Usually, protein domains with important biological functions, such as the ARM domain and TBC domain, are conserved during evolution [55, 56]. Here, we found that the 3D structures of the DEP domains are conserved between different mammals. This structural conservation guarantees the similar functions of the DEP domain in different proteins. The PPI network indicates that most DEP proteins participate in the mTOR signaling network, either directly or indirectly.

DEPTOR is the structural component of mTORC1 and mTORC2 [18]. DEPDC5 is the structural component of the GATOR1 complex, which interacts with and inactivates the RagA/C complex and ultimately inhibits the kinase activity of mTORC1 [19, 38]. PIKFYVE deficiency impairs lysosomal homeostasis, and that deficiency reduces the activity of mTORC1 [20]. PREX1/2 directly interacts with the mTOR protein in the mTORC2 complex and promotes cell migration [21, 57]. Collectively, these direct and indirect linkages of DEP protein with mTOR signaling contribute to their role in lactation regulation.

Conclusions

In the present work, we identified DEP family members in 22 species, including eight vertebrates, eight invertebrates and six fungi, and the molecular weights of fungal DEP proteins were significantly larger than those of invertebrates and vertebrates. Twenty DEP family members were identified in the sheep genome, and they can be divided into four major groups. Most DEP members contain two or more functional domains, which also contribute to their complicated biological functions. Ka/Ks calculation suggest that purifying selection is the main pressure acting on DEP genes, and this result also indicates the conservative molecular function of DEP genes in different species. Gene expression data show that in the late pregnancy and lactation period, the expression levels of 8 DEP family members exhibited significant differences in mammary parenchymal tissues. Moreover, nutritional conditions can exert a great influence on the expression of certain DEP members. In addition, we observed that the DEP family experienced more expansion than the overall average in the early evolutionary stage, and the DEP family members coevolved in biological evolution. Structural analyses suggest that the 3D structures of DEP domains are conserved between mammals. Last, protein–protein interaction networks show that the DEP family members are closely related to mTOR signaling, which implies that these DEP members may regulate lactation through mTOR signaling.

Abbreviations

DEP: Dishevelled, EGL-10, and pleckstrin; PRL: Prolactin; PPI: Protein–protein interaction; mTOR: Mechanistic target of rapamycin; Mw: Molecular weights; pI: Isoelectric point.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00336-w.

Additional file 1: Table S1. Ka/Ks calculation of the orthologous genes between sheep with horse. **Table S2.** Ka/Ks calculation of the orthologous genes between sheep with camel. **Table S3.** Ka/Ks calculation of the orthologous genes between sheep with camel.

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Author contributions

ZG, JZ and GG designed the study and wrote the manuscript; ZG, YW and JZ preformed research; YW, JM and SH analyzed data. All authors read and approved this manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interest.

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