



# Cloning, characterization and expression of Peking duck fatty acid synthase during adipocyte differentiation

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## ABSTRACT

**Background:** Fatty acid synthase (FAS) is a key enzyme of *de novo* lipogenesis (DNL), which has been cloned from several species: *Gallus gallus*, *Mus musculus*, *Homo sapiens*, but not from *Anas platyrhynchos*. The current study was conducted to obtain the full-length coding sequence of Peking duck FAS and investigate its expression during adipocyte differentiation.

**Results:** We have isolated a 7654 bp fragment from Peking duck adipocytes that corresponds to the FAS gene. The cloned fragment contains an open reading frame of 7545 bp, encodes a 2515 amino acid protein, and displays high nucleotide and amino acid homology to avian FAS orthologs. Twelve hour treatment of oleic acid significantly up-regulated the expression of FAS in duck preadipocytes ( $P < 0.05$ ). However, 1000  $\mu$ M treatment of oleic acid exhibited lipotoxic effect on cell viability ( $P < 0.05$ ). In addition, during the first 24 h of duck adipocyte differentiation FAS was induced; however, after 24 h its expression level declined ( $P < 0.05$ ).

**Conclusion:** We have successfully cloned and characterized Peking duck FAS. FAS was induced during adipocyte differentiation and by oleic acid treatment. These findings suggest that Peking duck FAS plays a similar role to mammalian FAS during adipocyte differentiation.

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## 1. Introduction

Fatty acid synthase (FAS, EC 2.3.1.85) is a key multifunctional enzyme that catalyzes the synthesis of saturated long-chain fatty acids, predominately palmitate, by using malonyl-CoA as a two-carbon donor [1]. It has been determined that FAS consists of two identical subunits, each of which contains seven unique functional domains:  $\beta$ -ketoacyl synthase (KS), malonyl/acetyl transferase (MAT), dehydratase (DH), enoyl reductase (ER),  $\beta$ -ketoacyl reductase (KR), acyl carrier protein (ACP), and the thioesterase (TE) [2]. Among them the TE domain of FAS plays an essential role in determining the final chain length of the product [3].

As FAS is critical to the synthesis of fatty acids, it is highly expressed in lipogenic tissues such as the liver, adipose tissue and lactating mammary glands [4]. In both rodents and mammals, there is a positive correlation between FAS mRNA expression level and body fat content [5,6,7]. Moreover, inhibition of FAS reduces food intake and facilitates weight loss

[8]. Therefore, FAS would be a reasonable therapeutic target for the treatment of obesity [5,9]. In chicken, FAS mRNA levels are strongly correlated with hepatic fat content [10]. Due to the low capacity of adipose tissue DNL in avian species, there is little information regarding the roles of FAS in adipocyte differentiation. However, down-regulation of FAS in 3T3L1 cells, either with inhibitors or by RNA interference, leads to decreased lipid droplet accumulation, demonstrating FAS plays a key role in adipocyte differentiation [11,12]. To date, it is unclear whether FAS can play a similar role in duck adipocyte differentiation.

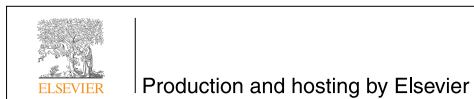
In avian species, the addition of fatty acids (mainly oleic acid) is needed to supplement the hormonal cocktail containing dexamethasone (DEX), insulin, and 3-isobutyl-1-methylxanthine (IBMX) to induce adipocyte differentiation in cell culture [13]. Compelling evidence, from studies conducted in chicken and mouse, has shown that oleic acid can significantly stimulate the expression of several marker genes related to adipocyte differentiation, such as FAS, adipocyte fatty acid-binding protein-4 (FABP4), and peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) [14,15]. Moreover, it has been identified that oleic acid by itself can also promote adipogenesis [15,16,17]. However, it is unknown whether oleic acid can play a similar role in duck adipocyte differentiation by stimulating adipocyte-related gene expression.

Until now, the FAS gene has been successfully cloned from several species, such as rat, chicken, pig, sheep, and human, but not duck [18,19,20]. In the current study, we cloned the full-length coding

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sequence of duck FAS gene and detected the expression level of FAS in duck preadipocytes exposed to oleic acid. Furthermore, the expression level of FAS during duck adipocyte differentiation was investigated. This work will have the potential to increase our understanding of the functional roles of FAS during duck adipocyte differentiation.

## 2. Materials and methods

### 2.1. Duck preadipocytes isolation and culture

Duck preadipocytes were isolated from Peking duck 1 week after hatching as previously described [13]. Briefly, Peking ducks were rapidly decapitated and subcutaneous adipose tissue was sterilely dissected from the leg. Then the adipose tissue was minced into fine sections using scissors and incubated in digestion buffer (PBS (-), 4% BSA (Gibco, USA), 0.1% collagenase type I (Gibco, USA)) at 37°C in a shaking water bath for 40–60 min. Growth medium (Dulbecco's modified Eagle's Medium/nutrient mixture F12 Ham's (V/V, 1:1; Gibco, USA), 10% fetal calf serum (Gibco, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco, USA) were used to end the digestion. The resulting mixture was filtered through 20 µm nylon screens to remove undigested tissue and large cell aggregates. These filtered cells were then centrifuged at 300 × g for 10 min at room temperature to separate floating adipocytes from the stromal-vascular cells. The preadipocytes were seeded at a density of  $1 \times 10^4$  cells/cm<sup>2</sup> and cultured in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air at 37°C. After the cells reached full confluence, oleic acid was added in the medium to induce preadipocyte differentiation. During this time, the FAS expression and the cell viability were measured.

### 2.2. Cloning the full-length coding sequence of FAS

Based on the FAS sequences of *Gallus gallus* (NM\_205155.2), *Meleagris gallopavo* (XM\_003211461.1), and *Anser cygnoides* (EU770327.1) deposited in the GenBank database, we designed and selected 11 pairs of gene-specific primers (namely FAS-P1 to FAS-P11) using DNAMAN 7.0, Primer Premier 5.0, and Oligo 7.0 software. PCR protocols are available upon request. The amplified products were verified by 1.5% agarose gel electrophoresis and purified with a gel extraction kit (Omega, USA). The purified products were then ligated into the pMD-19T vector (Biomed, China) and sequenced by Invitrogen Corporation (Applied Invitrogen, China). The primer sequences and PCR product lengths are listed in Table 1.

**Table 1**  
Primer sequences used in the current study.

Primer ID	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)
FAS-P1	CCGCCTACGCAGTAACAG	CTCACATTGGCAGAAGAC	980
FAS-P2	CAGCGGCAGTTGGTCAGT	ATCGCCCTCGCCAATAAG	1018
FAS-P3	AGATGAGGCTTTGAAGAACA	TGAACGAGGTTAGGGTGT	559
FAS-P4	TACCAGCCTGCCACAAC	TTCCCATTCCTGACACT	940
FAS-P5	TACCTGTGTGCTGGCTGG	CCTGTGACTGGTCATGTT	1062
FAS-P6	CTCCACCCCTGGAATAAT	AGACAGTTCACCATGCC	1053
FAS-P7	ATCCCTGCCAAACACC	AGTTTGCGGTGTCTTGCTC	1068
FAS-P8	AAGCAGCATTGCCATTG	CAAGCCCAATCCTCCTA	651
FAS-P9	ATGGTGTGGTAAAGCCCC	TTGATTGTAAGAAGTCGG	1093
FAS-P10	TTCCGGCACCCTCATCT	GCTGGGAGCACATTCAA	958
FAS-P11	GAGTCTGGCATCTATTA	GAAGAGTTCCTTGGGGTC	765
RT-FAS	TGGGAGTAACACTGATGCC	TCCAGGCTTGATACCACA	109
β-Actin	CAACGAGCGGTTTCAGGTGT	TGGAGTTGAAGGTGGTCTCG	92

Note: FAS-P1 to FAS-P11: Primers for gene cloning. RT-FAS: Primer for qRT-PCR. β-actin: Reference gene for data normalization.

### 2.3. RNA extraction and cDNA synthesis

Total RNA was extracted from the cultured cells at different differentiation stages using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions and quantified by spectrophotometric absorbance at 260 nm. First stand cDNA was synthesized from 10 µg of total RNA using a cDNA synthesis kit (TaKaRa, Japan). The newly synthesized cDNA product was immediately stored at -20°C for further study.

### 2.4. Cell viability assay

Duck preadipocytes were seeded on 96-well plates and treated with different concentrations of oleic acid for 24 h. Cell viability was determined using a commercial Cell Counting Kit-8 (CCK-8, Beijing Zomanbio biotechnology, China) as previously described [21]. 10 µL of CCK-8 reagent was added to each well and incubated at 37°C for 2–4 h until the media became yellow. Absorbance was measured at wavelength of 450 nm using a microplate reader.

### 2.5. Gene expression measurements

Quantitative real-time PCR (qRT-PCR) analysis was conducted by using SYBR PrimerScript™ RT-PCR kit (TaKaRa, Japan) in the CFX96™ Real-Time System (Bio-Rad, USA). The PCR was carried out in a 25 µL reaction volume, including 2.0 µL cDNA, 12.5 µL of SYBR Premix EX Taq, 8.5 µL of sterile water, and 1.0 µL of each gene-specific primer. The calibrator-normalized relative quantification method using the 2<sup>-ΔΔCT</sup> method was employed [22]. To normalize the target genes in similar cDNA samples, β-actin was selected as the reference gene. All reactions were completed in triplicate, and the data represents the mean of three independent experiments. The specific primers used are listed in Table 1.

### 2.6. Bioinformatics analysis

BLAST from NCBI was used to determine the similarity between nucleotide and protein sequences. Multiple alignments of the FAS sequences were conducted with the ClustalX multiple alignment software. MEGA 5.0 was used to generate the phylogenetic tree by the Neighbor-Joining (NJ) method based on the sequence of duck FAS and other known FAS sequences. The protein motif sequences and conserved domains were analyzed using NCBI CD-search tool in combination with SMART and PROSITE software.

### 2.7. Statistical analysis

Results are presented as the mean ± SD. The data were subjected to ANOVA testing, and the means were assessed for significance by Tukey's test using SPSS (version 17). *P* values less than 0.05 were considered significant in all statistical analysis.

## 3. Results

### 3.1. Cloning and sequence analysis of FAS from Peking duck

By sequencing and assembling the data, a 7654 bp mRNA of duck FAS was isolated by RT-PCR, which has been submitted to GenBank database with the accession number KF185112. The full-length coding sequence of duck FAS consists of 7545 nucleotides which encodes a 2525 amino acid protein with an estimated molecular mass of 275.0667 kDa and a theoretical isoelectric point of 6.07. The cloned FAS sequence is highly similar to turkey FAS, with 92% and 91% similarity at the nucleotide level and amino acid level, respectively (Table 2).

**Table 2**  
Homology of duck FAS with other species.

Species (Latin name)	Nucleotide (%)	Amino acid (%)
Duck ( <i>Anas platyrhynchos</i> )	99	99
Turkey ( <i>Meleagris gallopavo</i> )	92	91
Chicken ( <i>Gallus gallus</i> )	91	90
Pigeon ( <i>Columba livia</i> )	90	89
Green anole ( <i>Anolis carolinensis</i> )	76	77
Mouse ( <i>Mus musculus</i> )	69	65
Goat ( <i>Capra hircus</i> )	68	65
Human ( <i>Homo sapiens</i> )	66	65
Pig ( <i>Sus scrofa</i> )	66	64
Chimpanzee ( <i>Pan troglodytes</i> )	65	64

### 3.2. Phylogenetic analysis and alignment

A condensed phylogenetic tree was constructed based on the amino acid sequences of duck FAS and other organisms (Fig. 1). The overall topology of the tree showed that the duck FAS was most similar to those of the other avian species and also had a high similarity to FAS sequences of other organisms, especially *Anolis carolinensis*.

As indicated in Fig. 2a, multiple sequence alignment of duck FAS with other known FAS amino acid sequences revealed that they were highly conserved among different species, especially in the regions of FAS family signatures such as the KS domain, TE domain, and the ACP domain. The deduced FAS amino acid sequences were identified to have seven main functional domains by using SMART and NCBI CD-search software. Meanwhile, in the linear domain map of duck FAS (Fig. 2b), it could be seen that there were seven functional domains corresponding to KS (1–239, 243–360), AT (492–809), DH (865–1060), ER (1543–1859), KR (1890–2070), ACP (2129–2196), and TE (2245–2504).

### 3.3. Effect of different concentrations of oleic acid on duck FAS mRNA expression and cell viability

To investigate the effect of oleic acid on FAS gene expression and cell viability, duck preadipocytes were treated with different concentrations

of oleic acid for 12 h. As shown in Fig. 3, duck FAS mRNA expression level was up-regulated with increasing concentrations of oleic acid compared to the control group ( $P < 0.05$ ). However, FAS expression level decreased by treating the cells with 1000  $\mu\text{M}$  oleic acid ( $P < 0.05$ ). Meanwhile, it is shown in Fig. 4 that incubation of duck preadipocytes with growing concentrations of oleic acid led to a significant increase of cell viability ( $P < 0.05$ ). However, a high concentration of oleic acid (1000  $\mu\text{M}$ ) exhibited a lipotoxic effect by significantly reducing the cell viability ( $P < 0.05$ ).

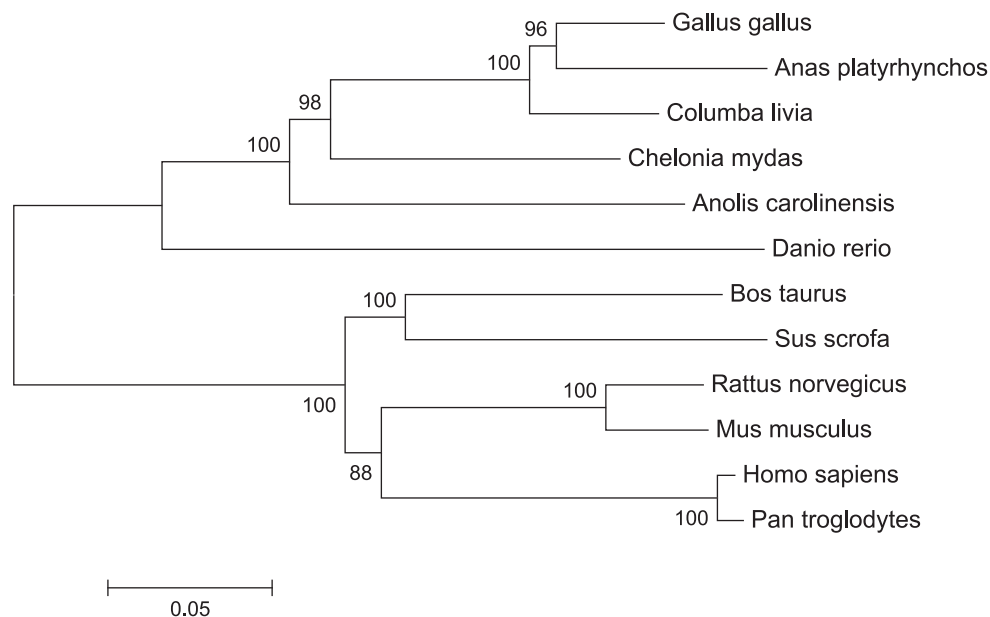
### 3.4. Expression of FAS mRNA during duck adipocyte differentiation

Duck preadipocytes were induced to differentiate by the addition of 300  $\mu\text{M}$  oleic acid and expression of FAS mRNA during adipogenesis was established. Results showed that duck preadipocytes began to differentiate at 12 h when treated with 300  $\mu\text{M}$  oleic acid (Fig. 5a–d). After 48 h of oleic acid treatment, most preadipocytes differentiated into mature adipocytes containing multiple lipid droplets (Fig. 5e).

As shown in Fig. 6, expression level of FAS mRNA gradually increased for 24 h when treated with oleic acid ( $P < 0.05$ ). However, expression level of FAS surprisingly decreased to a lower level after 24 h. There is a slight increase of FAS expression during 36–48 h post-oleic acid treatment ( $P < 0.05$ ).

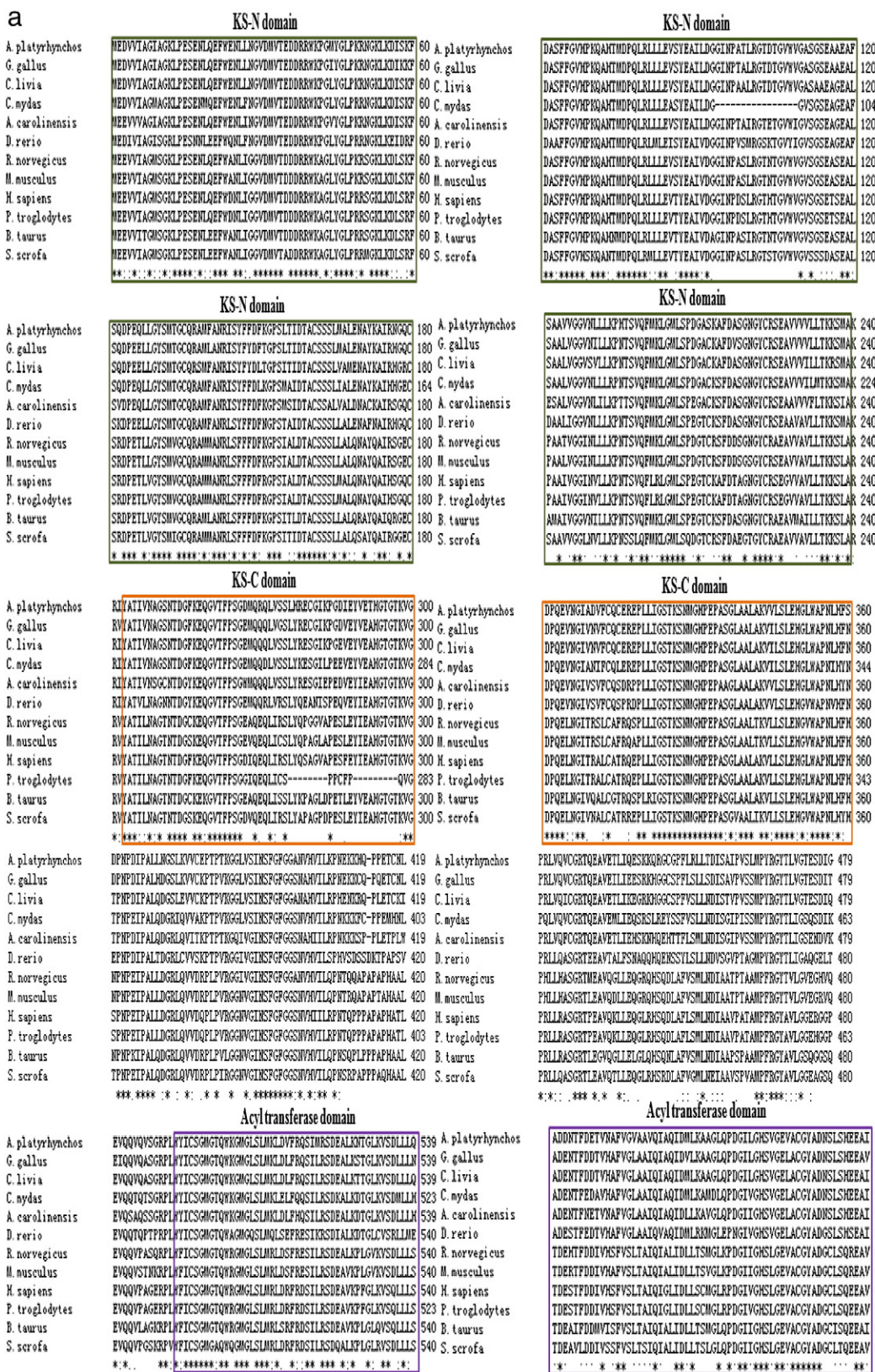
## 4. Discussion

Little is known about the role of FAS in duck. This study is the first to report the structures, expression, and possible functions of FAS during duck adipogenesis. Currently, we obtained the full-length coding sequence of duck FAS, which consists of 7515 nucleotides and encodes a 2505 amino acid protein. BLAST analysis revealed that the deduced amino acid sequence of FAS shared high identity with other known FAS sequences (65–92% identity in all the matches), especially with the *G. gallus* and *M. gallopavo* sequences. Based on the deduced amino acid sequence, we found that duck FAS, consistent with early reports [2,23], contained seven functional domains. The order of these functional domains is as follows: KS, MAT, DH, ER, KR, ACP, and TE. This gene has a molecular mass of 275.0667 kDa and a theoretical



**Fig. 1.** A phylogenetic tree of fatty acid synthase family members constructed using the neighbor-joining method. Numbers at each branch indicate the percentage of times a node was supported in 1000 bootstrap pseudoreplications by neighbor joining. The species names and the Genbank accession numbers of FAS are as follows: *Gallus gallus*, NP\_990486; *Homo sapiens*, AA473576; *Mus musculus*, NP\_032014; *Pan troglodytes*, XP\_511758; *Bos taurus*, XP\_005221054; *Sus scrofa*, NP\_001093400; *Danio rerio*, XP\_001923643; *Rattus norvegicus*, NP\_059028; *Columba livia*, EMC83573; *Chelonia mydas*, EMP32461; and *Anolis carolinensis*, XP\_003217337.





**Fig. 2.** (a) Multiple alignments of the deduced amino acid sequences of duck FAS with other species. The sequences were compared by ClustalX Multiple Sequence Alignment Program software. The numbers shown indicate the residue positions. Asterisks denote completely conserved residues; dashes indicate gaps introduced into the sequences to optimize the alignment; colons indicate conservative substitutions, and points indicate non-conservative substitutions. The species names and the GenBank accession numbers of FAS are as follows: *Gallus gallus*, NP\_990486; *Homo sapiens*, AAA73576; *Mus musculus*, NP\_032014; *Pan troglodytes*, XP\_511758; *Bos taurus*, XP\_005221054; *Sus scrofa*, NP\_001093400; *Danio rerio*, XP\_001923643; *Rattus norvegicus*, NP\_059028; *Columba livia*, EMC83573; *Chelonia mydas*, EMP32461; and *Anolis carolinensis*, XP\_003217337. (b) Linear domain map of duck FAS. The number of residues in each domain was indicated above the map. The specific locations of each domain were shown below. KS-N:  $\beta$ -ketoacyl synthase, N-terminal domain; KS-C:  $\beta$ -ketoacyl synthase, C-terminal domain; AT: Acyl transferase domain; DH: Dehydratase domain; ER: Enoyl reductase domain; KR:  $\beta$ -ketoacyl reductase domain; ACP: Acyl carrier protein; TE: Thioesterase domain.



Acyltransferase domain		Acyltransferase domain			
A. platyrhynchos	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	659	A. platyrhynchos	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	719
G. gallus	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	659	G. gallus	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	719
C. livia	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	659	C. livia	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	719
C. nydas	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	643	C. nydas	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	703
A. carolinensis	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	659	A. carolinensis	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	719
D. rerio	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	660	D. rerio	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	720
R. norvegicus	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	660	R. norvegicus	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	720
M. musculus	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	660	M. musculus	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	720
H. sapiens	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	660	H. sapiens	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	720
P. troglodytes	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	643	P. troglodytes	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	703
B. taurus	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	660	B. taurus	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	720
S. scrofa	LAATYRGRCVKEALPPGMAAVGLTVEECQRCPPVAVACHNSEDVTISGFLDSVNE	660	S. scrofa	FVAKLKDGVPFAKEVRSAGVAFHSTYMAFIAPALLSALKKVTIPHPKPSARWISTIPES	720
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Acyltransferase domain		Acyltransferase domain			
A. platyrhynchos	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	779	A. platyrhynchos	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	839
G. gallus	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	779	G. gallus	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	839
C. livia	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	779	C. livia	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	839
C. nydas	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	763	C. nydas	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	823
A. carolinensis	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	779	A. carolinensis	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	839
D. rerio	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	780	D. rerio	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	840
R. norvegicus	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	780	R. norvegicus	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	840
M. musculus	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	780	M. musculus	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	840
H. sapiens	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	780	H. sapiens	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	840
P. troglodytes	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	763	P. troglodytes	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	823
B. taurus	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	780	B. taurus	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	840
S. scrofa	QWQSLARSSAEYVNNLVNLPVLPQGLHCPENAVVEIAPHALLQALRLKPTCT	780	S. scrofa	ILPLMKDQKKNLEFFLTQAGKCHLTDGIVLGNMPPVEYVPGVPLISPIINQNSQ	839
* * * * *		* * * * *			
Dehydratase domain		Dehydratase domain			
A. platyrhynchos	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	A. platyrhynchos	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
G. gallus	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	G. gallus	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
C. livia	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	C. livia	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
C. nydas	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	883	C. nydas	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	942
A. carolinensis	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	A. carolinensis	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
D. rerio	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	900	D. rerio	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
R. norvegicus	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	R. norvegicus	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
M. musculus	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	M. musculus	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
H. sapiens	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	H. sapiens	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
P. troglodytes	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	882	P. troglodytes	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	941
B. taurus	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	899	B. taurus	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
S. scrofa	DWDVKAEDFPFGSGSASAVYNDVSPDSDNYLVGCHDGRVLPATGVLVLRWLT	898	S. scrofa	AGSLGNAMEQTAVNFEVTHQATILPKKGSQVLEVRIMPASHCFEVSQ-WGNLAVSGKI	958
* * * * *		* * * * *			
Dehydratase domain		Dehydratase domain			
A. platyrhynchos	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1017	A. platyrhynchos	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1077
G. gallus	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1017	G. gallus	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1077
C. livia	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1017	C. livia	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1077
C. nydas	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1001	C. nydas	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1061
A. carolinensis	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1015	A. carolinensis	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1075
D. rerio	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1018	D. rerio	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1078
R. norvegicus	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1015	R. norvegicus	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1075
M. musculus	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1015	M. musculus	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1075
H. sapiens	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1014	H. sapiens	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1074
P. troglodytes	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	997	P. troglodytes	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1057
B. taurus	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1018	B. taurus	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1078
S. scrofa	NLLENDALNFPQMLADPQS-QANVTANSGLMEDVQELHGLGVNPTQPGVLECKSE	1015	S. scrofa	GSAGKLVWGNVTFPLTLNMLVLAETGKSLPLTRISVCTDPLVLRQVQVQDQVE	1075
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A. platyrhynchos	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1137	A. platyrhynchos	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1196
G. gallus	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1137	G. gallus	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1196
C. livia	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1137	C. livia	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1196
C. nydas	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1121	C. nydas	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1190
A. carolinensis	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1135	A. carolinensis	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1194
D. rerio	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1138	D. rerio	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1198
R. norvegicus	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1135	R. norvegicus	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1196
M. musculus	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1135	M. musculus	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1196
H. sapiens	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1134	H. sapiens	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1191
P. troglodytes	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1117	P. troglodytes	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1174
B. taurus	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1138	B. taurus	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1195
S. scrofa	ALDVPDCLSLKAGGQVQGLHLSVAFRRQQRIPPTLEKFCPTPIYESDCLSSAQL	1135	S. scrofa	HATLEHCKGLKQKQVAVHVKLLHGLTQAG-AAAQSSPMQKGLHILAEICRLN	1192
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Fig. 2 (continued).

isoelectric point of 6.07. From this, we deduce that the sequence we cloned is the gene for Peking duck FAS.

Early studies have identified that expression of the FAS gene is controlled primarily at the level of transcription and is responsive to both nutritional and hormonal signals [24,25,26]. In diabetic mice,

insulin administration causes a significant and rapid induction of FAS mRNA expression. The dramatic induction of hepatic FAS mRNA by fasting/refeeding is prevented by cAMP and by streptozotocin induced diabetes [27]. Moreover, FAS mRNA expression decreased dramatically after fasting and rapidly restored to a level equal to or exceeding the



A. platyrhynchos	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1256	A. platyrhynchos	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1315
G. gallus	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1256	G. gallus	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1315
C. livia	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1256	C. livia	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1315
C. mydas	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1240	C. mydas	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1299
A. carolinensis	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1254	A. carolinensis	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1313
D. rerio	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1258	D. rerio	VFSQVSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1318
R. norvegicus	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1246	R. norvegicus	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1305
M. musculus	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1246	M. musculus	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1305
H. sapiens	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1251	H. sapiens	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1310
P. troglodytes	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1234	P. troglodytes	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1293
B. taurus	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1255	B. taurus	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1314
S. scrofa	GHLSELBQVITRENMHLDQDPLINGLLISSELKACLVDALENTSHRMKIVFALAGGR	1252	S. scrofa	LFSRVTSLINTQFLHLDITADCTLEALSANETELDAGHSFSQWDFSS-PFSGHLSNA	1311
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A. platyrhynchos	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1375	A. platyrhynchos	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1432
G. gallus	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1375	G. gallus	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1432
C. livia	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1375	C. livia	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1432
C. mydas	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1358	C. mydas	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1415
A. carolinensis	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1373	A. carolinensis	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1430
D. rerio	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1376	D. rerio	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1430
R. norvegicus	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1364	R. norvegicus	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1421
M. musculus	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1364	M. musculus	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1421
H. sapiens	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1370	H. sapiens	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1421
P. troglodytes	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1370	P. troglodytes	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1410
B. taurus	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1374	B. taurus	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1432
S. scrofa	DLVYVCTSVLHGTIKVSNLAAVKGFFVLLHTLGETLGETVFLSPILQAGHS	1369	S. scrofa	FLSQAGVWELPSKASLNTAMKSPFGSVFLCR--GSPATPFLPETHKHWES	1427
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A. platyrhynchos	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1491	A. platyrhynchos	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1551
G. gallus	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1491	G. gallus	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1551
C. livia	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1491	C. livia	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1551
C. mydas	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1474	C. mydas	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1534
A. carolinensis	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1474	A. carolinensis	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1549
D. rerio	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1483	D. rerio	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1553
R. norvegicus	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1480	R. norvegicus	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1540
M. musculus	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1480	M. musculus	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1540
H. sapiens	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1487	H. sapiens	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1547
P. troglodytes	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1470	P. troglodytes	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1530
B. taurus	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1491	B. taurus	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1530
S. scrofa	LKEILADQ-SEQPWLATSGHSGILGWNCRLLEAGHRCVFSVSLMFSSVAFSIS	1486	S. scrofa	LSSLEMQEIVQRLVWVYRDGKNGSFRMLPQQGQPELTETAYVNLTR-DLSSLSMT	1546
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ER domain					
A. platyrhynchos	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1610	A. platyrhynchos	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1670
G. gallus	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1610	G. gallus	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1669
C. livia	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1610	C. livia	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1669
C. mydas	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1593	C. mydas	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1652
A. carolinensis	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1608	A. carolinensis	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1667
D. rerio	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1612	D. rerio	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1671
R. norvegicus	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1600	R. norvegicus	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1659
M. musculus	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1599	M. musculus	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1658
H. sapiens	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1606	H. sapiens	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1665
P. troglodytes	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1589	P. troglodytes	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1648
B. taurus	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1610	B. taurus	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1669
S. scrofa	VSPILQHPYIT-TNFDVQLKRYTASLNFPRIMLATGKLSFDAIPGNWASQCMGWEPFSGR	1605	S. scrofa	DMAGREVMGLVPAKGLATVVDCKRFLKEVPENTLLEAASVPVYATAYSL-VVGRGV	1664
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ER domain					
A. platyrhynchos	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1730	A. platyrhynchos	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1790
G. gallus	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1729	G. gallus	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1789
C. livia	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1729	C. livia	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1789
C. mydas	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1712	C. mydas	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1772
A. carolinensis	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1727	A. carolinensis	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1787
D. rerio	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1731	D. rerio	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1791
R. norvegicus	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1719	R. norvegicus	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1779
M. musculus	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1718	M. musculus	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1778
H. sapiens	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1725	H. sapiens	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1785
P. troglodytes	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1704	P. troglodytes	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1784
B. taurus	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1729	B. taurus	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1789
S. scrofa	ERVVFLINSGGGQQAATIALSMGCRVPTTVGSABKRYLAQRFPLQDANSFASRN	1724	S. scrofa	TFPEQVHLVTVGKGVVVLNSLAEKLAQSLCLARHGRFLTEIGKFLDINSQNLGALF	1784
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Fig. 2 (continued).

original fed state by refeeding [28]. In goose primary hepatocytes, both insulin and glucose increase FAS activity, gene expression, and TG accumulation [29]. Also, it should be emphasized that FAS can be

activated by a combined effect of glucose and insulin in activating sterol regulatory element-binding protein-1c (SREBP-1c), which is an important transcription factor regulating lipid metabolic related gene



ER domain		KR domain	
A. platyrhynchos	LKNVSPHGLLDSIFEGNEEVSVKLLTGKINGVVPKLTSTVNDVEAFAFRMAQ 1850	A. platyrhynchos	GGTGKVMKQKEEKVPLRSEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1910
G. gallus	LKNVAFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1849	G. gallus	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1908
C. livia	LKNVAFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1849	C. livia	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1908
C. nydas	LKNVAFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1832	C. nydas	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1892
A. carolinensis	LKNVAFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1847	A. carolinensis	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1907
D. rerio	LKNVAFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1851	D. rerio	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1911
R. norvegicus	LKNVTFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1839	R. norvegicus	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1899
M. musculus	LKNVTFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1838	M. musculus	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1898
H. sapiens	LKNVTFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1845	H. sapiens	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1905
P. troglodytes	LKNVTFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1824	P. troglodytes	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1884
B. taurus	LKNVTFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1849	B. taurus	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1909
S. scrofa	LKNVTFHGLLDSIFEGNEEVSVKLLTGKIDGVVPLKRTTVKNEVEAFAFRMAQ 1844	S. scrofa	GGTGKVMKQKEEKVPLR-SEPVLSAISRSCPTTSSYITGGLGFLGAQLTI 1904
*****		*****	
KR domain		KR domain	
A. platyrhynchos	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1970	A. platyrhynchos	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2030
G. gallus	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1968	G. gallus	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2028
C. livia	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1968	C. livia	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2028
C. nydas	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1952	C. nydas	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2012
A. carolinensis	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1967	A. carolinensis	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2027
D. rerio	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1971	D. rerio	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2031
R. norvegicus	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1959	R. norvegicus	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2019
M. musculus	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1958	M. musculus	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2018
H. sapiens	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1965	H. sapiens	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2025
P. troglodytes	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1944	P. troglodytes	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2004
B. taurus	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1969	B. taurus	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2029
S. scrofa	ERGAQLVLTSSRGRTGVQAKVREYKALGIQVLTSDIGTLEGTQLLIEEALGLPV 1964	S. scrofa	GGTFNLAVLRDAMENQTPFLFVWPKATSGTLLMDVTRKCPDLDFVVFSSVSG 2024
*****		*****	
KR domain		ACP domain	
A. platyrhynchos	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2089	A. platyrhynchos	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2148
G. gallus	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2088	G. gallus	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2146
C. livia	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2087	C. livia	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2147
C. nydas	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2072	C. nydas	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2131
A. carolinensis	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2087	A. carolinensis	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2146
D. rerio	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2091	D. rerio	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2151
R. norvegicus	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2079	R. norvegicus	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2139
M. musculus	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2078	M. musculus	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2138
H. sapiens	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2085	H. sapiens	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2144
P. troglodytes	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2084	P. troglodytes	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2123
B. taurus	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2089	B. taurus	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2148
S. scrofa	RGNAGQNTYGFANSMERICERKRDGLPLAVQGAIGDV-ILKTGNTDVTGGTIP 2084	S. scrofa	QRISSCLEVLDLFLNQPHVMSFVLAERKSVK-SEGGSDRLVEAVHILGIRDLVSLN 2144
*****		*****	
ACP domain		Thioesterase domain	
A. platyrhynchos	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2207	A. platyrhynchos	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2267
G. gallus	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2205	G. gallus	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2265
C. livia	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2201	C. livia	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2254
C. nydas	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2190	C. nydas	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2250
A. carolinensis	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2203	A. carolinensis	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2263
D. rerio	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2208	D. rerio	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2267
R. norvegicus	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2199	R. norvegicus	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2258
M. musculus	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2198	M. musculus	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2257
H. sapiens	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2204	H. sapiens	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2264
P. troglodytes	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2183	P. troglodytes	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2243
B. taurus	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2208	B. taurus	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2268
S. scrofa	ADSTLADGLDLSUNVEVQTLERDVIINTMREITLITNKLRELSSTGTAEELP-S 2204	S. scrofa	QLLKTGFGEPPKLDLNLNLLVWPEGTITLNLVQSTERPLFLVHPTBGSTAVPTLASRI 2264
*****		*****	

Fig. 2 (continued).

expression [29,30]. In addition, dietary polyunsaturated fatty acids (PUFA) could suppress hepatic FAS gene expression, which is mediated by a pathway independent of peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) [31]. In goose preadipocytes, expression of FAS can be up-regulated by a high concentration of oleic acid [16]. Similarly, in our study, we found that duck FAS gene expression was also up-regulated with increasing concentrations of oleic acid ( $P < 0.05$ ) (Fig. 3). Additionally, our results showed that oleic acid enhanced duck preadipocyte viability, but at high concentrations, such as 1000  $\mu$ M, oleic acid decreased cell viability due to its lipotoxic effects ( $P < 0.05$ )

(Fig. 4). These results demonstrate that oleic acid would stimulate FAS mRNA expression in duck preadipocytes. However, high concentrations of oleic acid might decrease FAS gene expression due to its toxic effect on cell viability.

In poultry exogenous fatty acids are essential for adipogenesis. Compelling evidence has shown that oleic acid may promote adipogenic gene expression and lipid accumulation [16,17,32]. As a key gene in the DNL, FAS is of great importance for adipogenesis. In chicken, adipogenesis is induced by oleic acid and the expression level of FAS gradually increases for the first 24 h and remains high

Thioesterase domain

Thioesterase domain

A. platyrhynchos	HMPCYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2327	A. platyrhynchos	QNASHALNSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2387
G. gallus	HMPCYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2325	G. gallus	QNASHALNSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2385
C. livia	HMPCYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2314	C. livia	QNASHALNSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2374
C. mydas	SMFSGYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2310	C. mydas	QNFNSHLSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2370
A. carolinensis	KIPYGLQPTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2323	A. carolinensis	QNFNSHLSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2363
D. rerio	SVPCYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2327	D. rerio	HCFT---VETFLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2384
R. norvegicus	SVPTYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2318	R. norvegicus	QGFAPAHNHLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2378
M. musculus	SVPTYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2317	M. musculus	QGFAPAHNHLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2377
H. sapiens	SIPTYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2324	H. sapiens	QSFAPTHSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2384
P. troglodytes	SIPTYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2303	P. troglodytes	QSFAPTHSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2363
B. taurus	SIPTYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2328	B. taurus	QWAGPTNLSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2388
S. scrofa	SIPTYGLCTKAAPLDISQSLASTYIDCNQIQPEPGYTRIAGTSFGACVAFEMCSQLQAG	2324	S. scrofa	QSATPGHSLFLPDGSHSPVAATQSYRAKLTQGHAAETALCAFPVQQTGIEYHKL	2384

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Thioesterase domain

Thioesterase domain

A. platyrhynchos	ETLLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2447	A. platyrhynchos	LMRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2507
G. gallus	ETLLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2445	G. gallus	LMRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2505
C. livia	EVLLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2434	C. livia	LMRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2494
C. mydas	ETLLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2430	C. mydas	LMRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2490
A. carolinensis	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2443	A. carolinensis	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2503
D. rerio	ETLLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2444	D. rerio	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2504
R. norvegicus	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2438	R. norvegicus	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2498
M. musculus	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2437	M. musculus	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2497
H. sapiens	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2444	H. sapiens	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2504
P. troglodytes	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2423	P. troglodytes	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2483
B. taurus	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2448	B. taurus	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2508
S. scrofa	EALLPLKLEARVAAADLTQTHQINREALSPAASFYHMLKAADKTPESKTHGWT	2444	S. scrofa	LLRAKTHMEYBGLGQVYLSVCDGKSVVHVBGMRTLLRGGVVESTIGLINSIAEP	2504

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A. platyrhynchos	RYSVRBG	2514
G. gallus	RYSVRBG	2512
C. livia	RYSVRBG	2501
C. mydas	RYSVRBG	2497
A. carolinensis	RYSVRBG	2510
D. rerio	RYSVRBG	2511
R. norvegicus	RYSVRBG	2505
M. musculus	RYSVRBG	2504
H. sapiens	RYSVRBG	2511
P. troglodytes	RYSVRBG	2490
B. taurus	RYSVRBG	2515
S. scrofa	RYSVRBG	2511

\* \* \*

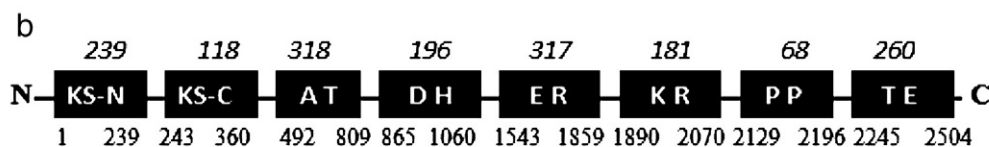
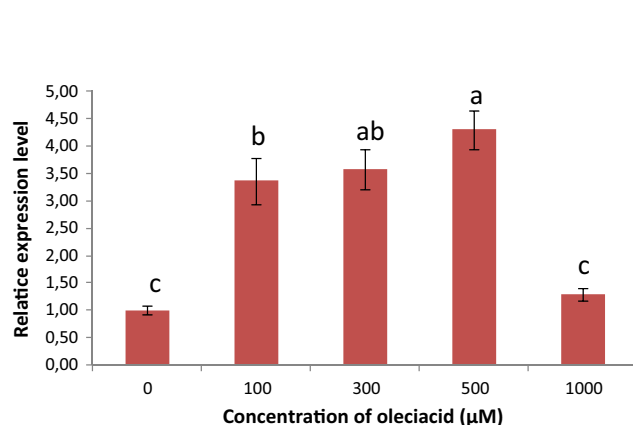
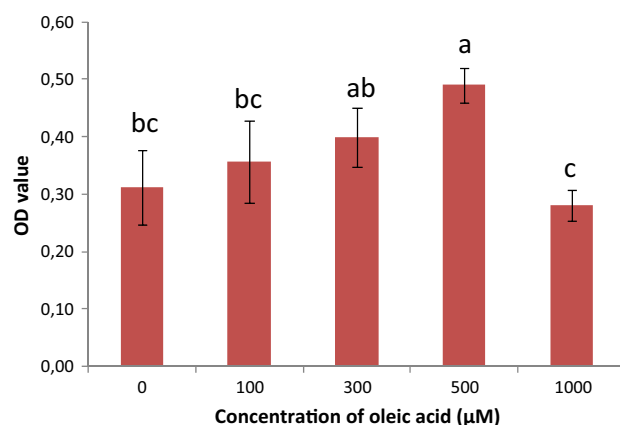


Fig. 2 (continued).

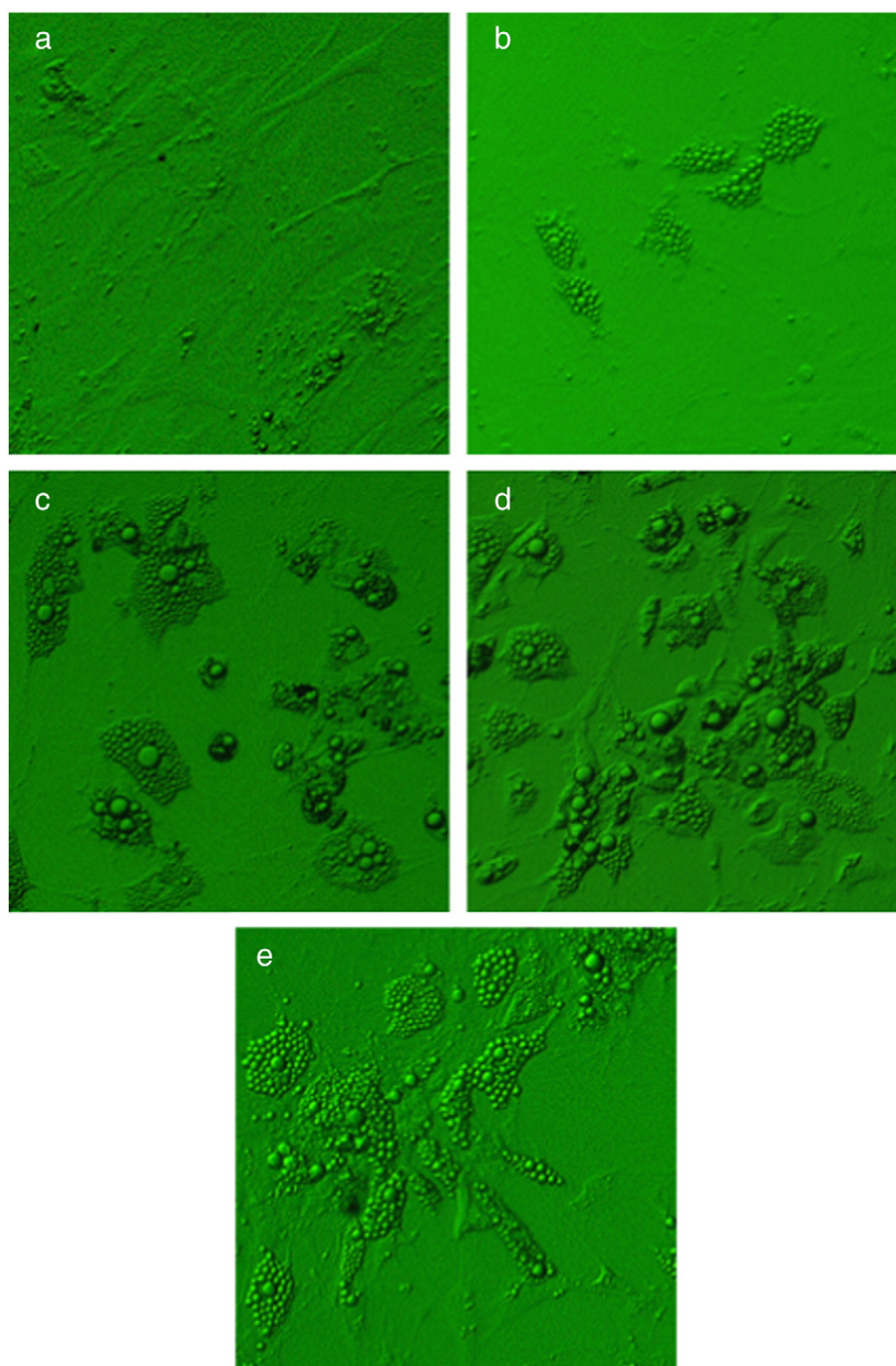


**Fig. 3.** Relative expression level of FAS gene in duck preadipocytes treated with different concentrations of oleic acid for 12 h. The data shown are mean  $\pm$  S.D. The mRNA expression level in the cells without oleic acid treatment was assigned as control. The different lowercase letters at the top of each bar indicate significant differences between the treatments ( $P < 0.05$ ).



**Fig. 4.** Viability of duck preadipocytes cultured in the medium supplemented with different concentrations of oleic acid for 12 h. The data shown are mean  $\pm$  S.D. Viability of the cells cultured in the medium in the absence of oleic acid was assigned as control. The different lowercase letters at the top of each bar indicate significant differences between the treatments ( $P < 0.05$ ).





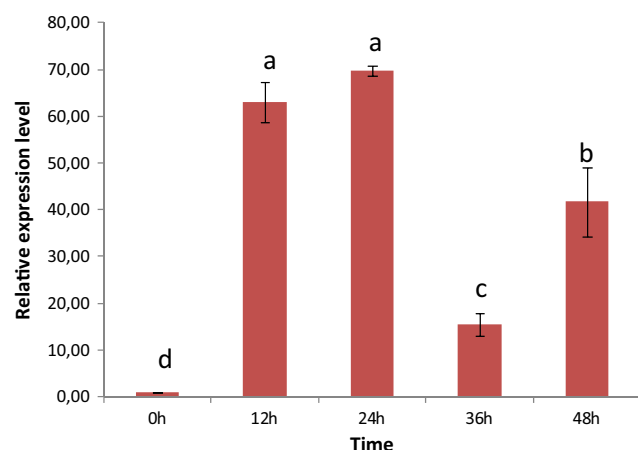
**Fig. 5.** Morphological changes of duck preadipocytes during differentiation. Duck preadipocytes were untreated (a), cultured with 300  $\mu\text{M}$  oleic acid for 12 h (b), 24 h (c), 36 h (d), and 48 h (e). Cells were observed by phase contrast microscopy at  $100\times$  magnification.

for 48 h [14]. Meanwhile, inhibition of FAS by standard inhibitors (such as cerumin or tannic acid) significantly suppresses the differentiation and lipid accumulation in 3T3-L1 preadipocytes [12,33]. These findings suggest that FAS is essential to promote adipocyte differentiation. In the present study, duck preadipocytes were induced to differentiate in the medium supplemented with 300  $\mu\text{M}$  oleic acid. We found that most preadipocytes have differentiated and accumulated huge lipid droplets after administration of oleic acid for 48 h (Fig. 5). During adipogenesis, the expression of FAS mRNA was notably up-regulated for the first 24 h; however, it declined to a lower level over 36–48 h (Fig. 6). Interestingly, one of our early studies showed that during the early growth stages, FAS mRNA in duck

subcutaneous adipose tissue exhibited a ‘rise and decline’ expression pattern. Moreover, the changes in FAS mRNA expression correlated to the changes in subcutaneous adipose tissue lipid content [34]. Altogether, these results indicate that FAS plays an important role in the early stage of duck adipogenesis. However, the molecular mechanism of how FAS promote duck adipogenesis is currently unknown and further research is needed.

## 5. Concluding remarks

For the first time we have successfully isolated the whole-length coding sequence of Peking duck FAS. Duck FAS encodes a 2515 amino



**Fig. 6.** Relative expression level of duck FAS gene in the preadipocytes during differentiation. The data are shown as mean  $\pm$  S.D. The mRNA expression level in the cells without oleic acid treatment was assigned as control. The different lowercase letters at the top of each bar indicate significant differences between different time points ( $P < 0.05$ ).

acid protein and shows high nucleotide and amino acid homology to other avian species. In addition, oleic acid was able to significantly stimulate FAS mRNA expression in duck preadipocytes. However, a high level of oleic acid decreased FAS expression due to its toxic effect on the cell viability. Furthermore, we found that FAS mRNA was highly expressed in the early stage of duck adipogenesis. Taken together, these results indicate that FAS plays an important role for duck adipocyte differentiation.

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### Author contributions

Proposed the theoretical frame: JW, FD; Conceived and designed the experiments: FD, JW; Contributed materials and analysis tools: QL, WS, CG; Performed the experiments: QL, HH, CS; Analyzed the data: FD, XY; Wrote the paper: FD.

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