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Overexpression of SREBF chaperone (SCAP) enhances nuclear SREBP1 translocation to upregulate fatty acid synthase (*FASN*) gene expression in bovine mammary epithelial cells

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ABSTRACT

Fatty acid synthase is a key enzyme for the synthesis of milk fat in the ruminant mammary gland. In nonruminants, sterol regulatory element binding protein 1 (SREBP1) is a regulator of *FASN* gene expression, and SREBF chaperone (SCAP) is essential for SREBP1 maturation and activity. However, the role of SCAP on the regulation of *FASN* gene expression in ruminants is unknown. The objective of this study was to investigate the transcriptional regulation of *FASN* by overexpressing SCAP in bovine mammary epithelial cells. A bovine SCAP expression vector, SREBP1 expression vector, and the promoter of *FASN* were cloned. The transcription factor binding sites of *FASN* promoter were predicted using bioinformatics analysis. After transfection with *FASN* promoter vectors in the immortalized bovine mammary epithelial cell line MAC-T, we co-overexpressed the SCAP + SREBP1 expression vector with pcDNA3.1 vector as control. The effect of SCAP + SREBP1 overexpression on the regulation of *FASN* was investigated using luciferase assay, immunofluorescence, Western blot, real-time PCR, and lipid droplet staining. We observed that co-overexpression of SCAP + SREBP1 significantly increased activity of the *FASN* promoter containing a sterol response element binding site. The *FASN* mRNA abundance and lipid droplet formation increased due to co-overexpression of SCAP + SREBP1. Compared with overexpression of SREBP1 alone, co-overexpression of SCAP + SREBP1 enhanced the nuclear translocation and nuclear SREBP1 protein abundance. Overall, as in nonruminants cells, results indicate that SCAP is essential for promoting nuclear translocation of SREBP1 and activation of *FASN* gene transcription, leading to lipid droplet formation in bovine mammary epithelial cells.
Key words: lactation, lipogenesis, milk fat

INTRODUCTION

The lactating mammary gland is the major lipid-synthesizing organ in the body (Rudolph et al., 2010). Fatty acid synthase (**FASN**), as a key metabolic enzyme that catalyzes the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA, is essential for functional development of mammary gland and milk fat production (Suburu et al., 2014). The bovine *FASN* gene is mapped to chromosome 19 and several SNP in the 5′ flanking region are significantly associated with milk fat content in dairy cattle (Roy et al., 2006; Ordovás et al., 2008). Therefore, studying the regulation of *FASN* will enhance mechanistic knowledge and may provide tools for practical manipulation of milk fat synthesis.

The regulation of *FASN* is primarily dependent on transcription factors that bind to the specific *cis*-acting elements located on the promoter (Griffin et al., 2007). Several potential transcriptional regulatory elements have been identified in the 5′ flanking region of rat and human *FASN* promoter that mediate both positive and negative effects on its abundance (Hsu et al., 1996; Oskouian et al., 1997).

Sterol regulatory element binding protein 1 (**SREBP1**) is a key transcription factor whose transcriptional activation coincides with copious milk fat synthesis at the onset and throughout lactation in dairy cows (Bionaz and Loor, 2008; Loor et al., 2013). The SREBP1 are synthesized as inactive precursors in the endoplasmic reticulum (**ER**) and form complexes with SREBF chaperone (**SCAP**; an ER-Golgi transporter). In the Golgi apparatus, SREBP1 are hydrolyzed by proteases and the transcriptional fragments of nuclear SREBP1 released to activate lipogenic genes (Moon et al., 2012; Moon, 2017). In vitro work with the nonsecretory MAC-T cell line indicated that abundance of *FASN* could be regulated by SREBP (Ma and Corl,

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2012). In fact, the role of SREBP in lipid synthesis has been well established in bovine mammary epithelial cells (Li et al., 2014).

At least in nonruminants, the SCAP protein is essential for the activation of SREBP, and in its absence the protease fails to cleave SREBP and prevents the transcriptionally active fragments of SREBP from entering the cell nucleus to activate gene expression (Rawson et al., 1999). Inhibition of SCAP function reduced SREBP-regulated gene expression and de novo lipogenesis in rhesus monkeys (Jensen et al., 2016). In ob/ob mice, knockdown of the hepatic *SCAP* gene reduced nuclear SREBP expression and produced a dramatic decrease in fatty acid (**FA**) synthesis (Moon, 2017). However, little is known about the role of SCAP in the transcriptional regulation of FASN abundance in ruminant mammary cells.

We hypothesized that SCAP might enhance SREBP1 activation of *FASN* gene abundance. To investigate this hypothesis, we constructed a bovine SREBP1 vector, a SCAP vector, and a *FASN* promoter vector and assessed the effect of overexpression of SCAP on the *FASN* promoter activity, mRNA expression, and lipid droplet formation in bovine mammary epithelial cells.

MATERIALS AND METHODS

Plasmid Construction

Total RNA was extracted from bovine mammary tissue samples using TRIzol reagent (15596, Invitrogen, Carlsbad, CA) and was reverse-transcribed to cDNA as a template for PCR. To construct the SCAP and SREBP1 expression plasmid, full-length open reading frame sequences were amplified from cDNA using PCR and subcloned into the restriction sites of the pcDNA3.1 (+) vector. The PCR primer pair of SCAP and SREBP1 encoded His-tag and Myc-tag protein sequences at the N terminus, respectively (primers are listed in Table 1).

FASN Promoter Reporter Construction and Characterization

Bovine genomic DNA was extracted from blood and used as a template for amplifying the *FASN* gene promoter. Three different 5′ flanking fragments of various lengths containing *FASN* promoter region (GenBank Accession Number: AF285607) were amplified by PCR. Promoters of 399, 255, and 177 bp were produced using PCR primers designed to hybridize at the corresponding positions and used with the common downstream primer (primers are listed in Table 1). The PCR fragment of *FASN* promoter was gel purified and digested with *Kpn*I and *Hin*dIII restriction enzymes to clone into the pGL3-Basic Vectors (E1741, Promega, Madison, WI). Plasmid DNA from selected clones was identified by restriction enzyme and then sequenced. The potential transcription factor binding sites were predicted using online bioinformatics software (Roy et al., 2005; Li et al., 2015). The sterol response element (**SRE**) sequence of the bovine *FASN* promoter was aligned to that of human (GenBank: AF250144), rat (GenBank: X54671), and goat (GenBank: kp749922) using the BioXM2.6 software (Institute of Rice Research, Nanjing Agricultural University, Nanjing, China).

Cell Culture and Transient Transfection

Experiments were performed using the immortalized bovine mammary epithelial cell line MAC-T. The MAC-T cell line was produced from primary bovine mammary

Table 1. Primers used for cloning the open reading frame of SREBP1, open reading frame of SCAP, the FASN promoter and *FASN* mRNA

Gene symbol and GenBank accession number	Primer[1] sequence (5′ – 3′)	Product length (bp)
SCAP	F: CCCAAGCTTATGCATCATCACCATCACCATACCCTGACTGAAAG	3,876
NM_001101889	R: ATTTGCGGCCGCTCAGTCCAGCTTCTCCA	
SREBP1	F: CTAGCTAGCATGGAACAAAAACTCATCTCAGAAGAGGATCTGGACGAGCC	3,486
NM_001113302.1	R: TGCTCTAGACTAGCTGGAGGTCACAGTGGTCCCA	
FASN[2]	*FASN* 3F: CGGGGTACCGGGAGGCGTGGAGCACGGAA	399
AF285607	*FASN* 2F: CGGGGTACCGCATCACCCCACTGGCGGC	255
	FASN 1F: CGGGGTACCCTGTCAGCCCATGTGGCGTGTC	177
	FASN R: CCCAAGCTTTGGCCGCTTGTACCTGGTCTGG	
	Q F: ACCTCGTGAAGGCTGTGACTCA	92
	Q R: TGAGTCGAGGCCAAGGTCTGAA	

[1]F = forward, R = reverse; restriction sites and protecting bases are underlined.

[2]*FASN*3 F, *FASN*2 F, and *FASN*1 F were used to amplify 3 different lengths containing the *FASN* promoter region. Q denotes the PCR primer for *FASN* mRNA.