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# Interactions of the ubiquitous octamer-binding transcription factor-1 with both the signal transducer and activator of transcription 5 and the glucocorticoid receptor mediate prolactin and glucocorticoid-induced β-casein gene expression in mammary epithelial cells

Xi Qian, Feng-Qi Zhao*

*Laboratory of Lactation and Metabolic Physiology, Department of Animal Science, University of Vermont, Burlington, VT 05405, USA*

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ABSTRACT

Regulation of milk protein gene expression by lactogenic hormones (prolactin and glucocorticoids) provides an attractive model for studying the mechanisms by which protein and steroid hormones synergistically regulate gene expression. β-Casein is one of the major milk proteins and its expression in mammary epithelial cells is stimulated by lactogenic hormones. The signal transducer and activator of transcription 5 and glucocorticoid receptor are essential downstream mediators of prolactin and glucocorticoid signaling, respectively. Previous studies have shown that mutating the octamer-binding site of the β-casein gene proximal promoter dramatically reduces the hormonal induction of the promoter activity. However, little is known about the underlying molecular mechanisms. In this report, we show that lactogenic hormones rapidly induce the binding of octamer-binding transcription factor-1 to the β-casein promoter and this induction is not mediated by either increasing the expression of octamer-binding transcription factor-1 or inducing its translocation to the nucleus. Rather, lactogenic hormones induce physical interactions between the octamer-binding transcription factor-1, signal transducer and activator of transcription 5, and glucocorticoid receptor to form a ternary complex, and these interactions enhance or stabilize the binding of these transcription factors to the promoter. Abolishing these interactions significantly reduces the hormonal induction of β-casein gene transcription. Thus, our study indicates that octamer-binding transcription factor-1 may serve as a master regulator that facilitates the DNA binding of both signal transducer and activator of transcription 5 and glucocorticoid receptor in hormone-induced β-casein expression, and defines a novel mechanism of regulation of tissue-specific gene expression by the ubiquitous octamer-binding transcription factor-1.



## 1. Introduction

Transcriptional regulation of gene expression is largely dependent on the interactions of transcription factors with the corresponding *cis*-DNA elements located in the promoter or enhancer region of a gene. Octamer-binding transcription factor-1 (Oct-1) was originally discovered for its ability to bind the conserved octamer motif (ATGCAAAT), which is located in the promoter and enhancer sequences of the histone H2B, immunoglobulin, and U2 small nuclear RNA (snRNA) genes (Sive and Roeder, 1986). As a member of the POU (Pit-1, Oct and Unc-86) family of homeodomain transcription factors, Oct-1 contains a POU specific domain ($POU_S$) in addition to a POU homeodomain ($POU_H$), which is distantly related to the classic homeodomain encoded by homeobox genes (Kang et al., 2009b; Zhao, 2013). Oct-1 has been implicated in many important biological processes, including embryogenesis (Range and Lepage, 2011; Sebastiano et al., 2010), immune/inflammatory responses (Cheng et al., 2012; Ren et al., 2011), metabolic responses to stress (Goettsch et al., 2011; Malhas et al., 2009; Wang et al., 2009), and tumorigenicity (Kang et al., 2009b; Shakya et al., 2009). The genes regulated by Oct-1 include a wide variety of both

*Abbreviations:* DTT, dithiothreitol; EGF, epidermal growth factor; EMSA, electrophoresis mobility shift assay; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; GR, glucocorticoid receptor; GRE, glucocorticoid response elements; HP, hydrocortisone (glucocorticoids) and prolactin; IP, immunoprecipitation; MECs, mammary epithelial cells; Oct-1, octamer-binding transcription factor-1; PMSF, phenylmethylsulfonyl fluoride; POU, Pit-1, Oct and Unc-86; $POU_H$, POU homeodomain; $POU_S$, POU-specific domain; PrlR, prolactin receptor; qChIP, quantitative chromatin immunoprecipitation; qPCR, quantitative PCR; qRT-PCR, quantitative reverse transcription PCR; snRNA, small nuclear RNA; STAT5, signal transducer and activator of transcription 5; TBP, TATA box-binding protein; WT, wild-type.

* Corresponding author at: Department of Animal Science, University of Vermont, 211 Terrill Building, 570 Main Street, Burlington, VT 05405, USA.
Tel.: +1 802 656 0786; fax: +1 802 656 8196.
*E-mail address:* fzhao@uvm.edu (F.-Q. Zhao).

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ubiquitously expressed genes and tissue-specific genes. Oct-1 regulates these genes via DNA binding-dependent or -independent mechanisms. Both of the POU-domains are required for the high-affinity, site-specific binding to the octamer motif and are involved in protein–protein interactions with other transcription factors and co-factors (Kang et al., 2009b; Ren et al., 2011; Robinson et al., 2011).

β-Casein is a major milk protein, that is expressed via stimulation by lactogenic hormones, including prolactin and glucocorticoids (HP) (Rosen et al., 1999). There are three highly conserved regions in the proximal promoter of the casein genes, which are referred to as blocks A, B, and C (Yoshimura and Oka, 1990). Blocks A and B have been intensively studied and have been shown to be the binding sites of HP downstream molecules, signal transducer and activator of transcription 5 (STAT5) and glucocorticoid receptor (GR) (Groner et al., 1994). Following mammary epithelial cell stimulation with lactogenic hormones, both STAT5 and GR are phosphorylated, translocate from the cytoplasm to the nucleus, recognize and bind to the corresponding binding sites in blocks A and B, and synergistically stimulate β-casein gene transcription (Lechner et al., 1997). Less is known about the mechanisms by which block C contributes to β-casein gene regulation. We have previously demonstrated that block C contains an octamer-binding site and that both its integrity and orientation are critical for the hormonal induction of β-casein gene promoter activity (Dong and Zhao, 2007; Dong et al., 2009).

In this study, we explored the molecular mechanisms by which Oct-1 participates in the hormonal induction of β-casein gene expression in mammary epithelial cells. Quantitative chromatin immunoprecipitation (qChIP) experiments indicated that Oct-1 indeed binds to the β-casein gene promoter in mammary epithelial cells and that this binding activity is hormonally regulated. Transfection experiments revealed that Oct-1 knockdown inhibits while overexpression stimulates β-casein gene expression induced by lactogenic hormones. Additionally, we demonstrated that in response to lactogenic hormones, Oct-1 physically interacts with STAT5 and GR, which facilitates the DNA binding of both STAT5 and GR to the β-casein gene promoter. Our data provide new insight into the molecular mechanisms by which the ubiquitously expressed Oct-1 contributes to the hormonal regulation of mammary epithelial cell-specific β-casein gene expression.

## 2. Materials and methods

### 2.1. Materials

Prolactin (L6520), hydrocortisone (one of glucocorticoids, H6909), insulin (I0516), and murine epidermal growth factor (EGF) (E4127) were purchased from Sigma (St. Louis, MO). Heat-inactivated fetal calf serum (1082-147), RPMI 1640 medium (31800-022), gentamicin (15750-060), and antibiotic–antimycotic solution (15240-062) were purchased from Invitrogen (Carlsbad, CA). Dynabeads® Protein A (100-01D) for ChIPs and immunoprecipitations (IPs) and Dynabeads® M-280 Streptavidin (112-05D) for DNA pull-down assays were also obtained from Invitrogen. Charcoal-stripped horse serum (52-0745) was purchased from Cocalico Biologicals (Reams Town, PA). Growth factor reduced matrigel (354230) and dispase (354235) were obtained from BD Biosciences (Franklin Lakes, NJ). The mouse Oct-1B (mOct-1B/pcDNA3.1), GR (mGR/pcDNA3.1), STAT5a (mSTAT5a/pcDNA3.1), and prolactin receptor (PrlR) expression plasmids as well as the wild-type (WT) mouse β-casein promoter (−258/+7)/luciferase construct (LHRRWT/pGL3) have been described previously (Dong and Zhao, 2007). The *Renilla* luciferase control plasmid (phRL-CMV) was purchased from Promega (Madison, WI). The anti-TATA box binding protein (TBP) (sc-273), anti-actin (sc-1615-R), anti-STAT5 (sc-1081), and anti-GR (sc-1004) antibodies were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). The anti-Oct-1 (A310-610A) antibody was provided by Bethyl Laboratories (Montgomery, TX). Normal rabbit IgG (10500C) was obtained from Invitrogen.

### 2.2. Cell cultures, transfection, and luciferase assays

The murine mammary epithelial cell line, HC11, was cultured as previously described (Kabotyanski et al., 2006). HC11 Lux cells, which are HC11 cells stably transfected with a β-casein promoter luciferase construct (p-344/-1βc-Lux), were obtained from Dr. Hynes (Friedrich Miescher Institute, Switzerland) (Wartmann et al., 1996) and cultured as described for HC11 cells. Primary mouse mammary epithelial cells (MECs) were isolated following the procedures described by Watkin and Streuli (2002). Briefly, the mammary glands from mid-pregnant C57BL/6 mice were pooled, minced, and digested by collagenase. Next, the epithelial cells were enriched via centrifugation, plated on 60-mm dishes that were pre-coated with matrigel, and cultured in complete growth medium (D-MEM/F-12 supplemented with 10% fetal calf serum, 5 μg/ml bovine insulin, 10 ng/ml EGF, 1 μg/ml hydrocortisone, 1× antibiotic–antimycotic solution, and 50 μg/ml gentamicin). After 2 days of confluence, the cells were incubated in hormone-priming medium (D-MEM/F-12 medium supplemented with 10% charcoal-treated horse serum, 5 μg/ml bovine insulin, 1× antibiotic–antimycotic solution, and 50 μg/ml gentamicin) for 24 h and then incubated for 24 h in hormone-treatment medium (priming medium supplemented with 1 μg/ml hydrocortisone and 5 μg/ml prolactin).

The methods applied for the transfection and luciferase assays have been described previously (Dong and Zhao, 2007). In the Oct-1-overexpression studies, HC11 cells were transfected with either 0.2 pmol of pcDNA3.1 or mOct-1B/pcDNA3.1, 0.2 pmol of LHRRWT/pGL3, and 0.004 pmol of phRL-CMV using Lipofectamine 2000 (Invitrogen). In the siRNA transfection experiments, HC11 Lux cells were transfected with either 40 pmol of Oct-1 siRNA #1 (Santa Cruz Biotechnologies, siRNA #sc-36120), Oct-1 siRNA #2 [Ambion (Austin, TX), siRNA #68842], or control siRNA (Ambion, siRNA #4611). In the co-transfection studies, HC11 cells were transfected with 0.07 pmol of the Oct-1B, GR, or STAT5 expression plasmid or various combinations of these constructs along with 0.2 pmol of LHRRWT/pGL3 and 0.004 pmol of phRL-CMV. In all groups, the total molar amount of DNA was balanced using pcDNA3.1. After 10–12 h, the transfection medium was replaced with hormone medium (RPMI1640 supplemented with 10% charcoal-treated horse serum, 50 μg/ml gentamicin, 1 μg/ml hydrocortisone, 5 μg/ml bovine insulin, and 5 μg/ml prolactin). Luciferase activities were examined after 24 h of hormone treatment. The *Renilla* luciferase control plasmid was used to normalize transfection efficiency. In HC11 Lux cells, the luciferase activity levels were normalized to protein concentrations.

### 2.3. qChIP

ChIP was performed as described previously (Kabotyanski et al., 2006) with a few modifications. Formaldehyde was added to the growth medium at a final concentration of 1% to crosslink the chromatin and interacting proteins. After sonication, the chromatin suspension was precleared with Dynabeads® Protein A. Before performing the IP, 1% of the total sheared chromatin was kept as a total input control. Next, the designated antibody was added to precipitate the sheared chromatin. The immuno-complexes were then captured with Dynabeads® Protein A. After reverse cross-linking and DNA purification, 2 μl of the final precipitated DNA was used