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Gonadotropin gene transcription is activated by menin-mediated effects on the chromatin

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ABSTRACT

The genes encoding luteinizing hormone and follicle stimulating hormone are activated by gonadotropin-releasing hormone (GnRH), and we hypothesized that this involves GnRH-induction of various histone modifications. At basal conditions in an immature gonadotrope-derived cell line, the hormone-specific β-subunit gene promoters are densely packed with histones, and contain low levels of H3K4 trimethylation (H3K4me3). GnRH both induces this modification and causes histone loss, creating a more active chromatin state. The H3K4me3 appears to be mediated by menin and possibly catalyzed by the menin-mixed-lineage leukemia (MLL) 1/2 methyl transferase complex, as inhibition of MLL recruitment or menin knockdown reduced gene expression and the levels of H3K4me3 on all three promoters. Menin recruitment to the β-subunit gene promoters is increased by GnRH, possibly involving transcription factors such as estrogen receptor α and/or steroidogenic factor 1, with which menin interacts. Menin also interacts with ring finger protein 20, which ubiquitylates H2BK120 (H2BK120ub), which was reported to be a pre-requisite for H3K4me3 at various gene promoters. Although levels of H2BK120ub are increased by GnRH in the coding regions of these genes, levels at the promoters do not correlate with those of H3K4me3, nor with gene expression, suggesting that H3K4me3 is not coupled to H2BK120ub in transcriptional activation of these genes.

1. Introduction

The gonadotropin hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH) control mammalian reproduction. They are regulated by a number of factors along the hypothalamic–pituitary–gonadal axis, the most dominant of which is the hypothalamic gonadotropin releasing hormone (GnRH), which binds a G-protein coupled receptor on the gonadotropes to exert its effects [1]. In accordance with the various stages of reproductive activity during the lifespan and through changes in reproductive seasons, the altered levels of GnRH synthesis and release determine the expression of the three gonadotropin subunit genes, *Lhβ*, *Fshβ* and that encoding the common α subunit: *αGsu*, and the production and release of these hormones. Notably however, the *αGsu*, which is also expressed in the thyrotropes as it comprises part of the thyroid stimulating hormone, is expressed at higher levels at all stages of development and is under less stringent control than the hormone-specific β-subunit genes, whose expression is considered the rate-limiting step in production of the active hormones.

The mechanisms through which these genes are activated in basal conditions and via induction of various signaling cascades and gene-specific transcription factors have been described in some detail [1–6]. However, in order for these proteins to bind the gene promoters and transcription to be activated, the DNA must be made accessible through altering the structure of the chromatin. We have previously reported that GnRH induces the removal of histone deacetylases (HDACs) and some corepressors from the *Lhβ* and *Fshβ* gene promoters to facilitate the expression of these genes [7,8]. This suggests that in their basal state, the chromatin at the β-subunit genes may be repressed as a result of deacetylation. However nothing more is known about the chromatin packaging of these genes in their basal states, nor which histone modifications can be specifically induced by GnRH to activate their transcription.

A number of histone modifications are associated with actively transcribed genes, one of the most notable being trimethylation of histone H3 at lysine 4 (H3K4me3), which is seemingly present at the promoters of all active genes and considered essential for transcription initiation [9–14]. This modification interacts with and recruits various factors, including TFIID, histone acetyltransferases and chromatin remodelers to the transcriptional start site to facilitate transcription [15–19]. However its presence is not necessarily sufficient to induce transcription, as bivalent promoters carry both H3K4me3 and the repressive mark of trimethylation at H3K27 (H3K27me3), which allows

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this category of genes to be poised and ready for either entering a repressed state following the loss of H3K4me3, or activation following the loss of H3K27me3 [12].

In yeast, H3K4me3 is catalyzed by the Set1/COMPASS histone methyl transferase (HMT) complex whose recruitment and activity are regulated by the RNA polymerase II (RNAPII)-associated PAF complex, such that H3K4me3 occurs only subsequent to RNAPII recruitment [20–23]. This modification is dependent on prior ubiquitylation of histone H2B at lysine 123 (H2BK123ub; [20,21]), although in mammalian cells, the role of H2BK120ub (analogous to the yeast H3K123ub) at the promoter and such a cross-talk between these modifications is more controversial [24–28].

The complexity in the regulation of H3K4me3 and its cross-talk with H2BK120ub is greater in mammalian cells than in yeast because there are at least six Set1-related HMTs in mammals which can catalyze H3K4me3. These include Set1A/B, and the mixed-lineage leukemia (MLL) proteins, MLL1 to 4 which are found in COMPASS-like complexes (reviewed by [14,29]). Although the Set1/COMPASS complex appears to be responsible for the majority of H3K4me3, the MLL/COMPASS-like complexes catalyze this modification at specific subsets of genes, the reasons for which are not clear [29,30]. Targeting is presumably directed, at least in part, by some of the more specific components of these complexes, and it seems likely that the dependency on H2BK120ub which has been disputed in mammals, may also depend on the H3K4me3 HMT complex involved.

In this study, we hypothesized that the transcriptional activation of the gonadotropin genes requires the induction of various histone modifications at the gene promoters, including most likely H3K4me3, and possibly H2BK120ub. We sought to characterize some of the changes in these modifications following GnRH treatment, and to elucidate the HMT complex responsible for catalyzing H3K4me3 at these genes. We propose a mechanism for the likely recruitment of this HMT complex through a novel protein–protein interaction, and also provide evidence suggesting that H2BK120ub is likely not coupled to H3K4me3 at these genes.

2. Materials and methods

2.1. Cell culture

Cells were cultured and transfected essentially as reported previously [31]. Briefly, the immature male murine gonadotrope αT3-1 cells were cultured in minimum essential media, supplemented with 10% fetal bovine serum, 10 mM HEPES, 1 mM sodium pyruvate solution, 0.1 mM minimum essential medium non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin. The mature female murine gonadotrope LβT2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% dialyzed fetal bovine serum, 10 mM HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin (all from Biological Industries, Beit HaEmek). Primary pituitary cells were extracted from C57BL/6J mice (held at the Technion Animal Facility and handled humanely, in accordance with institutional and IACUC guidelines), and dispersed (in 0.25% collagenase, 0.25% trypsin–EDTA, 25 mM HEPES pH 7.2 in PBS: 40 min at 37 °C), before culture in 96-well plates in DMEM with addition of 10% certified fetal bovine serum, 10 mM HEPES, 100 units/ml penicillin, and 100 μg/ml streptomycin (as in [32]). The treatment was begun after 24 h. In experiments with αT3-1 cells involving GnRH treatment, they were generally serum starved for 16 h prior to addition of the GnRH (100 nM; Sigma). The MI-2 hydrochloride (Cayman Chemicals, Michigan) was dissolved in absolute ethanol and added at <0.1% final volume. Transfections were carried out at 50–60% confluency using GenePorter2 Transfection Reagent (Genelantis), according to the manufacturer's instructions and as described [6].

The HA-SF1 WT and mutant expression plasmids, as well as FLAG-SF1 and HA-Pitx1 have been reported previously [31]. The FLAG-menin plasmid was produced similarly by PCR amplification of the coding sequence, using primers shown in Table 1, and its insertion into BamHI and NotI restriction sites of the pcDNA5/FRT/TO vector (Invitrogen) together with an N-terminal FLAG tag. Stable transfections were carried out using linearized (Xmn1-digested) pSUPER-GFP/neo plasmid (Oligoengine) containing the following target sequences (menin: CTCT TCAGCTTCATCACAG and SF1: CAGTCCAGAACAACAAGCA), and positive clones selected using G418 (500 μg/ml) which was maintained during subsequent culturing.

2.2. RNA extraction, reverse transcriptase PCR and real-time PCR

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA (2 μg) was reverse transcribed using either Moloney murine leukemia virus reverse transcriptase (ImPro-II reverse transcriptase; Promega, Madison, WI) and random oligo(dT)VN primers (Sigma, Rehovot) or "High efficiency" reverse transcriptase and random hexamers (Applied Biosystems). The cDNA sample was used as the

Table 1
Primers used for PCR amplifications.

Gene target	Sequence (all 5′ to 3′)
qPCR for expression analysis	
αGsu (100 bp: +98 to +197)	ATGGATTACTACAGAAAATATGCAG and CCTGAATAATAAAGTCTCCATCAGG
Lhβ (145 bp: +176 to +327)	CTAGCATGGTCCGAGTACTG and CTGAGGGCTACAGGAAAGGA
Fshβ (159 bp: +100 to +258)	GCTGGAGAGCAATCTGCTGCCA and TATTGGGCCGAGCTGGGTCCTTA
β-actin (198 bp: +470 to +667)	GCCATGTACGTAGCCATCCA and ACGCTCGGTCAGGATCTTCA
RPLP0 (19–103 of CDS: NM_007475.5)	GCGACCTGGAAGTCCAACTA and ATCTGCTTGGAGCCCACAT
GAPDH (78 to 288 of CDS: NM_001289726.1)	ATGGTGAAGGTCGGTGTGAA and TCCTGGAAGATGGTGATGGG
qPCR for chromatin immunoprecipitation (bp from tss)	
αGsu (−269 to −61)	GCCAAATGCTCTCTCTTCATAAGC and CCAGCAGAGTAATACAAATTGC
Lhβ (−122 to −2)	GTCTGTGTCTCGCCCCCAAAGA and CTTCCCTACCTTGGGCACCTG
Fshβ (−161 to −66)	TTCTGCTCTGTGGCATTTAGA and CCAATACCAACATAAAGCCTGCTG
p27 (−622 to −503)	CTACATAGCAGAGACTTCTGGG and AGATTCAGGAAACCTTGGCTC
Atoh1 (−353 to −149)	CCCTCACTCAGGTCGCCTG and CGTGCGAGGAGCCAATCA
Gapdh (−226 to −148)	GGAAGCAGCATTCAGGTCTC CAGGATAGGACTCAGGGAATACAG
αGsu (121 bp; chr4: 34896306 + 34896426)	GATTTAGCCACAAACTCCCT and GAAACAGCAATGAAACTCACAC
Lhβ (189 bp; chr7: 45421329 + 45421517)	CCAGTCTGCATCACCTTCAC and AAGCCCTCTTCTGAGTGTCC
OR (196 bp; chr7: 45421546 + 45421741)	TGCCGGCTGCTTTGCCTCCT and CAGGCCATTGGTTGAGTCCT
Fshβ (249 bp; chr2: 107058033–107058281)	ATTTCTAGGGCTATCAGTTAGG and CTGAACCTTTGTAACATGCT
Cystallin (78 bp; chr17: 31678124–31678201)	TCCACCATCAGCCCCTACTA and GTTGCATCTTACCTCAGAGA
FLAG-menin construct	
Menin orf	GCATGGATCCATGGGGCTGAAGGCCGCC and GCATGCGGCCGCTCAGAGGCCTTTGCGCTG