Enhancing gonadotrope gene expression through regulatory lncRNAs

Tal Refael\textsuperscript{1} and Philippa Melamed\textsuperscript{1#}

\textsuperscript{1}Faculty of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel

\textsuperscript{*}Corresponding author

Disclosure statement: The authors have nothing to disclose.

**Funding:** Funding for this work was received from the Israel Science Foundation (1850/17) and Ministry of Science and Technology through a Levi Eshkol Doctoral Fellowship (TR)
Abstract

The world of long non-coding RNAs (lncRNAs) has opened up massive new prospects in understanding the regulation of gene expression. Not only are there seemingly almost infinite numbers of lncRNAs in the mammalian cell, but they have highly diverse mechanisms of action. In the nucleus, some are chromatin-associated, transcribed from transcriptional enhancers (eRNAs) and/or direct changes in the epigenetic landscape with profound effects on gene expression. The pituitary gonadotrope is responsible for activation of reproduction through production and secretion of appropriate levels of the gonadotropic hormones. As such, it exemplifies a cell whose function is defined through changes in developmental and temporal patterns of gene expression, including those that are hormonally-induced. Roles for diverse distal regulatory elements and eRNAs in gonadotrope biology have only just begun to emerge. Here, we will present an overview of the different kinds of lncRNAs that alter gene expression, and what is known about their roles in regulating some of the key gonadotrope genes. We will also review various screens that have detected differentially expressed pituitary lncRNAs associated with changes in reproductive state, and those whose expression is found to play a role in gonadotrope-derived non-functioning pituitary adenomas. We hope to shed light on this exciting new field, emphasize the open questions, and encourage research to illuminate the roles of lncRNAs in various endocrine systems.

Keywords: lncRNA; eRNA; enhancer; transcription; chromatin; epigenetic; gonadotrope; gonadotropin; LH; FSH; GnRHR; NR5A1; reproduction; NFPA; adenoma.
Introduction

Transcriptional enhancers are defined by function, as cis regulatory DNA elements which increase basal levels of transcription and can mediate cell-specific transcription. They bind regulatory proteins which, following DNA looping, are brought in close proximity with the target gene promoter, to facilitate recruitment of the general RNAPII-associated machinery, leading to increased rates of transcription\(^1\). The role and importance of enhancers have been recognized for many years, but the massive advances in genome-wide RNA sequencing in recent years have shed a new light on their actions. It is now clear that enhancers can act as transcriptional units and are transcribed to, and/or associated with long non-coding (lncRNA) and enhancer RNAs (eRNAs), which share both similar and distinct characteristics (Table 1)\(^3\)–\(^6\). This activity, together with the discovery that much of the genome is transcribed to lncRNAs, many of which affect gene expression and some directly associated with the chromatin, has opened up an entire new field in understanding gene regulation\(^7\).

Despite evidence on the common occurrence and crucial importance and of these elements, functions have yet to be assigned to most lncRNAs and their distinct roles as compared to eRNAs is not yet clear. Furthermore, it is still not known exactly how these elements are activated, although key characteristics embedded in the regulatory DNA are beginning to emerge and may hold some clues.

The endocrine system is characterized by multiple hormonal signals and feedback mechanisms that converge on individual hormone-coding genes, most of which are expressed in a cell-specific or restricted manner. The pituitary gonadotropes form the center of the endocrine axis regulating reproduction. At puberty they are stimulated by the hypothalamic gonadotropin releasing hormone (GnRH) to synthesize luteinizing hormone (LH) and follicle stimulating hormone (FSH). These two gonadotrophic hormones, comprising a common α subunit (encoded by Cga) and a hormone specific β-subunit (encoded by Lhb or Fshb), then travel through the circulation to stimulate steroidogenesis and germ cell maturation. In the female mammal, these hormones are responsible for the estrous or
menstrual cycle, involving regulation through feedback from the gonadal steroids and by activin/inhibin signaling pathways\textsuperscript{8–10}.

Given the unique function of gonadotropes: production of the gonadotropin hormones at specific times and required levels, and in response to diverse hormonal signals, multiple transcriptional enhancers and other regulatory elements would be expected to play key roles in both the cell-specific expression and hormonal responsiveness of many of these genes. In this review, after briefly introducing enhancer elements, eRNAs and other regulatory IncRNAs, we will discuss what is known about how they direct the cell-specific and/or hormone responsiveness of some of the key gonadotropic genes. We will also examine the growing number of reports of IncRNAs whose levels in the pituitary have been found associated with activity of the reproductive axis, or development of gonadotrope derived non-functioning adenomas, in which regulatory functions of certain IncRNAs also in normal gonadotrope function have been indicated.

1. Enhancers and associated long non-coding RNAs

2.1 Enhancer regulatory elements

Transcriptional enhancers comprise regulatory elements that can activate genes nearby or at long-distances, and their characteristics have been reviewed extensively (e.g.\textsuperscript{1,11–14}). DNA looping, as shaped by the boundaries of topologically associated domains (TADs: Fig 1, 2A), allows these elements (Fig 2B) to interact physically with their target gene promoters\textsuperscript{13,15}. The large number of putative enhancers that have been detected suggests that most genes can likely utilize several of these elements in distinct contexts, such as in diverse cell types and in response to different signals (Fig 1)\textsuperscript{16,17}. These elements can function additively or synergistically\textsuperscript{18}, while some are associated with corepressors and act as silencers to repress transcription of target genes\textsuperscript{19}. Enhancer-like elements are also sometimes clustered into super-enhancers (Fig 2C)\textsuperscript{20,21} spanning extensive several
kbp regions in which they bind numerous transcription factors (TFs) and work additively or co-operatively to enhance gene expression to direct cell lineages\textsuperscript{20,22}. It has further been proposed that in this context, they function by driving a liquid-phase separation due to the concentration of proteins and possibly RNAs\textsuperscript{22–25}.

Although enhancers generally function independently of their location, frequently being located far up- or down-stream from the transcriptional start site (TSS), they are often found within the first 1 kb of first introns (Fig 2D)\textsuperscript{26}. These intronic enhancers were noted to regulate commonly genes with tissue-specific functions, as compared to housekeeping genes which tend to utilize intergenic enhancers; moreover, their usage often changes during development\textsuperscript{27}. Intronic enhancer elements activate transcription strongly and are thought to affect only the gene in which they are located\textsuperscript{26}. The mechanisms through which these enhancers function are not well understood, and presumably differ from those of distal regulatory elements, especially if close to the TSS; they might include trapping and recycling of unproductive RNA polymerase II (RNAPII) to the gene promoter, as proposed for an intronic enhancer of Fgf5\textsuperscript{22}.

Beyond their typically open chromatin and association with transcription factors and cofactors, characteristics that are shared by active gene promoters, enhancers are usually marked by monomethylation of histone H3 at Lysine 4 (H3K4me1), which contrasts with its trimethylation (H3K4me3) at protein-coding gene promoters\textsuperscript{14,28}. Given that many enhancers are transcribed to eRNAs by RNAPII, it is not clear why H3K4me3 is not required, but presumably relates to distinct mechanisms of recruitment of the transcription machinery, possibly due to characteristics determined by DNA sequence\textsuperscript{17,29–31}. Notably, while protein-coding gene promoters often include dense regions of CpGs, forming CpG islands (CpGIs), enhancers rarely contain CpGs, and those that do, form a distinct class with unique features of eRNA transcription and transcription-factor binding\textsuperscript{31}. Analysis with machine learning of thousands of transcribed enhancers and promoters from different cell contexts was able to distinguish promoters from enhancers based on their
sequence, and predicted enhancer activity, while also indicating functionally relevant differences in enhancer and promoter GC content beyond the influence of CpG islands.\textsuperscript{30}

The above study by Colbran\textsuperscript{30} trained on counts of 6-bp-long sequences and focused on TF binding sites. However, some G-rich sequences form non-canonical DNA structures which are enriched at regulatory regions of the genome (Fig 2E).\textsuperscript{32,33} At transcriptional enhancers, these structures appear to direct cell specific gene-expression and differentiation\textsuperscript{33–35}. G-quadruplexes (G4s) are formed from stretches of guanines on a single DNA strand which, depending on precise sequence, fold into various parallel or anti-parallel secondary structures through Hoogsteen hydrogen base-pairing.\textsuperscript{36} On the C-rich complementary strand, an iMotif (iM) can form through stacking of intercalating hemiprotected C-neutral C base pairs, though this formation is highly sensitive to ambient pH.\textsuperscript{34} G4 and possibly iM structures appear to serve as beacons for moderating transcription: both bind diverse proteins including chromatin architectural and modifying proteins like nucleolin, high-mobility group (HMG) proteins, DNA and histone methyltransferases, as well as numerous helicases and transcription factors which mediate some of their effects.\textsuperscript{33,36,37}

2.2 The bidirectional eRNAs

Bidirectional transcription from the central non-transcribed region of active enhancers characteristically produces divergent eRNAs (Fig 2F).\textsuperscript{12,16,38–42} These eRNAs are typically relatively short (800-2000 nt), unstable, and neither spliced nor poly-adenylated. Their increased transcription following the same stimulation that up-regulates the target gene, together with effects observed on target gene expression after eRNA knockdown, can indicate a functional role for these eRNAs.

Accordingly, transcription of one of the eRNAs may be more favorable than the other for activation of the target gene.\textsuperscript{43–45} It is not yet clear how this directionality is regulated, but it was reported to be determined at the region where the RNA polymerase II complex assembles.\textsuperscript{43}
Functions for the eRNAs have been demonstrated to include inducing activating histone modifications, changes in chromatin conformation and stabilization of enhancer-promoter looping.\textsuperscript{41,44,46–48} The eRNAs have been shown to interact with various proteins (e.g. mediator,\textsuperscript{46} CBP,\textsuperscript{45,49} cohesin,\textsuperscript{41} NELF\textsuperscript{50}) which mediate some of their functions. However, the diversity of eRNA sequences and structures suggests that eRNAs have multiple different roles and in some cases, the act of transcription itself might also play a role in enhancer activity and/or chromatin organization.\textsuperscript{12,51}

2.3 Regulatory IncRNAs: distinct processing endows unique features

Asides from the bidirectional eRNAs, other IncRNAs are often found in close proximity to active enhancers and can regulate gene expression via various mechanisms (Fig 2G-J), including by mediating activity of the enhancer on its target gene.\textsuperscript{52–56} These IncRNAs are divided into various classes according to the regions of the genome from which they are transcribed and how they are processed.\textsuperscript{3,7,54,57} The promoter regions encoding these RNAs are usually marked with H3K4me3, and the IncRNAs are often both capped and polyadenylated, providing them the stability that characterizes these transcripts.\textsuperscript{53} However, distinct processing of IncRNAs can endow them with unique features and functions.\textsuperscript{5,53,58} For example, stabilization can be achieved by unusual mechanisms, such as RNase P cleavage to generate mature 3' ends that fold on themselves to form a triple-helical knot,\textsuperscript{59,60} and capping by small nucleolar RNA protein complexes at the 5'- or both ends of the RNA.\textsuperscript{61–63} Directs export of the IncRNAs into the cytosol. The processing of IncRNAs appears to be determined, at least in part, by their sequence: in IncRNAs with long introns, the processing is often weaker and the editing inefficient, so the IncRNAs remain in the nucleus where they act in a regulatory capacity.\textsuperscript{3,53} The retention of IncRNAs in the nucleus, was proposed to be due to their binding U1 small nuclear ribonucleoprotein (snRNP) which is via a specific binding motif.\textsuperscript{64,65}

In the cytosol, IncRNAs are found in various cell compartments, including mitochondria, exosomes, bound to ribosomes or to distinct RNA binding proteins (RBPs), for their various functions which
include regulating gene expression post-transcriptionally\textsuperscript{53,66–68}. Some IncRNA can function as competing endogenous RNAs (ceRNAs), by acting as sponges for miRNAs\textsuperscript{69–71}. These include pseudogenes, and also circular RNAs (circRNAs) derived from back splicing of several spliced exons, or from excised introns, termed circular intronic RNAs (ciRNAs)\textsuperscript{72–74}. CircRNAs (Fig 2J) are highly stable and have multiple functions in the cell, as well as sometimes being secreted\textsuperscript{72,75–77}. They are also found in the nucleus and have been reported to regulate RNAPII transcription and can interfere with gene splicing\textsuperscript{78–80}. The circRNAs and ciRNAs regulate expression of their parent genes while also exerting effects on other genes in \textit{trans}\textsuperscript{81}.

\textbf{2.4 Roles of chromatin-associated IncRNAs}

Nuclear IncRNAs are found both associated with the chromatin and in subnuclear compartments\textsuperscript{5,57,66,67}. They affect nuclear and chromatin organization, regulate gene expression in \textit{cis} or in \textit{trans} including through altering transcription factor activity, as well as via post-transcriptional regulation\textsuperscript{53,54,57}. At the chromatin, IncRNAs interact directly with various chromatin modifying and chromatin-associated proteins to activate or repress transcription of nearby genes, and with CTCF proteins to mediate long distance interaction\textsuperscript{54,82,83}. Many IncRNAs interact with the polycomb complex 2 (PRC2: Fig 2I), and this interaction was shown to facilitate gene expression by preventing the repressive PRC2-mediated methylation of H3K27\textsuperscript{84}. However other IncRNAs, such as \textit{XIST} and \textit{HOTAIR} repress gene expression through activating this same mechanism. The switch that determines whether PRC2 is activated or repressed by different IncRNAs is reportedly regulated by RNA-RNA bridging which alters the IncRNA structure\textsuperscript{85}. Thus the activity of PRC2, which preferentially binds to single-stranded RNA containing G-tracts and G-quadruplexes\textsuperscript{86}, is also determined by sequence through the potential for such bridging.

Some IncRNAs also interact with the DNA to form R-Loops or RNA-DNA triplexes, which recruit protein complexes to regulate expression of nearby genes (Fig 2H)\textsuperscript{87}. When R-loops form on the opposite strand from G-quadruplexes, they stabilize the structure and promote antisense
transcription, and purportedly can act as intrinsic Pol II promoters. RNA-DNA triplex formation was shown to mediate the effects of lncRNA MEG3 at distal elements on TGFβ pathway genes and was suggested to be a general characteristic of target gene recognition by chromatin-interacting lncRNAs. The bi-directional transcription at transcriptional enhancers might thus also be facilitated and directed by G4 structures and R-loops formed from chromatin-associated lncRNAs and eRNAs transcribed in these regions. The particularly high concentrations of lncRNAs and eRNAs at super-enhancers indicates that they might play additional roles, for example in the condensate formation that purportedly mediates super-enhancer activity. Thus, a positive feedback network might exist whereby the G4/iM promotes lncRNA transcription, resulting in stabilization of the G4/iM structures, while both the lncRNAs and the intrinsically-disordered regions of proteins binding these structures contribute also to condensate formation.

3. Regulation of key gonadotrope genes by enhancers, eRNAs and regulatory lncRNAs

3.1 A distal enhancer and its eRNA determine the chromatin landscape of Cga

The first distal gonadotropin gene enhancer whose function was shown to involve transcribed eRNAs was that of the chorionic gonadotropin alpha (Cga) subunit gene. Earlier studies showed that regulatory sequences positioned far upstream of the Cga gene control its cell-specific expression and were sufficient to drive Cga expression in gonadotropes. Deletion analysis in transgenic mice located a region between 4.6 and 3.7 kbp upstream of the Cga gene as an enhancer responsible for high levels of gene expression specifically in gonadotropes and thyrotropes. This study revealed that the tissue-specific activity of the enhancer requires also a proximal region near the Cga promoter, implying direct protein-protein or protein-nucleic acid interactions.

Subsequently, this upstream regulatory region was seen to be enriched with histone marks typical of an enhancer (H3K4me1 and H3K27ac), and found to drive bi-directional transcription to two
The more distal eRNA, transcribed in the reverse direction from that of Cga, is responsible for chromatin remodeling at the enhancer and proximal promoter, involving looping of the DNA (Fig 3). Knockdown of this distal eRNA led to reduction in activating H3K27 acetylation and its replacement with repressive H3K27 trimethylation at the enhancer and promoter. Promoter H3K4me3 was also reduced and the chromatin more compact following the eRNA knockdown, and the nucleosome depleted region was diminished, seemingly due to loss of the Chd1 chromatin remodeler. The eRNA knockdown also led to a major decrease in Cga expression levels which progressed over some weeks, reaching levels that were below 5% those in control cells. Thus the Cga distal enhancer, through this eRNA, plays a crucial role in determining gene expression levels by shaping the chromatin landscape. Notably, however, the gene could still be stimulated by GnRH, suggesting the presence of additional, yet to be identified enhancers that might mediate this hormonal response.

3.2 Fshb is regulated in cis by a distal enhancer and possibly in trans by miRNA-sponging lncRNAs

A novel distal enhancer 26 kbp upstream of the human Fshb gene was recently reported. This region comprises gonadotrope-specific open chromatin which was observed in a number of studies, strongly indicating its regulatory function. Moreover, genome-wide association studies (GWAS) revealed that this region contains a significant single nucleotide polymorphism (SNP) which is associated with polycystic ovary syndrome. In mouse gonadotrope cell lines and even whole pituitaries, the region was seen to be enriched with typical enhancer histone modifications. This region was also found to contain several TF binding motifs which affected its activity in reporter assays in which a ~450 bp conserved sequence was placed upstream of the FSHB promoter. A functional binding site for Nr5a1 overlapped the SNP and was seen to play a role in enhancer activity in these reporter assays, while the SNP increased Nr5a1 binding and FSHB promoter activity. Although the reporter gene assays demonstrated that this element increases FSHB-activated transcription, similar effects were seen on other gene promoters, and it was concluded that its
function is not promoter-specific, while its reversibility might be context-dependent\textsuperscript{95}. However, the study did not map the eRNAs/IncRNAs associated with the enhancer, and the DNA encoding these was presumably not included in the 450 bp sequence studied; these regions might well impart some of its target-specific function and could also explain the relevance of its orientation.

Several IncRNAs have been proposed to regulate \textit{Fshb} expression, mostly by acting as “sponges” for various miRNAs. The IncRNA-m433s1 was seen to upregulate \textit{Fshb} levels and FSH secretion in the rat pituitary by interacting with miR-433, and reducing its inhibition of \textit{Fshb} mRNA\textsuperscript{100}. Another IncRNA, this time a circular RNA, circAkap17b, was seen to function similarly, by neutralizing miR-7 which also suppresses FSH secretion\textsuperscript{101}. Knockdown of this cirRNA suppressed \textit{Fshb} mRNA and FSH secretion, while its over-expression had the opposite effect\textsuperscript{101}. However, circAkap17b levels in the anterior pituitary were shown to drop quite dramatically during the first two weeks after birth\textsuperscript{101}, such that its function in regulating FSH might be limited. Additional circRNAs were found differentially expressed in pituitaries from immature and mature rats (Section 4), and predicted to interact with various miRNAs reported to regulate \textit{Fshb}\textsuperscript{102} although their function has yet to be demonstrated.

3.3 Regulatory IncRNAs upstream of the \textit{Lhb} gene?

Gene-specific transcriptional enhancers for \textit{Lhb} have yet to be described, and the major regulatory elements required to drive \textit{Lhb} expression have long been thought to be present in the proximal ~500 bp region upstream of the TSS, which is highly conserved across species\textsuperscript{103–108}. The genomic locus of the \textit{Lhb} gene is complex in humans due to duplication events leading to multiple copies of the \textit{CGB} genes, but immediately upstream of \textit{LHB} there are several regions enriched with H3K4me1 (ENCODE data). This same region is reported in both human and mouse, to be transcribed to one or two IncRNAs, with only partial sequence similarity across species. The longer of the putative transcripts has an open reading frame (potentially encoding 310 [mouse] or 334 aa [human]), but is annotated as a spliced IncRNA which is associated with the polycomb complex\textsuperscript{109}. As noted above,
many IncRNAs interact with PRC2, but this association can lead to diverse outcomes depending on sequence and local context. Our previous studies showed that there is some H3K27me3 on the proximal Lhb promoter in αT3-1 cells\textsuperscript{110,111}, though levels are considerably lower at the region encoding the 3’ end of this IncRNA\textsuperscript{111}. Work will be required to determine whether this and other IncRNAs regulate Lhb transcription, as well as identifying distal enhancer elements and eRNAs responsible for its tissue-specific and hormonally-induced expression.

3.4 The intronic enhancers of NR5A1

The transcription factor NR5A1 (SF-1) is responsible for differentiation of the gonadotrope cell lineage, and its expression is restricted to steroidogenic tissues. In mice, Nr5a1 expression was shown to be regulated by several intronic and one upstream enhancer which work with the basal promoter to direct its cell-specific expression, and whose activities are regulated by DNA methylation\textsuperscript{112,113}. A pituitary gonadotrope-specific enhancer which binds pituitary homeobox 2 (Pitx2), is located in the sixth intron of the gene\textsuperscript{114}, and is hypomethylated in gonadotropes, but hypermethylated in VMH neurons and in the adrenal cortex\textsuperscript{113}. Interestingly however, in non-gonadotrope pituitary cells (i.e. not Gnrhr-expressing cells or their progeny\textsuperscript{115}), it was found less hypermethylated than in other tissues\textsuperscript{113}, perhaps underlying its potential expression in these cells which might lead to the transdifferentiation between distinct hormonal cell types and/or multi-hormonal pituitary cells that have been reported\textsuperscript{116–118}.

Studies in mouse gonadotrope-derived cell lines thought to represent gonadotropes at various stages of differentiation, found that methylation of this gonadotrope-specific intronic enhancer correlates with levels of gene expression, being unmethylated in LβT2 cells and heavily methylated in αT1-1 cells, while partially methylated in the αT3-1 gonadotrope precursor-derived cell line and the AtT20 corticotrope cell line\textsuperscript{119}. In these cells, an additional putative Nr5a1 enhancer was also identified in the fourth intron, which was suggested to play a transient role in early gonadotrope differentiation, and its temporary chromatin accessibility was evident in 12.5-14.5 d mouse
embryonic pituitary cells\textsuperscript{120}. Activity of this enhancer in the gonadotrope cell lines was seen to be directed by estrogen receptor α (ERα)-mediated chromatin remodeling, and ERα protected the enhancer from repressive DNA methylation\textsuperscript{120}. Given that chromatin compaction, histone modifications and DNA methylation/hydroxymethylation can vary between model cell lines and primary cells\textsuperscript{121,122}, genome editing approaches will be useful to confirm the functions of these elements \textit{in vivo}.

3.5 Distal regulation of the Gnrhr gene is not conserved

Several distal regulatory elements have been reported to drive expression the Gnrhr gene in the gonadotropes where it regulates all three gonadotropin genes, although some of these are highly species specific\textsuperscript{123}. The rat Gnrhr gene is activated by Lhx2, Isl1 and Gata2 at a distal enhancer \textasciitilde1 kbp upstream of the TSS that interacts with the Nr5a1-bound proximal promoter\textsuperscript{124}. However, this element is not conserved in other species. The human GNRHR contains several silencer elements around \textasciitilde1-1.7 kbp upstream of the TSS which appear to function in a cell specific manner, as well as more distal activating elements that are utilized differently in gonadotrope and placental cell lines\textsuperscript{123,125}. In ovarian cancer cell lines, an upstream alternative promoter was also reported\textsuperscript{126}. The ability to utilize these diverse sites to enhance GNRHR transcription presumably underlies its aberrant expression in many cancers\textsuperscript{127,128} where mis-regulated open chromatin permits access to these potential regulatory elements.

Although its direct regulation by IncRNAs has yet to be elucidated, the GNRHR gene is regulated post-transcriptionally by miRNAs which reportedly mediate up-regulation of GnRH protein by Leptin\textsuperscript{129}. Thus “sponging” IncRNAs, including possibly cirRNAs, might well play an indirect role to stimulate GnRHR expression by reducing effects of these repressive miRNAs. Elucidation of how GnRHR expression is controlled by these various, as yet elusive, IncRNAs is important for understanding its regulation in the pituitary gonadotrope and its aberrant expression in tumor cells. There is clearly still much to explore in how diverse classes of IncRNAs regulate this gene.
4. Screening for functional pituitary lncRNAs associated with sexual maturation and reproductive function

The identification of lncRNAs that are specific to gonadotrope function is hampered by the small numbers of these cells in the pituitary. One approach to identify them and determine their possible roles has been to examine lncRNA levels in whole pituitaries at various stages of sexual maturation and the estrous cycle. In one such study, RNA sequencing of the anterior pituitary of immature and mature rats, led to the identification of 7039 lncRNAs, including lincRNAs (58.9%), antisense lncRNAs (12.7%), intronic lncRNAs (22.5%) and sense lncRNAs (5%), which together corresponded to 4442 lncRNA genes. Of these, 1181 transcripts were differentially expressed (DE), a similar number being up- and down-regulated. Possible functions were inferred by searching for protein-coding genes within 100 kb of the lncRNAs, and three lncRNAs (MSTRG.80236.1, MSTRG.80236.2 and MSTRG.80236.3) that were predicted to interact with Fshb, showed similarly increased expression.

This group also screened for functional circRNAs using a similar approach, and detected 32 DE circRNAs in the sexually immature and mature rat anterior pituitaries. They predicted interactions of some of the circRNAs and miRNAs, which lead to the discovery of several circRNAs with potential to regulate Fshb by acting as miRNA sponges as detailed above (Section 3.2.

Several comparable studies in sheep pituitaries were performed to explore the functions of lncRNAs in sexual maturation and reproductive function. Comparison of the lncRNAs in pituitaries of immature and mature rams revealed 2417 known lncRNAs and 1256 new lncRNAs, including 193 that were DE, while 1407 DE mRNAs were identified. For the DE protein-coding genes (DEGs) related to growth, reproduction or steroid synthesis, interactions with the lncRNAs were predicted and networks constructed. Short-interfering RNA (siRNA) knockdown of one of these lncRNAs (TCONS_00066406) in sheep pituitary cells led to a decrease in expression of its predicted target Hsd17b12, as well as in Lhb and Fshb mRNA levels, although the specificity of this effect was not reported. The same group used strand-specific RNA-seq to profile lncRNAs and mRNAs in highly and poorly prolific sheep, which revealed 57 DE lncRNAs and 298 DE mRNAs. Co-expression
networks of these IncRNAs and their putative target genes highlighted Smad2, and the inhibitory effect of one of the IncRNAs (MSTRG.259847.2) on Smad2 as well as Lhb, though not Fshb, mRNA levels was shown in cultured sheep pituitary cells.\(^\text{132}\)

The involvement of IncRNAs in seasonal estrus was also examined in sheep pituitaries and 995 IncRNAs were identified, of which 335 were DE during estrus and anestrus\(^\text{133}\). Prediction of the target genes of these IncRNAs, together with the function of the encoded proteins in hormone synthesis and metabolism, led the authors to suggest possible functions for some of these IncRNAs in estrous.\(^\text{132}\) The same group profiled also the circRNAs expressed in estrus and anestrus sheep pituitary\(^\text{134}\) and those expressed in prenatal and postnatal sheep pituitaries\(^\text{135}\). Both studies revealed a large number of DE circRNAs, and the latter study predicted that some of these interact with pituitary-specific miRNAs\(^\text{135}\).

These screens in the model animal pituitaries have produced large datasets of IncRNA and circRNAs that might play a role in pituitary and possibly gonadotrope function. However, this data needs to be integrated and functions analyzed, and it is not yet clear how many of these RNAs are conserved across species. Accessing human pituitary and gonadotropes specific IncRNAs is obviously more challenging, though a useful resource and online tool is available in the Long non-coding RNA Knowledgebase (IncRNAKB; http://www.lncrnakb.org/), which comprises a catalog of IncRNAs expressed in human pituitary as well as 30 other normal human tissues\(^\text{136}\). The database contains co-expression modules which point to possible IncRNA functions, and expression quantitative trait loci with tissue-specific IncRNA-trait associations from 323 GWAS studies, and thus should comprise a highly useful resource for study of IncRNAs particularly in the context of human disease.
5. Regulatory IncRNAs in non-functioning pituitary and gonadotrope adenomas

Altered expression of IncRNAs is likely to underlie diverse cell phenotypes and behavior including oncogenesis, and several studies have examined their differential expression and possible functions in gonadotrope or non-functioning adenomas (NFPAs) which are primarily of gonadotrope origin\textsuperscript{137,138}. Analysis of the co-expression networks for IncRNAs and mRNAs in these tissues, compared with normal pituitary tissue, suggested that each IncRNA might have a large number of mRNA targets\textsuperscript{139,140} and exposed IncRNAs that appear to play a role in tumor formation or invasiveness, while some of their targets are known to play roles in normal gonadotrope function.

The maternally expressed gene 3 (\textit{MEG3}) locus is characteristically strongly repressed in NFPAs. This region encodes multiple imprinted genes, which are expressed from only one of the alleles, depending on its parent of origin, the second allele being repressed by DNA methylation\textsuperscript{141}. \textit{MEG3} encodes a noncoding RNA expressed only from the maternal-allele, and this \textit{locus} also encodes three genes expressed only from the paternal allele, which include Protein delta homolog 1 (\textit{DLK1}). Both \textit{MEG3} and \textit{DLK1} are virtually silenced in NFPAs, though not in functioning adenomas\textsuperscript{139,140,142,143}; this is associated with increased DNA methylation at an intergenic control region upstream of \textit{MEG3} and at the \textit{MEG3} promoter\textsuperscript{143–145}. \textit{DLK1} is expressed highly in the pituitary, and mutations on the paternal allele are associated with central precocious puberty\textsuperscript{146}, while \textit{Dlk1} knockout mice have reduced FSH and LH levels\textsuperscript{147,148}. Although known to encode a receptor for Notch signaling, its precise function in the gonadotropes and a role in suppressing tumor formation or invasiveness have yet to be determined.

\textit{MEG3} is a particularly large IncRNA found as multiple isoforms, some of which induce cell cycle arrest and apoptosis via interactions with p53 protein\textsuperscript{149,150}, but \textit{MEG3} can also target the chromatin directly. It was found in breast cancer cells to bind, together with PRC2, to distal GA-rich regulatory elements involving RNA-DNA triplex formation. In this way it represses expression of various genes in the \textit{TGFβ/activin} pathway, including \textit{SMAD2} in an apparent feedback loop\textsuperscript{91}. Thus its silencing in
NFPAs might activate these pathways. However, other studies found reduced SMAD3 expression in NFPAs compared to normal pituitaries\textsuperscript{151–153}, and one of these projected that this is due to a subset of miRNAs targeting the TGFβ signaling pathway\textsuperscript{153}. These findings point to diverse mechanisms through which MEG3 might affect TGFβ and activin signaling. Elucidation of its role in modulating these signaling pathways, which regulate gonadotropin gene expression and normal gonadotrope function\textsuperscript{154–156}, awaits further study.

Expression of another lncRNA, \textit{C5orf66-AS1}, was also found reduced in NFPAs as compared to normal pituitary tissue, was lower in invasive adenomas compared to those that were non-invasive, and its expression levels negatively correlated with maximum tumor diameter\textsuperscript{157}. This spliced lncRNA is transcribed from a region immediately upstream of \textit{Pitx1} whose role in pituitary development and gene expression, including of gonadotropes, is well recognized\textsuperscript{158–161}. \textit{C5orf66-AS1} is predicted to interact with the \textit{Pitx1} proximal promoter and first intron, and these RNAs show similar patterns of expression which is in a limited number of tissues (Fig 4). Moreover, they were differentially expressed in an additional cohort of NFPAs\textsuperscript{157}. Supporting a possible regulatory role, a correlation was also seen in head and neck carcinoma between increased methylation at this locus and lower expression of the lncRNA, with reduced \textit{Pitx1} expression in tumor tissue compared to surrounding normal tissue\textsuperscript{162}. The first exon of \textit{C5orf66-AS1} and the first intron of \textit{Pitx1} contain G-quadruplex forming sequences (predicted by pqsfinder), and the genomic region is enriched for PRC2 (ENCODE; Fig 4). Together these findings point to this lncRNA as a putative regulator of \textit{Pitx1}, possibly via its interactions with the PRC2 complex, and whose activity might be controlled by DNA methylation in normal tissues as well as tumors.

Additional screens of lncRNAs differentially-expressed in gonadotrope adenomas compared to normal pituitaries revealed high levels of several lncRNAs that regulate expression of \textit{HMGA1} and
HMGA2 whose roles in pituitary development are established\textsuperscript{163,164}. The lncRNAs (ceRNAs) block the inhibitory effects of miRNAs that target the \textit{HMGA} genes. \textit{RPSAP52}, which is antisense to \textit{HMGA2}, was found highly up-regulated in gonadotrope and prolactin-secreting pituitary adenomas and its expression was correlated with that of HMGA2 in the tumors\textsuperscript{165}. This lncRNA was seen to regulate also other HMGA family members, and possibly plays a role in the cell cycle\textsuperscript{166}. Another study found that two pseudogenes \textit{HMGA1P6} and \textit{HMGA1P7} also act as ceRNAs, protecting HMGA mRNAs from miRNAs, and their expression also correlated with HMGA1 levels in tumors\textsuperscript{166}. Moreover, over-expression of these pseudogenes caused enhanced proliferation and migration of a tumor cell line, suggesting that they might contribute to the tumor behavior\textsuperscript{166}.

Several studies have looked at circRNAs in NFPAs, making comparisons between invasive vs. non-invasive tumors, and in tumors that are recurrent vs. at first surgery. Putative circRNA-miRNA networks were then produced to predict function\textsuperscript{167–170}. These large datasets on the differential expression of lncRNAs are not easy to interpret, both because lncRNAs have multiple targets mediated via numerous mechanisms, and because of the heterogeneity of NFPAs tissues. Single cell-RNA-seq should help decipher which lncRNAs are associated with the individual cell phenotype and behavior and lead to a better understanding of their function. More extensive work will then be required to understand their roles in regulating gene expression and tumorigenesis.

6. Outlook and Concluding Comments

The regulation of gonadotropin gene transcription has been studied for many years, promoter elements defined, and more recently the role of the chromatin addressed. Now, however, the field of regulatory biology has undergone a paradigm shift. RNAs are transcribed not only from classical enhancer-elements, but potential regulatory regions have expanded almost infinitely to include massive inter- and intra-genic regions of the genome that can function to regulate transcription.
While the magnitude of this is clear, we have barely begun to understand the intricacies of these mechanisms and how they are implemented. Considerable work lies ahead to understand the mechanisms through which these diverse classes of enhancing and repressing lncRNAs are controlled and, in turn, how they regulate their target genes. This will lead to an understanding of hitherto unexplained aspects of gene expression, and could well also provide novel targets for manipulating gene expression in health and disease.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.
References

1. Long HK, Prescott SL, Wysocka J. Ever-Changing Landscapes: Transcriptional Enhancers in Development and Evolution. Cell. 2016;167(5):1170-1187. doi:10.1016/j.cell.2016.09.018

2. Shlyueva D, Stampfel G, Stark A. Transcriptional enhancers: From properties to genome-wide predictions. Nat Rev Genet. 2014;15(4):272-286. doi:10.1038/nrg3682

3. Wu H, Yang L, Chen L-LL. The Diversity of Long Noncoding RNAs and Their Generation. Trends Genet. 2017;33(8):540-552. doi:10.1016/j.tig.2017.05.004

4. Andersson R, Sandelin A, Danko CG. A unified architecture of transcriptional regulatory elements. Trends Genet. 2015;31(8):426-433. doi:10.1016/j.tig.2015.05.007

5. Gil N, Ulitsky I. Regulation of gene expression by cis-acting long non-coding RNAs. Nat Rev Genet. 2020;21(2):102-117. doi:10.1038/s41576-019-0184-5

6. Guttman M, Amit I, Garber M, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009;458(7235):223-227. doi:10.1038/nature07672

7. Mattick JS, Rinn JL. Discovery and annotation of long noncoding RNAs. Nat Struct Mol Biol. 2015;22(1):5-7. doi:10.1038/nsmb.2942

8. Styne DM, Grumbach MM. Chapter 25 – Physiology and Disorders of Puberty. Thirteenth. Elsevier Inc.; 2016. doi:10.1016/B978-0-323-29738-7.00025-3

9. Plant TM. Neuroendocrine control of the onset of puberty. Front Neuroendocrinol. 2015;38:73-88. doi:10.1016/j.yfrne.2015.04.002

10. Shalev D, Melamed P. The role of the hypothalamus and pituitary epigenomes in central activation of the reproductive axis at puberty. Mol Cell Endocrinol. 2020;518:111031.
11. Buffry AD, Mendes CC, McGregor AP. The Functionality and Evolution of Eukaryotic Transcriptional Enhancers. *Adv Genet*. 2016;96:143-206. doi:10.1016/bs.adgen.2016.08.004

12. Hou TY, Kraus WL. Spirits in the Material World: Enhancer RNAs in Transcriptional Regulation. *Trends Biochem Sci*. 2020. doi:10.1016/j.tibs.2020.08.007

13. Schoenfelder S, Fraser P. Long-range enhancer–promoter contacts in gene expression control. *Nat Rev Genet*. 2019;20(8):437-455. doi:10.1038/s41576-019-0128-0

14. Andersson R, Sandelin A. Determinants of enhancer and promoter activities of regulatory elements. *Nat Rev Genet*. 2020;21(2):71-87. doi:10.1038/s41576-019-0173-8

15. Dixon JR, Selvaraj S, Yue F, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*. 2012;485(7398):376-380. doi:10.1038/nature11082

16. Natoli G, Andrau JC. Noncoding transcription at enhancers: General principles and functional models. *Annu Rev Genet*. 2012;46:1-19. doi:10.1146/annurev-genet-110711-155459

17. Levine M, Cattoglio C, Tjian R. Looping back to leap forward: Transcription enters a new era. *Cell*. 2014;157(1):13-25. doi:10.1016/j.cell.2014.02.009

18. Choi J, Lysakovskai K, Stik G, et al. Evidence for additive and synergistic action of mammalian enhancers during cell fate determination. *Elife*. 2021;10. doi:10.7554/eLife.65381

19. Huang Z, Liang N, Goñi S, et al. The corepressors GPS2 and SMRT control enhancer and silencer remodeling via eRNA transcription during inflammatory activation of macrophages. *Mol Cell*. 2021;81(5). doi:10.1016/j.molcel.2020.12.040

20. Whyte WA, Orlando DA, Hnisz D, et al. Master transcription factors and mediator establish
super-enhancers at key cell identity genes. *Cell*. 2013;153(2):307-319.
doi:10.1016/j.cell.2013.03.035

21. Hnisz D, Abraham BJ, Lee TI, et al. XSuper-enhancers in the control of cell identity and
disease. *Cell*. 2013;155(4):934. doi:10.1016/j.cell.2013.09.053

22. Thomas HF, Kotova E, Jayaram S, et al. Temporal dissection of an enhancer cluster reveals
distinct temporal and functional contributions of individual elements. *Mol Cell*. 2021;81(5).
doi:10.1016/j.molcel.2020.12.047

23. Sabari BR, Dall’Agnese A, Boija A, et al. Coactivator condensation at super-enhancers links
phase separation and gene control. *Science (80-)*. 2018;361(6400):eaar3958.
doi:10.1126/science.aar3958

24. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA. A Phase Separation Model for
Transcriptional Control. *Cell*. 2017;169(1):13-23. doi:10.1016/j.cell.2017.02.007

25. Cho WK, Spille JH, Hecht M, et al. Mediator and RNA polymerase II clusters associate in
transcription-dependent condensates. *Science (80-)*. 2018;361(6400):412-415.
doi:10.1126/science.aar4199

26. Rose AB. Introns as Gene Regulators: A Brick on the Accelerator. *Front Genet.*
2019;9(FEB):672. doi:10.3389/fgene.2018.00672

27. Borsari B, Villegas-Mirón P, Laayouni H, et al. Intronic enhancers regulate the expression of
genes involved in tissue-specific functions and homeostasis. *bioRxiv*. August
2020:2020.08.21.260836. doi:10.1101/2020.08.21.260836

28. Gasperini M, Tome JM, Shendure J. Towards a comprehensive catalogue of validated and
target-linked human enhancers. *Nat Rev Genet*. 2020;21(5):292-310. doi:10.1038/s41576-
019-0209-0
29. Koch F, Fenouil R, Gut M, et al. Transcription initiation platforms and GTF recruitment at tissue-specific enhancers and promoters. *Nat Struct Mol Biol*. 2011;18(8):956-963. doi:10.1038/nsmb.2085

30. Colbran LL, Chen L, Capra JA. Sequence characteristics distinguish transcribed enhancers from promoters and predict their breadth of activity. *Genetics*. 2019;211(4):1205-1217. doi:10.1534/genetics.118.301895

31. Steinhaus R, Gonzalez T, Seelow D, Robinson PN. Pervasive and CpG-dependent promoter-like characteristics of transcribed enhancers. *Nucleic Acids Res*. 2020;48(10):5306-5317. doi:10.1093/nar/gkaa223

32. Hänsel-Hertsch R, Beraldi D, Lensing S V., et al. G-quadruplex structures mark human regulatory chromatin. *Nat Genet*. 2016;48(10):1267-1272. doi:10.1038/ng.3662

33. Spiegel J, Adhikari S, Balasubramanian S. The Structure and Function of DNA G-Quadruplexes. *Trends Chem*. 2020;2(2):123-136. doi:10.1016/j.trechm.2019.07.002

34. Zeraati M, Langley DB, Schofield P, et al. I-motif DNA structures are formed in the nuclei of human cells. *Nat Chem*. 2018;10(6):631-637. doi:10.1038/s41557-018-0046-3

35. Varshney D, Spiegel J, Zyner K, Tannahill D, Balasubramanian S. The regulation and functions of DNA and RNA G-quadruplexes. *Nat Rev Mol Cell Biol*. 2020;21(8):459-474. doi:10.1038/s41580-020-0236-x

36. Mao SQ, Ghanbarian AT, Spiegel J, et al. DNA G-quadruplex structures mold the DNA methylome. *Nat Struct Mol Biol*. 2018;25(10):951-957. doi:10.1038/s41594-018-0131-8

37. Guilbaud G, Murat P, Recolin B, et al. Local epigenetic reprogramming induced by G-quadruplex ligands. *Nat Chem*. 2017;9(11):1110-1117. doi:10.1038/NCHEM.2828

38. Kim TK, Hemberg M, Gray JM, et al. Widespread transcription at neuronal activity-regulated
39. de Santa F, Barozzi I, Mietton F, et al. A large fraction of extragenic RNA Pol II transcription sites overlap enhancers. *PloS Biol.* 2010;8(5). doi:10.1371/journal.pbio.1000384

40. Sartorelli V, Lauberth SM. Enhancer RNAs are an important regulatory layer of the epigenome. *Nat Struct Mol Biol.* 2020;27(6):521-528. doi:10.1038/s41594-020-0446-0

41. Li W, Notani D, Ma Q, et al. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature.* 2013;498(7455):516-520. doi:10.1038/nature12210

42. Melamed P, Yosefzon Y, Rudnizky S, Pnueli L. Transcriptional enhancers: Transcription, function and flexibility. *Transcription.* 2016;7(1):26-31. doi:10.1080/21541264.2015.1128517

43. Kristjánsdóttir K, Dziubek A, Kang HM, Kwak H. Population-scale study of eRNA transcription reveals bipartite functional enhancer architecture. *Nat Commun.* 2020;11(1). doi:10.1038/s41467-020-19829-z

44. Pnueli L, Rudnizky S, Yosefzon Y, Melamed P. RNA transcribed from a distal enhancer is required for activating the chromatin at the promoter of the gonadotropin α-subunit gene. *Proc Natl Acad Sci U S A.* 2015;112(14):4369-4374. doi:10.1073/pnas.1414841112

45. Carullo NVN, Phillips RA, Simon RC, et al. Enhancer RNAs predict enhancer–gene regulatory links and are critical for enhancer function in neuronal systems. *Nucleic Acids Res.* 2020;48(17):9550-9570. doi:10.1093/nar/gkaa671

46. Lai F, Orom UA, Cesaroni M, et al. Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. *Nature.* 2013;494(7438):497-501. doi:10.1038/nature11884

47. Melo CA, Drost J, Wijchers PJ, et al. ERNAs Are Required for p53-Dependent Enhancer Activity and Gene Transcription. *Mol Cell.* 2013;49(3):524-535. doi:10.1016/j.molcel.2012.11.021
48. Hsieh CL, Fei T, Chen Y, et al. Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proc Natl Acad Sci U S A*. 2014;111(20):7319-7324. doi:10.1073/pnas.1324151111

49. Bose DA, Donahue G, Reinberg D, Shiekhattar R, Bonasio R, Berger SL. RNA Binding to CBP Stimulates Histone Acetylation and Transcription. *Cell*. 2017;168(1-2):135-149.e22. doi:10.1016/j.cell.2016.12.020

50. Schaukowitch K, Joo JY, Liu X, Watts JK, Martinez C, Kim TK. Enhancer RNA facilitates NELF release from immediate early genes. *Mol Cell*. 2014;56(1):29-42. doi:10.1016/j.molcel.2014.08.023

51. van Steensel B, Furlong EEM. The role of transcription in shaping the spatial organization of the genome. *Nat Rev Mol Cell Biol*. 2019;20(6):327-337. doi:10.1038/s41580-019-0114-6

52. Gil N, Ulitsky I. Production of Spliced Long Noncoding RNAs Specifies Regions with Increased Enhancer Activity. *Cell Syst*. 2018;7(5):537-547.e3. doi:10.1016/j.cels.2018.10.009

53. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol*. 2021;22(2):96-118. doi:10.1038/s41580-020-00315-9

54. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. *Nat Cell Biol*. 2019;21(5):542-551. doi:10.1038/s41556-019-0311-8

55. Luo S, Lu JY, Liu L, et al. Divergent IncRNAs regulate gene expression and lineage differentiation in pluripotent cells. *Cell Stem Cell*. 2016;18(5):637-652. doi:10.1016/j.stem.2016.01.024

56. Kim TK, Shiekhattar R. Diverse regulatory interactions of long noncoding RNAs. *Curr Opin Genet Dev*. 2016;36:73-82. doi:10.1016/j.gde.2016.03.014
57. Sun Q, Hao Q, Prasanth K V. Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression. *Trends Genet.* 2018;34(2):142-157. doi:10.1016/j.tig.2017.11.005

58. Engreitz JM, Haines JE, Perez EM, et al. Local regulation of gene expression by IncRNA promoters, transcription and splicing. *Nature.* 2016;539(7629):452-455. doi:10.1038/nature20149

59. Wilusz JE, JnBaptiste CK, Lu LY, Kuhn CD, Joshua-Tor L, Sharp PA. A triple helix stabilizes the 3’ ends of long noncoding RNAs that lack poly(A) tails. *Genes Dev.* 2012;26(21):2392-2407. doi:10.1101/gad.204438.112

60. Brown JA, Bulkley D, Wang J, et al. Structural insights into the stabilization of MALAT1 noncoding RNA by a bipartite triple helix. *Nat Struct Mol Biol.* 2014;21(7):633-640. doi:10.1038/nsmb.2844

61. Yin QF, Yang L, Zhang Y, et al. Long Noncoding RNAs with snoRNA Ends. *Mol Cell.* 2012;48(2):219-230. doi:10.1016/j.molcel.2012.07.033

62. Wu H, Yin QF, Luo Z, et al. Unusual Processing Generates SPA LncRNAs that Sequester Multiple RNA Binding Proteins. *Mol Cell.* 2016;64(3):534-548. doi:10.1016/j.molcel.2016.10.007

63. Zhang XO, Yin QF, Wang H Bin, et al. Species-specific alternative splicing leads to unique expression of sno-IncRNAs. *BMC Genomics.* 2014;15(1). doi:10.1186/1471-2164-15-287

64. Yin Y, Lu JY, Zhang X, et al. U1 snRNP regulates chromatin retention of noncoding RNAs. *Nature.* 2020;580(7801):147-150. doi:10.1038/s41586-020-2105-3

65. Azam S, Hou S, Zhu B, et al. Nuclear retention element recruits U1 snRNP components to restrain spliced IncRNAs in the nucleus. *RNA Biol.* 2019;16(8):1001-1009. doi:10.1080/15476286.2019.1620061
66. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet*. 2016;17(1):47-62. doi:10.1038/nrg.2015.10

67. Chen LL. Linking Long Noncoding RNA Localization and Function. *Trends Biochem Sci*. 2016;41(9):761-772. doi:10.1016/j.tibs.2016.07.003

68. Studniarek C, Egloff S, Murphy S. Noncoding RNAs Set the Stage for RNA Polymerase II Transcription. *Trends Genet*. 2021;37(3):279-291. doi:10.1016/j.tig.2020.09.013

69. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: The rosetta stone of a hidden RNA language? *Cell*. 2011;146(3):353-358. doi:10.1016/j.cell.2011.07.014

70. Tay Y, Kats L, Salmena L, et al. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. *Cell*. 2011;147(2):344-357. doi:10.1016/j.cell.2011.09.029

71. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature*. 2014;505(7483):344-352. doi:10.1038/nature12986

72. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495(7441):384-388. doi:10.1038/nature11993

73. Kalyana-Sundaram S, Kumar-Sinha C, Shankar S, et al. Expressed pseudogenes in the transcriptional landscape of human cancers. *Cell*. 2012;149(7):1622-1634. doi:10.1016/j.cell.2012.04.041

74. Taulli R, Loretelli C, Pandolfi PP. From pseudo-ceRNAs to circ-ceRNAs: A tale of cross-talk and competition. *Nat Struct Mol Biol*. 2013;20(5):541-543. doi:10.1038/nsmb.2580

75. Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013;495(7441):333-338. doi:10.1038/nature11928

76. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-Type Specific Features of Circular...
RNA Expression. *PLoS Genet*. 2013;9(9). doi:10.1371/journal.pgen.1003777

77. Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood. *PLoS One*. 2015;10(10). doi:10.1371/journal.pone.0141214

78. Li Z, Huang C, Bao C, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol*. 2015;22(3):256-264. doi:10.1038/nsmb.2959

79. Zhang XO, Wang H Bin, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. *Cell*. 2014;159(1):134-147. doi:10.1016/j.cell.2014.09.001

80. Ashwal-Fluss R, Meyer M, Pamudurti NR, et al. CircRNA Biogenesis competes with Pre-mRNA splicing. *Mol Cell*. 2014;56(1):55-66. doi:10.1016/j.molcel.2014.08.019

81. Zhang Y, Zhang XO, Chen T, et al. Circular Intronic Long Noncoding RNAs. *Mol Cell*. 2013;51(6):792-806. doi:10.1016/j.molcel.2013.08.017

82. Zhao J, Ohsumi TK, Kung JT, et al. Genome-wide Identification of Polycomb-Associated RNAs by RIP-seq. *Mol Cell*. 2010;40(6):939-953. doi:10.1016/j.molcel.2010.12.011

83. Khalil AM, Guttman M, Huarte M, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A*. 2009;106(28):11667-11672. doi:10.1073/pnas.0904715106

84. Zhang Q, McKenzie NJ, Warneford-Thomson R, et al. RNA exploits an exposed regulatory site to inhibit the enzymatic activity of PRC2. *Nat Struct Mol Biol*. 2019;26(3):237-247. doi:10.1038/s41594-019-0197-y

85. Balas MM, Hartwick EW, Barrington C, et al. Establishing RNA-RNA interactions remolds IncRNA structure and promotes PRC2 activity. *Sci Adv*. 2021;7(16):eabc9191. doi:10.1126/sciadv.abc9191
86. Wang X, Goodrich KJ, Gooding AR, et al. Targeting of Polycomb Repressive Complex 2 to RNA by Short Repeats of Consecutive Guanines. *Mol Cell.* 2017;65(6):1056-1067.e5. doi:10.1016/j.molcel.2017.02.003

87. Niehrs C, Luke B. Regulatory R-loops as facilitators of gene expression and genome stability. *Nat Rev Mol Cell Biol.* 2020;21(3):167-178. doi:10.1038/s41580-019-0206-3

88. Pefanis E, Wang J, Rothschild G, et al. RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity. *Cell.* 2015;161(4):774-789. doi:10.1016/j.cell.2015.04.034

89. Crossley MP, Bocek M, Cimprich KA. R-Loops as Cellular Regulators and Genomic Threats. *Mol Cell.* 2019;73(3):398-411. doi:10.1016/j.molcel.2019.01.024

90. Tan-Wong SM, Dhir S, Proudfoot NJ. R-Loops Promote Antisense Transcription across the Mammalian Genome. *Mol Cell.* 2019;76(4):600-616.e6. doi:10.1016/j.molcel.2019.10.002

91. Mondal T, Subhash S, Vaid R, et al. MEG3 long noncoding RNA regulates the TGF-β pathway genes through formation of RNA-DNA triplex structures. *Nat Commun.* 2015;6. doi:10.1038/ncomms8743

92. Kendall SK, Gordon DF, Birkmeier TS, et al. Enhancer-mediated high level expression of mouse pituitary glycoprotein hormone alpha-subunit transgene in thyrotropes, gonadotropes, and developing pituitary gland. *Mol Endocrinol.* 1994;8(10):1420-1433. doi:10.1210/mend.8.10.7531821

93. Brinkmeier ML, Gordon DF, Dowding JM, et al. Cell-specific expression of the mouse glycoprotein hormone α-subunit gene requires multiple interacting DNA elements in transgenic mice and cultured cells. *Mol Endocrinol.* 1998;12(5):622-633. doi:10.1210/mend.12.5.0103
94. Rudnizky S, Bavly A, Malik O, Pnueli L, Melamed P, Kaplan A. H2A.Z controls the stability and mobility of nucleosomes to regulate expression of the LH genes. *Nat Commun*. 2016;7(1):12958. doi:10.1038/ncomms12958

95. Bohaczuk SC, Thackray VG, Shen J, Skowronski-Krawczyk D, Mellon PL. FSHB Transcription is Regulated by a Novel 5’ Distal Enhancer with a Fertility-Associated Single Nucleotide Polymorphism. *Endocrinol (United States)*. 2021;162(1). doi:10.1210/endcr/bqaa181

96. Ruf-Zamojski F, Fribourg M, Ge Y, et al. Regulatory architecture of the LβT2 gonadotrope cell underlying the response to gonadotropin-releasing hormone. *Front Endocrinol (Lausanne)*. 2018;9(FEB). doi:10.3389/fendo.2018.00034

97. Mayran A, Sochdolsky K, Khetchoumian K, et al. Pioneer and nonpioneer factor cooperation drives lineage specific chromatin opening. *Nat Commun*. 2019;10(1):3807. doi:10.1038/s41467-019-11791-9

98. Perry JRB, Murray A, Day FR, Ong KK. Molecular insights into the aetiology of female reproductive ageing. *Nat Rev Endocrinol*. 2015;11(12):725-734. doi:10.1038/nrendo.2015.167

99. Hayes MG, Urbanek M, Ehrmann DA, et al. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun*. 2015;6. doi:10.1038/ncomms8502

100. Han DX, Sun XL, Wang CJ, et al. Differentially expressed lncRNA-m433s1 regulates FSH secretion by functioning as a miRNA sponge in male rat anterior pituitary cells. *Biol Reprod*. 2019;101(2):416-425. doi:10.1093/biolre/ioz100

101. Wang CJ, Gao F, Huang YJ, et al. Circakap17b acts as a mir-7 family molecular sponge to regulate fsh secretion in rat pituitary cells. *J Mol Endocrinol*. 2020;65(4):135-148. doi:10.1530/JME-20-0036
102. Han DX, Wang CJ, Sun XL, et al. Identification of circular RNAs in the immature and mature rat anterior pituitary. *J Endocrinol*. 2019;240(3):393-402. doi:10.1530/JOE-18-0540

103. Tremblay JJ, Drouin J. Egr-1 Is a Downstream Effector of GnRH and Synergizes by Direct Interaction with Ptx1 and SF-1 To Enhance Luteinizing Hormone β Gene Transcription. *Mol Cell Biol*. 1999;19(4):2567-2576. doi:10.1128/mcb.19.4.2567

104. Halvorson LM, Kaiser UB, Chin WW. The Protein Kinase C System Acts through the Early Growth Response Protein 1 to Increase LHβ Gene Expression in Synergy with Steroidogenic Factor-1. *Mol Endocrinol*. 1999;13(1):106-116. doi:10.1210/mend.13.1.0216

105. Kaiser UB, Halvorson LM, Chen MT. Sp1, Steroidogenic Factor 1 (SF-1), and Early Growth Response Protein 1 (Egr-1) Binding Sites Form a Tripartite Gonadotropin-Releasing Hormone Response Element in the Rat Luteinizing Hormone-β Gene Promoter: an Integral Role for SF-1. *Mol Endocrinol*. 2000;14(8):1235-1245. doi:10.1210/mend.14.8.0507

106. Wolfe MW, Call GB. Early growth response protein 1 binds to the luteinizing hormone-β promoter and mediates gonadotropin-releasing hormone-stimulated gene expression. *Mol Endocrinol*. 1999;13(5):752-763. doi:10.1210/mend.13.5.0276

107. Melamed P, Zhu Y, Siew HTHT, Xie M, Koh M. Gonadotropin-releasing hormone activation of C-jun, but not early growth response factor-1, stimulates transcription of a luteinizing hormone β-subunit gene. *Endocrinology*. 2006;147(7):3598-3605. doi:10.1210/en.2006-0022

108. Melamed P, Kadir MNANA, Wijeweera A, Seah S. Transcription of gonadotropin β subunit genes involves cross-talk between the transcription factors and co-regulators that mediate actions of the regulatory hormones. *Mol Cell Endocrinol*. 2006;252(1-2):167-183. doi:10.1016/j.mce.2006.03.024

109. Borowsky M, Lee J, Zhao J, Sarma K, Ohsumi T. Polycomb-Associated Non-Coding RNAs;
110. Wijeweera A, Haj M, Feldman A, Pnueli L, Luo Z, Melamed P. Gonadotropin gene transcription is activated by menin-mediated effects on the chromatin. *Biochim Biophys Acta*. 2015;1849(3):328-341. doi:10.1016/j.bbagrm.2015.01.001

111. Yosefzon Y, David C, Tsukerman A, et al. An epigenetic switch repressing Tet1 in gonadotropes activates the reproductive axis. *Proc Natl Acad Sci U S A*. 2017;114(38):10131-10136. doi:10.1073/pnas.1704393170

112. Hoivik EA, Bjanesoy TE, Bakke M. Epigenetic regulation of the gene encoding steroidogenic factor-1. *Mol Cell Endocrinol*. 2013;371(1-2):133-139. doi:10.1016/j.mce.2012.12.028

113. Hoivik EA, Bjanesoy TE, Mai O, et al. DNA methylation of intronic enhancers directs tissue-specific expression of steroidogenic factor 1/adrenal 4 binding protein (SF-1/Ad4BP). *Endocrinology*. 2011;152(5):2100-2112. doi:10.1210/en.2010-1305

114. Shima Y, Zubair M, Komatsu T, et al. Pituitary Homeobox 2 Regulates Adrenal4 Binding Protein/Steroidogenic Factor-1 Gene Transcription in the Pituitary Gonadotrope through Interaction with the Intronic Enhancer. *Mol Endocrinol*. 2008;22(7):1633-1646. doi:10.1210/me.2007-0444

115. Wen S, Schwarz JR, Niculescu D, et al. Functional Characterization of Genetically Labeled Gonadotropes. *Endocrinology*. 2008;149(6):2701-2711. doi:10.1210/en.2007-1502

116. Villalobos C, Núñez L, García-Sancho J. Phenotypic characterization of multi-functional somatotropes, mammotropes and gonadotropes of the mouse anterior pituitary. *Pflugers Arch Eur J Physiol*. 2004;449(3):257-264. doi:10.1007/s00424-004-1337-7

117. Childs G V. Development of gonadotropes may involve cyclic transdifferentiation of growth hormone cells. In: *Archives of Physiology and Biochemistry*. Vol 110. Arch Physiol Biochem;
118. Fontaine R, Ciani E, Haug TM, et al. Gonadotrope plasticity at cellular, population and structural levels: A comparison between fishes and mammals. *Gen Comp Endocrinol*. 2020;287. doi:10.1016/j.ygcen.2019.113344

119. Laverrière JN, L’Hôte D, Tabouy L, Schang AL, Quérat B, Cohen-Tannoudji J. Epigenetic regulation of alternative promoters and enhancers in progenitor, immature, and mature gonadotrope cell lines. *Mol Cell Endocrinol*. 2016;434:250-265. doi:10.1016/j.mce.2016.07.010

120. Pacini V, Petit F, Querat B, Laverriere JN, Cohen-Tannoudji J, L’Hôte D. Identification of a pituitary ERα-activated enhancer triggering the expression of Nr5a1, the earliest gonadotrope lineage-specific transcription factor. *Epigenetics and Chromatin*. 2019;12(1). doi:10.1186/s13072-019-0291-8

121. Liu F, Wang L, Perna F, Nimer SD. Beyond transcription factors: How oncogenic signalling reshapes the epigenetic landscape. *Nat Rev Cancer*. 2016;16(6):359-372. doi:10.1038/nrc.2016.41

122. Novakovic B, Gordon L, Wong NC, et al. Wide-ranging DNA methylation differences of primary trophoblast cell populations and derived cell lines: Implications and opportunities for understanding trophoblast function. *Mol Hum Reprod*. 2011;17(6):344-353. doi:10.1093/molehr/gar005

123. Schang AL, Quérat B, Simon V, et al. Mechanisms underlying the tissue-specific and regulated activity of the Gnrhr promoter in mammals. *Front Endocrinol (Lausanne)*. 2012;3(DEC). doi:10.3389/fendo.2012.00162

124. Pincas H, Amoyel K, Counis R, Laverrière JN. Proximal cis-acting elements, including
steroidogenic factor 1, mediate the efficiency of a distal enhancer in the promoter of the rat gonadotropin-releasing hormone receptor gene. *Mol Endocrinol.* 2001;15(2):319-337.
doi:10.1210/mend.15.2.0593

125. Ngan ESW, Leung PCK, Chow BKC. Interplay of pituitary adenylate cyclase-activating polypeptide with a silencer element to regulate the upstream promoter of the human gonadotropin-releasing hormone receptor gene. *Mol Cell Endocrinol.* 2001;176(1-2):135-144.
doi:10.1016/S0303-7207(01)00402-6

126. Cheng CK, Yeung CM, Chow BKC, Leung PCK. Characterization of a new upstream GnRH receptor promoter in human ovarian granulosa-luteal cells. *Mol Endocrinol.* 2002;16(7):1552-1564. doi:10.1210/mend.16.7.0869

127. Gründker C, Emons G. The role of gonadotropin-releasing hormone in cancer cell proliferation and metastasis. *Front Endocrinol (Lausanne).* 2017;8(AUG).
doi:10.3389/fendo.2017.00187

128. Limonta P, Marelli MM, Mai S, Motta M, Martini L, Moretti RM. GnRH receptors in cancer: From cell biology to novel targeted therapeutic strategies. *Endocr Rev.* 2012;33(5):784-811.
doi:10.1210/er.2012-1014

129. Odle AK, Akhter N, Syed MM, et al. Leptin regulation of gonadotrope gonadotropin-releasing hormone receptors as a metabolic checkpoint and gateway to reproductive competence. *Front Endocrinol (Lausanne).* 2018;8(JAN). doi:10.3389/fendo.2017.00367

130. Han DX, Sun XL, Fu Y, et al. Identification of long non-coding RNAs in the immature and mature rat anterior pituitary. *Sci Rep.* 2017;7(1). doi:10.1038/s41598-017-17996-6

131. Yang H, Ma J, Wang Z, et al. Genome-wide analysis and function prediction of long noncoding RNAs in sheep pituitary gland associated with sexual maturation. *Genes (Basel).* 2020;11(3).
132. Zheng J, Wang Z, Yang H, et al. Pituitary transcriptomic study reveals the differential regulation of IncRNAs and mRNAs related to prolificacy in different FecB genotyping sheep. *Genes (Basel)*. 2019;10(2). doi:10.3390/genes10020157

133. Li X, Li C, Xu Y, et al. Analysis of pituitary transcriptomics indicates that IncRNAs are involved in the regulation of sheep estrus. *Funct Integr Genomics*. 2020;20(4):563-573. doi:10.1007/s10142-020-00735-y

134. Li X, Li C, Wei J, et al. Comprehensive Expression Profiling Analysis of Pituitary Indicates that circRNA Participates in the Regulation of Sheep Estrus. *Genes (Basel)*. 2019;10(2). doi:10.3390/genes10020090

135. Li C, Li X, Ma Q, et al. Genome-wide analysis of circular RNAs in prenatal and postnatal pituitary glands of sheep. *Sci Rep.* 2017;7(1). doi:10.1038/s41598-017-16344-y

136. Seifuddin F, Singh K, Suresh A, et al. IncRNAKB, a knowledgebase of tissue-specific functional annotation and trait association of long noncoding RNA. *Sci Data*. 2020;7(1). doi:10.1038/s41597-020-00659-z

137. Taniguchi-Ponciano K, Andonegui-Elguera S, Peña-Martínez E, et al. Transcriptome and methylome analysis reveals three cellular origins of pituitary tumors. *Sci Rep.* 2020;10(1). doi:10.1038/s41598-020-76555-8

138. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and Pathological Aspects of Silent Pituitary Adenomas. *J Clin Endocrinol Metab*. 2019;104(7):2473-2489. doi:10.1210/jc.2018-00688

139. Li J, Li C, Wang J, et al. Genome-wide analysis of differentially expressed IncRNAs and mRNAs in primary gonadotrophin adenomas by RNA-seq. *Oncotarget*. 2017;8(3):4585-4606.
140. Xing W, Qi Z, Huang C, et al. Genome-wide identification of lncRNAs and mRNAs differentially expressed in non-functioning pituitary adenoma and construction of an lncRNA-mRNA co-expression network. *Biol Open*. 2019;8(1). doi:10.1242/bio.037127

141. Tucci V, Isles AR, Kelsey G, et al. Genomic Imprinting and Physiological Processes in Mammals. *Cell*. 2019;176(5):952-965. doi:10.1016/j.cell.2019.01.043

142. Li Z, Li C, Liu C, Yu S, Zhang Y. Expression of the long non-coding RNAs MEG3, HOTAIR, and MALAT-1 in non-functioning pituitary adenomas and their relationship to tumor behavior. *Pituitary*. 2015;18(1):42-47. doi:10.1007/s11102-014-0554-0

143. Zhao J, Dahle D, Zhou Y, … XZ-TJ of C, 2005 undefined. Hypermethylation of the Promoter Region Is Associated with the Loss of MEG3 Gene Expression in Human Pituitary Tumors. academic.oup.com https://academic.oup.com/jcem/article-abstract/90/4/2179/2836810. Accessed March 31, 2021.

144. Gejman R, Batista DL, Zhong Y, et al. Selective loss of MEG3 expression and intergenic differentially methylated region hypermethylation in the MEG3/DLK1 locus in human clinically nonfunctioning pituitary adenomas. *J Clin Endocrinol Metab*. 2008;93(10):4119-4125. doi:10.1210/jc.2007-2633

145. Cheunsuchon P, Zhou Y, Zhang X, et al. Silencing of the imprinted DLK1-MEG3 locus in human clinically nonfunctioning pituitary adenomas. *Am J Pathol*. 2011;179(4):2120-2130. doi:10.1016/j.ajpath.2011.07.002

146. Dauber A, Cunha-Silva M, Macedo DB, et al. Paternally Inherited DLK1 Deletion Associated With Familial Central Precocious Puberty. *J Clin Endocrinol Metab*. 2017;102(5):1557-1567. doi:10.1210/jc.2016-3677
147. Puertas-Avendaño RA, González-Gómez MJ, Ruvira MD, et al. Role of the Non-Canonical Notch Ligand Delta-Like Protein 1 in Hormone-Producing Cells of the Adult Male Mouse Pituitary. *J Neuroendocrinol*. 2011;23(9):849-859. doi:10.1111/j.1365-2826.2011.02189.x

148. Cheung LYM, Rizzoti K, Lovell-Badge R, Le Tissier PR. Pituitary Phenotypes of Mice Lacking the Notch Signalling Ligand Delta-Like 1 Homologue. *J Neuroendocrinol*. 2013;25(4):391-401. doi:10.1111/jne.12010

149. Zhou Y, Zhong Y, Wang Y, et al. Activation of p53 by MEG3 non-coding RNA. *J Biol Chem*. 2007;282(34):24731-24742. doi:10.1074/jbc.M702029200

150. Uroda T, Anastasakou E, Rossi A, et al. Conserved Pseudoknots in IncRNA MEG3 Are Essential for Stimulation of the p53 Pathway. *Mol Cell*. 2019;75(5):982-995.e9. doi:10.1016/j.molcel.2019.07.025

151. Zhenye L, Chu Zhong L, You Tu W, et al. The expression of TGF-β1, Smad3, phospho-Smad3 and Smad7 is correlated with the development and invasion of nonfunctioning pituitary adenomas. *J Transl Med*. 2014;12(1). doi:10.1186/1479-5876-12-71

152. Liu C, Li Z, Wu D, Li C, Zhang Y. Smad3 and phospho-Smad3 are potential markers of invasive nonfunctioning pituitary adenomas. *Onco Targets Ther*. 2016;9:2265-2271. doi:10.2147/OTT.S99699

153. Butz H, Likó I, Czirják S, et al. MicroRNA profile indicates downregulation of the TGFβ pathway in sporadic non-functioning pituitary adenomas. *Pituitary*. 2011;14(2):112-124. doi:10.1007/s11102-010-0268-x

154. Bilezikjian LM, Justice NJ, Blackler AN, Wiater E, Vale WW. Cell-type specific modulation of pituitary cells by activin, inhibin and follistatin. *Mol Cell Endocrinol*. 2012;359(1-2):43-52. doi:10.1016/j.mce.2012.01.025
155. Schang G, Ongaro L, Schultz H, et al. Murine FSH Production Depends on the Activin Type II
Receptors ACVR2A and ACVR2B. Endocrinol (United States). 2020;161(7).
doi:10.1210/endocr/bqaa056

156. Fortin J, Ongaro L, Li Y, et al. Minireview: Activin signaling in gonadotropes: What does the
FOX say …to the SMAD? Mol Endocrinol. 2015;29(7):963-977. doi:10.1210/me.2015-1004

157. Yu G, Li C, Xie W, et al. Long non-coding RNA C5orf66-AS1 is downregulated in pituitary null
cell adenomas and is associated with their invasiveness. Oncol Rep. 2017;38(2):1140-1148.
doi:10.3892/or.2017.5739

158. Drouin J, Lamolet B, Lamonerie T, Lanctôt C, Tremblay JJ. The PTX family of homeodomain
transcription factors during pituitary developments. In: Molecular and Cellular Endocrinology.
Vol 140. Mol Cell Endocrinol; 1998:31-36. doi:10.1016/S0303-7207(98)00026-4

159. Tremblay JJ, Lanctot C, Drouin J. The pan-pituitary activator of transcription, Ptx1 (pituitary
homeobox 1), acts in synergy with SF-1 and Pit1 and is an upstream regulator of the Lim-
homeodomain gene Lim3/Lhx3. Mol Endocrinol. 1998;12(3):428-441.
doi:10.1210/mend.12.3.0073

160. Szeto DP, Rodriguez-Esteban C, Ryan AK, et al. Role of the Bicoid-related homeodomain
factor Pitx1 in specifying hindlimb morphogenesis and pituitary development. Genes Dev.
1999;13(4):484-494. doi:10.1101/gad.13.4.484

161. Luo Z, Wijeweera A, Oh Y, Liou Y-C, Melamed P. Pin1 Facilitates the Phosphorylation-
Dependent Ubiquitination of SF-1 To Regulate Gonadotropin β-Subunit Gene Transcription.
Mol Cell Biol. 2010;30(3):745-763. doi:10.1128/mcb.00807-09

162. Sailer V, Charpentier A, Dietrich J, et al. Intragenic DNA methylation of PITX1 and the adjacent
long non-coding RNA C5orf66-AS1 are prognostic biomarkers in patients with head and neck
squamous cell carcinomas. *PLoS One*. 2018;13(2). doi:10.1371/journal.pone.0192742

163. Fedele M, Palmieri D, Fusco A. HMGA2: A pituitary tumour subtype-specific oncogene? *Mol Cell Endocrinol*. 2010;326(1-2):19-24. doi:10.1016/j.mce.2010.03.019

164. Portovedo S, Gaido N, de Almeida Nunes B, et al. Differential Expression of HMGA1 and HMGA2 in pituitary neuroendocrine tumors. *Mol Cell Endocrinol*. 2019;490:80-87. doi:10.1016/j.mce.2019.04.010

165. D’Angelo D, Mussnich P, Sepe R, et al. RPSAP52 IncRNA is overexpressed in pituitary tumors and promotes cell proliferation by acting as miRNA sponge for HMGA proteins. *J Mol Med*. 2019;97(7):1019-1032. doi:10.1007/s00109-019-01789-7

166. Esposito F, De Martino M, D’Angelo D, et al. HMGA1-pseudogene expression is induced in human pituitary tumors. *Cell Cycle*. 2015;14(9):1471-1475. doi:10.1080/15384101.2015.1021520

167. Wang J, Wang D, Wan D, et al. Circular RNA In Invasive and Recurrent Clinical Nonfunctioning Pituitary Adenomas: Expression Profiles and Bioinformatic Analysis. *World Neurosurg*. 2018;117:e371-e386. doi:10.1016/j.wneu.2018.06.038

168. Guo J, Wang Z, Miao Y, et al. A two-circRNA signature predicts tumour recurrence in clinical non-functioning pituitary adenoma. *Oncol Rep*. 2019;41(1):113-124. doi:10.3892/or.2018.6851

169. Hu Y, Zhang N, Zhang S, et al. Differential circular RNA expression profiles of invasive and non-invasive non-functioning pituitary adenomas: A microarray analysis. *Med (United States)*. 2019;98(26). doi:10.1097/MD.00000000000016148

170. Du Q, Hu B, Feng Y, et al. Circoma1-mediated miR-145-5p suppresses tumor growth of nonfunctioning pituitary adenomas by targeting TPT1. *J Clin Endocrinol Metab*.
171. Barrett SP, Salzman J. Circular RNAs: Analysis, expression and potential functions. *Dev.* 2016;143(11):1838-1847. doi:10.1242/dev.128074

172. Li D, Lv B, Zhang H, Lee JY, Li T. Disintegration of cruciform and G-quadruplex structures during the course of helicase-dependent amplification (HDA). *Bioorganic Med Chem Lett.* 2015;25(8):1709-1714. doi:10.1016/j.bmcl.2015.02.070

173. Melamed P, Haj M, Yosefzon Y, et al. Multifaceted Targeting of the Chromatin Mediates Gonadotropin-Releasing Hormone Effects on Gene Expression in the Gonadotrope. *Front Endocrinol (Lausanne).* 2018;9(FEB):58. doi:10.3389/fendo.2018.00058
### Table 1: Commonly used long non-coding RNA terminology

| Term         | Name in full                       | Features                                                                 | Further Information |
|--------------|------------------------------------|---------------------------------------------------------------------------|---------------------|
| LncRNA       | Long non-coding RNA                | >200 nt RNA that does not encode a protein, capped, with or without polyA; located in nucleus or cytosol. Multiple functions and sub-groups. | 3,5,7,53,54,57      |
| LincRNA      | Long intergenic non-coding RNA     | As above, transcribed from genomic regions located between protein-coding genes | 3,5,7,53,54,57      |
| eRNA         | Enhancer RNA                       | Commonly 200-2000 nt, unstable, capped and no polyA; transcribed bi-directionally from central untranscribed region | 16,40               |
| ceRNA        | Competing/competitive endogenous RNA | LncRNA of various origins that compete for the miRNAs and act as miRNA sponges. | 72,74               |
| circRNA      | Circular RNA                       | Produced by back-splicing circularization of several exons; very stable, located throughout the cell and sometimes secreted. Produced slowly and associated with fast rates of transcription. | 75,171              |
| ciRNA        | Circular intronic RNA              | Derived from excised introns; formation depends on specific sequence elements; predominantly nuclear. | 72,75,81,172        |
Figure 1: Differential usage of enhancer elements. Multiple enhancers, activated by various signals, can be used in distinct contexts to regulate expression of genes within the same topologically associated domain (TAD).
Figure 2: Regulatory enhancers and lncRNAs discussed in this review. CTCF-Cohesin complex (A) closes the DNA domain into a loop containing various transcription units, which allows the regulatory elements to interact physically with their target gene promoters. Transcriptional enhancers (B) located up- or down-stream from the TSS of their target genes, sometimes clustered as super-enhancers (C) or enriched within the first introns (D), bind tissue specific TFs to enhance or repress transcription. Enhancers are characteristically marked by H3K4me1, unlike promoters marked by H3K4me3. The central non-transcribed regions of active enhancers often contain non-canonical structures like G-quadruplexes (E), and produce bidirectional eRNAs (F) or lncRNAs (G) which might interact with the DNA to form R-loops (H). The lncRNAs enriched with G-rich sequences can also form non-canonical DNA structures which are often bound by PRC2 complex (I). Other lncRNAs can form circRNAs (J) which are found in the nucleus and cytosol and sometimes secreted from the cell.
Figure 3. The Cga distal enhancer and its eRNA. A distal regulatory region located upstream the Cga gene is marked by typical histone marks of an enhancer (H3K4me1 and H4K27ac), and drives bi-directional transcription of two eRNAs. The more distal eRNA (opposite) plays a role in the DNA looping which brings the enhancer close to the proximal promoter, to maintain the epigenetic landscape and high rates of Cga transcription\textsuperscript{44,173}. 
Figure 4. The expression of IncRNA C5orf66-AS1 and its connections with Pitx1. The IncRNA C5orf66-AS1 is expressed in the pituitary from the region located immediately upstream (~4.5 kbp) of the Pitx1 gene (the IncRNA C5orf66 transcribed from the opposite strand is also expressed in the pituitary). Gene expression from GTEx RNA-seq (from UCSC Genome Browser) shows a similar pattern of expression for Pitx1 and C5orf66-AS1. C5orf66-AS1 is predicted by GeneHancer to interact with three regions, one of which is at the Pitx1 proximal promoter and the second in Pitx1 first intron. The first exon of C5orf66-AS1 and the first intron of Pitx1 contain G-quadruplex forming sequences (as predicted by pqsfinder). ChIP seq data from ENCODE reports high occupancy of EZH2 and SUZ12 proteins (i.e. the PRC2 complex) at this genomic region.