DISSERTATIONES
BIOLOGICAE
UNIVERSITATIS
TARTUENSIS
382

ANTTI MATVERE

Studies on aryl hydrocarbon receptor in murine granulosa cells and human embryonic stem cells

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
382

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
382

ANTTI MATVERE

Studies on aryl hydrocarbon receptor in murine granulosa cells and human embryonic stem cells

UNIVERSITY OF TARTU
Press

Chair of Cell Biology, Institute of Molecular and Cell Biology, University of Tartu, Estonia

Dissertation was accepted for the commencement of degree of Doctor of Philosophy (in Cell Biology) on February 23th, 2021 by the Council of the Institute of Molecular and Cell Biology, University of Tartu, Estonia

Supervisors: Prof. Toivo Maimets, PhD
Prof. Arnold Kristjuhan, PhD
Chair of Cell Biology
Institute of Molecular and Cell Biology
University of Tartu, Estonia

Reviewer: Prof. Ants Kurg, PhD
Chair of Biotechnology
Institute of Molecular and Cell Biology
University of Tartu, Estonia

Opponent: Prof. Xavier Coumoul, PhD
INSERM UMR-S 1124 - T3S
Faculty of Fundamental and Biomedical Sciences
University Paris Descartes, France

Commencement: Room No 105, Riia 23B, Tartu, on March 30, 2021, at 12:15

The publication of this dissertation is granted by the Institute of Molecular and Cell Biology, University of Tartu.

ISSN 1024-6479
ISBN 978-9949-03-562-5 (print)
ISBN 978-9949-03-563-2 (pdf)

University of Tartu Press
www.tyk.ee

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis contains the following publications, which are referred in the text by Roman numerals:

I. Teino, Indrek; **Matvere, Antti**; Kuuse, Sulev; Ingerpuu, Sulev; Maimets, Toivo; Kristjuhan, Arnold; Tiido, Tarmo (2014). Transcriptional repression of the Ahr gene by LHCGR signalling in preovulatory granulosa cells is controlled by chromatin accessibility. Molecular and Cellular Endocrinology, 382 (1), 292–301. **https://doi.org/10.1016/j.mce.2013.10.011.**

II. **Matvere, Antti**; Teino, Indrek; Varik, Inge; Kuuse, Sulev; Tiido, Tarmo; Kristjuhan, Arnold; Maimets, Toivo (2019). FSH/LH-Dependent Up-regulation of Ahr in Murine Granulosa Cells Is Controlled by PKA Signalling and Involves Epigenetic Regulation. International Journal of Molecular Sciences, 20 (12), 3068. **https://doi.org/10.3390/ijms20123068.**

III. Teino, Indrek; **Matvere, Antti**; Pook, Martin; Varik, Inge; Pajusaar, Laura; Uudeküll, Keyt; Vaher, Helen; Trei, Annika; Kristjuhan, Arnold; Org, Tõnis; Maimets, Toivo (2020). Impact of AHR Ligand TCDD on Human Embryonic Stem Cells and Early Differentiation. International Journal of Molecular Sciences, 21 (23), 9052. **https://doi.org/10.3390/ijms21239052.**

My contribution to these publications is as follows:

Ref. I – Participated in conceptualisation and experimental design, contributed to performing the experiments and data analysis.

Ref. II – Participated in conceptualisation and experimental design, performing the experiments, data analysis and manuscript preparation.

Ref. III – Contributed to conceptualisation and experimental design, participated in performing the experiments, data analysis and manuscript preparation.

LIST OF ABBREVIATIONS

3-MC	–	3-methylcholanthrene
AC	–	adenylyl cyclase
ActD	–	actinomycin D
AHR	–	aryl hydrocarbon receptor
AHRR	–	aryl hydrocarbon receptor repressor
AIP	–	aryl hydrocarbon receptor interacting protein
ARNT	–	aryl hydrocarbon receptor nuclear translocator
ATAC-seq	–	assay for transposase accessible chromatin followed by high-throughput sequencing
cAMP	–	cyclic adenosine monophosphate
CHART-PCR	–	chromatin accessibility by real-time polymerase chain reaction
ChIP-seq	–	chromatin immunoprecipitation followed by high-throughput sequencing
CHX	–	cycloheximide
CREB	–	cAMP response element
DMSO	–	dimethyl sulfoxide
eCG	–	equine chorionic gonadotropin
ED	–	embryonic day
ERK1/2	–	extracellular signal-regulated kinase 1/2
FICZ	–	6-formylindolo[3,2b]carbazole
FSH	–	follicle stimulating hormone
FSHR	–	follicle stimulating hormone receptor
Fsk	–	forskolin
GC	–	granulosa cell
GnRH	–	gonadotropin releasing-hormone
GO	–	gene ontology
hCG	–	human chorionic gonadotropin
HDAC	–	histone deacetylase
hESC	–	human embryonic stem cell
hnRNA	–	heteronuclear RNA
HPOA	–	hypothalamus-pituitary-ovary axis
HSC	–	hematopoietic stem cell
HSP90	–	heat shock protein 90
I3C	–	indole-3-carbinole
ICM	–	inner cell mass
IDO	–	indoleamine 2,3-dioxygenase
Kyn	–	kynurenine
LHCGR	–	luteinising hormone and chorionic gonadotropin receptor
LH	–	luteinising hormone
LHR	–	luteinising hormone receptor
miRNA	–	microRNA
MSI2	–	musashi-2

PAS – Per-Arnt-Sim
PER – period homolog 1 (*Drosophila*)
p-CREB – phosphorylated cAMP response element
PD-1 – programmed cell death 1
PD-L1 – programmed cell death ligand 1
PGC – primordial germ cell
PKA – protein kinase A
PMSG – pregnant mare's serum gonadotropin
PND – postnatal day
SIM – single-minded homolog (*Drosophila*)
SR1 – StemRegenin 1
TAD – transactivation domain
TCDD – 2,3,7,8-tetrachlorodibenzo-p-dioxin
TDO – tryptophan-2,3-dioxygenase
TH – tyrosine hydroxylase
TME – tumour microenvironment
TSA – trichostatin A
TSH – thyroid-stimulating hormone
TSS – transcription start site

1. INTRODUCTION

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor. It was initially discovered as a mediator of toxic effects of various environmental chemicals, which are often secreted into the atmosphere as organic waste. These chemicals include a wide range of polycyclic/halogenated aromatic hydrocarbons, of which the most well-recognized is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is also known as the highest-affinity AHR ligand. However, research in AHR biology over the past few decades has revealed that this protein has a broad range of functions other than being a sensor to toxic substances.

Evidence on the importance of this receptor in normal physiology has emerged from studies using knock-out animal models as well as the result of studying AHR in human tissues. It has been discovered that AHR has a crucial role in the immune system and liver function. AHR has also been shown to have a fundamental role in several tissues serving a barrier function, including skin and lung tissues, but also in the placenta. Moreover, the role of AHR in the gut immune system has been demonstrated, being a sensor to a variety of dietary molecules, whether produced by stomach acid or microbial metabolism. Additional evidence has shown the importance of AHR in the brain, several cancers, but also in reproductive tissues. Several studies investigating the role of this protein have found AHR to function as a regulator of ovarian follicle development. Furthermore, increasing evidence of AHR in embryogenesis has emerged over the past years, suggesting that AHR may regulate developmental potency, including pluripotency and differentiation of stem cells.

Nowadays, the role of AHR in cellular homeostasis has also been established by discoveries of numerous endogenous AHR ligands, including tryptophan catabolite kynurenine. Clinical sampling of AHR expression as well as basic research studying the role of AHR has paved way to the prospect of using this protein as a therapeutic target. Indeed, today's research in AHR biology is widely focused on its potential to serve as a marker in various diseases, but also as being a cellular target in modulation of treatment outcomes. However, the knowledge about the exact functions of this protein is far from complete. In addition, many studies have pointed out the complex nature of AHR by having tissue- and cell-specific functions. Additionally, the health outcomes of AHR regulation are highly dependent on the timing and threshold of the expression as well as changes in the activity of this protein.

Collectively, there is much research to be done in elucidating the mechanisms by which the expression of AHR is regulated. For exploiting the potential of AHR in therapeutic targeting and treatment of several diseases, it is imperative to work on obtaining a detailed understanding on the cellular signalling of AHR. This study focused on investigating the expression and regulation of the aryl hydrocarbon receptor in cells with high developmental potential, including murine ovarian granulosa cells and human embryonic stem cells.

2. OVERVIEW OF LITERATURE

2.1. Early development and folliculogenesis

2.1.1. Stem cells

During the very early stages of embryonic development, a small number of cells are known as totipotent stem cells and have the potential to differentiate into any cell types of the organism, including extraembryonic tissues. As embryonic development progresses, cells in the embryo reorganise, specialise and gradually start losing their differentiation capacity. Pluripotent stem cells, arising from the inner cell mass (ICM) of the blastocyst, are cells that have the capacity to self-renew and differentiate into multiple types of cells, excluding extraembryonic tissues. The differentiation of these cells gives rise to three germ layers – ectoderm, mesoderm and endoderm.

In addition to embryonic stem cells, some adult stem cells have the capacity to develop into a limited range of cell types. The primary function of these cells is to replace lost cells in mature tissues. As stem cells differentiate, vast changes in the gene expression, signalling network and morphology take place (Bai et al., 2012). In pluripotent stem cells, the state of self-renewal is governed by core pluripotency factors OCT4, SOX2 and NANOG (Boyer et al., 2005; Loh et al., 2006). These factors have the capacity to enhance their own expression and suppress the expression of genes essential for differentiation, thus assuring the state of self-renewal. The differentiation of pluripotent cells is generally accompanied by the downregulation of these pluripotency factors and upregulation of lineage-specific genes.

2.1.2. Ovarian development and folliculogenesis

Ovarian development begins from a bipotential gonad during embryogenesis. In mouse, primordial germ cells (PGCs) colonize the genital ridge around ED 10.5 (Edson et al., 2009; Hirshfield, 1991; Pepling, 2006; Tingen et al., 2009). Germ cells go through rapid proliferation and form clusters by remaining attached by tubular intercellular bridges due to incomplete cytokinesis (Hirshfield, 1991; Merchant & Zamboni, 1972; Pepling & Spradling, 1998). Germ cell clusters are surrounded by a single layer of somatic cells, forming germ cell nests (Guigon & Magre, 2006; Hirshfield & DeSanti, 1995). Germ cells are mitotically arrested until ED 12.5, when they enter meiosis and become known as oocytes (Pepling, 2006). The progression of oocytes through the meiotic prophase I continues until ED 17.5, when they become arrested in the diplotene stage. A substantial number of germ cells are lost during this period in a process called attrition (Tilly, 1996). Although attrition occurs in both mitotic and meiotic germ cells, most of the PGCs are lost post-mitotically at ED 13.5 and postnatal day (PND) 5 (Ghafari et al., 2007; Hirshfield, 1991; McClellan et al., 2003; Pepling & Spradling, 2001).

Surviving oocytes assemble into primordial follicles, which contain an oocyte surrounded by a basement membrane and a single layer of flattened granulosa cells (Figure 1). These granulosa cells are often referred to as pre-granulosa cells. Primordial follicle formation is known to vary between species. While the first primordial follicle formation has been reported to occur on postnatal day 2 in mice, these follicles form 4.5 months after fertilization in humans (Hirshfield, 1991; Peters, 1969).

Follicle formation is accompanied by rapid proliferation of somatic cells. However, somatic cell division ceases once primordial follicles have formed. Additionally, lack of differentiation has been reported in this quiescent state (Guraya & Guraya, 1985). Proliferation of pre-granulosa cells will not resume until further follicular growth and meiosis of the oocytes remains incomplete until ovulation.

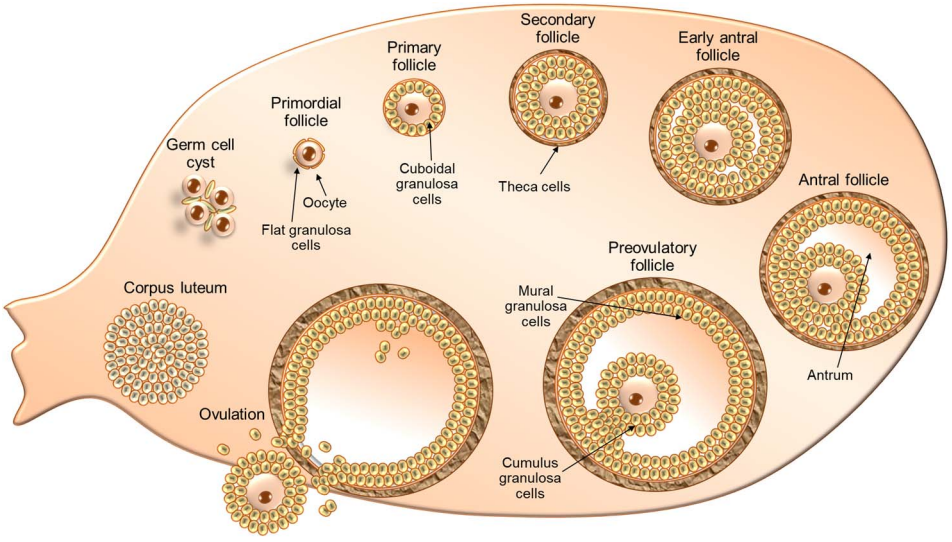

Figure 1. Schematic representation of different stages of mammalian folliculogenesis. Primordial follicles are derived from germ cell cyst. The oocyte in primordial follicles is surrounded by basement membrane and flattened granulosa cells (GCs). In primary follicles, the oocyte becomes surrounded by cuboidal GCs. By reaching secondary stage, two layers of GCs surround the oocyte. Preantral follicle development does not require gonadotropin stimulus. At puberty, follicle growth becomes dependent on FSH stimulus. At this stage, a fluid filled cavity (antrum) is formed. Further antral follicle growth is accompanied with GC proliferation and differentiation. Ovulation occurs in response to rising concentration of LH. After ovulation, remaining granulosa and theca cells undergo terminal differentiation and form the corpus luteum.

Most of the follicles remain in the quiescent state for a long time. First signs of reawakened follicles appear after resumption of granulosa cell proliferation. This process is followed by enlargement of the oocyte and change of granulosa cells to a cuboidal state (Figure 1) (Hirshfield, 1991). During further progression of

follicles, the growth of the oocyte ceases, whereas granulosa cells continue extensive proliferation. The follicles becomes surrounded by steroidogenic cells (theca interna) and connective tissue cells (theca externa) (Hirshfield, 1991). In mouse, first thecal cells occur after the oocyte has reached its full size and is surrounded by 2-3 layers of granulosa cells (Peters, 1969). Further follicular development is characterised by the formation of fluid-filled cavity (i.e. antrum) between granulosa cells. As follicular fluid accumulates, the antral cavity increases rapidly until ovulation (Figure 1).

The last stages of follicle maturation are characterised by significant changes in somatic cells, which have gone through differentiation. Granulosa cells in these follicles are heterogeneous, varying by morphological, but also by functional characteristics (Zoller & Weisz, 1979). Mural granulosa cells, which reside in the outer part of the follicle, develop more steroidogenic features. This is supported by evidence showing these cells to have fragile lysosomes (Zoller & Weisz, 1980). Additionally, characteristic changes in the theca layer take place. The inner layer of thecal cells is interlaced with vascular channels and have multiple characteristics identifying them as steroidogenic cells (O'Shea et al., 1978). Theca externa layer is mainly formed by cells resembling fibroblasts, but also smooth-muscle cells (O'Shea, 1970). The fully-grown follicle contains granulosa and theca cells, which are highly differentiated and possess tissue-specific features. The most prominent feature of granulosa cells is the presence of follicle stimulating hormone (FSH) receptors (FSHR) and luteinising hormone (LH) receptors (LHR) (Nimrod et al., 1976; Zeleznik et al., 1981). In addition, granulosa cells contain a large number of steroidogenic enzymes, including aromatase, which is involved in oestradiol production (Fortune et al., 1986). Theca cells, on the other hand, are the main source for the production of androgens (Howard et al., 1988).

2.1.3. Hypothalamus/pituitary/ovary axis

Hypothalamus-pituitary-ovary axis (HPOA) forms the central part of folliculogenesis signalling cascade. HPOA is essential for the regulation of hormonal balance and contains a tightly regulated feedback system (Freeman, 2006). Hypothalamus is responsible for the synthesis and release of gonadotropin-releasing hormone (GnRH) from neurons in the anterior hypothalamic areas, medial preoptic and arcuate nucleus to the anterior pituitary gland. GnRH stimulates the pituitary to produce and release FSH and LH from the gonadotrophic cells. FSH binds to corresponding FSH receptors in the ovarian granulosa cells, thus stimulating follicle growth and steroidogenesis. LH binds to LH receptors and its main function is to stimulate ovulation. Ovarian steroid hormones (i.e. oestrogen, progesterone), which are produced in response to gonadotropin stimulation, affect hypothalamus and pituitary in a feedback mechanisms to suppress the release of GnRH, FSH and LH (Neill et al., 2006).

2.1.4. Hormonal signalling

Ovarian follicles display first signs of functional maturation after the beginning of oestradiol secretion, which is produced in response to FSH (Luderer & Schwartz, 1994). Oestradiol at the initial stages of follicle maturation stimulates granulosa cell proliferation (Robker & Richards, 1998). Moreover, oestradiol increases the sensitivity of the pituitary to GnRH, resulting in increased FSH production (Quiñones-Jenab et al., 1996). As a result of a feedback loop, FSH stimulates oestrogen production by binding to FSH receptors on the surface of proliferating granulosa cells. Reaching to antral stage, follicular granulosa cells begin to express LH receptors, another distinct feature of the functional maturation process. Precisely controlled hormonal balance between gonadotropins and steroid hormones supports further progress of follicular maturation, leading to a significant increase in the number of LH receptors on granulosa cells. Then, a rapid increase in LH concentrations occurs, resulting in ovulation of the oocyte from the follicle (Edson et al., 2009).

2.1.5. Gonadotropins and steroid hormones. Hormone receptors.

The family of glycoprotein hormones consists of FSH, LH, human chorionic gonadotropin (hCG) and thyroid-stimulating hormone (TSH). Hormones have a heterodimeric structure, comprising of alpha-subunit and beta-subunit (Pierce & Parsons, 1981). It is established that the alpha-subunits of these hormones are very similar. However, the N-terminal residue of the beta-subunit is unique to each hormone and is responsible for the specificity in biological activity (Loumaye et al., 1998). The alpha-subunit of FSH consists of 89 amino acids and the beta-subunit consists of 111 amino acids. LH shares the amino acid length of the alpha-subunit, whereas the beta-subunit is reported to be composed of 112 to 115 amino acids (McClamrock, 2003). A single gene located on chromosome 6 is responsible for encoding the alpha-subunit. Beta-subunits for FSH and LH are encoded by genes located in chromosome 11 and 19, respectively (Jameson et al., 1991). Additional difference between FSH and LH is the number of glycosylation sites with FSH containing four and LH containing three asparagine-linked glycosylation sites. The distinct features of these hormones in the biological activity may thus be influenced by the differences in carbohydrate moieties known to affect pharmacokinetics (Shoham & Insler, 1996).

Hormonal signalling and transcytosis in the ovary are mediated by gonadotropin receptors. FSHR and LHR belong to the subgroup of G-protein coupled receptors (GPCRs). These receptors contain a large extracellular domain, which is responsible for the binding of hormones (Milgrom et al., 1997). Characterisation of the amino sequences of these receptors by cloned cDNA sequencing has revealed that they contain a seven times transmembrane domain. The N-terminal extracellular domain is composed of leucine-rich repeats and is the binding site for hormones. The intracellular C-terminal domain of the receptor is

relatively short and is involved in downstream GPCR signalling by being a target to G-protein receptor kinase (Milgrom et al., 1997).

hCG is primarily produced by differentiated syncytiotrophoblasts and is important in the maintenance of pregnancy. Alpha-subunit of this hormone shares similarity to FSH, LH and TSH, whereas the beta-subunit is 80–85% homologous to LH (Nwabuobi et al., 2017). In the organism, hCG and LH act on the same receptor (LHR, also known as LHCGR). However, differences in the cellular and molecular response and activation of the signalling cascades have been reported previously (Casarini et al., 2012).

In the ovary, FSH receptors are expressed on granulosa cells at every stage of follicular maturation. LH receptors, on the other hand, are limited to theca and granulosa cells of follicles, which have reached the antral stage (Bao & Garverick, 1998; Lapolt et al., 1992). Activation of FSH and LH receptors by binding of the gonadotropins is known to stimulate adenylyl cyclase, which results in an increase of intracellular cAMP levels. This, in turn, activates protein kinase A (PKA) (Carlone & Richards, 1997; Mukherjee et al., 1996). Most of the actions of FSH on granulosa cell differentiation have been shown to be mediated by PKA (Puri et al., 2016). PKA, in turn, is directly involved in phosphorylation of cAMP response element (CREB) or histone H3 in granulosa cells (Salvador et al., 2001).

2.2. The aryl hydrocarbon receptor (AHR)

2.2.1. AHR background

The aryl hydrocarbon receptor (AHR) was initially discovered in the middle of 70s as a receptor for binding environmental pollutants and mediating the induction of enzyme responsible for the metabolism of xenobiotics (Poland et al., 1976). Earlier studies thus mainly focused on investigating the role of AHR in detoxification of these substances. It took years before it was discovered that this protein might have important physiological roles. The evidence for this was gained using knock-out animals, but also investigation of the *Ahr* gene sequence, which revealed its old evolutionary origin (Fernandez-Salguero et al., 1995; Hahn, 2002; Schmidt et al., 1996). During the past few decades, this ligand-activated transcription factor has been extensively studied for its endogenous roles, as well as for its potential as a therapeutic target in treatment of multiple diseases (Opitz et al., 2011; Sadik et al., 2020; Safe et al., 2020; Wang et al., 2021).

2.2.2. AHR structure

AHR belongs to the basic helix-loop-helix (bHLH) family of proteins. Cloning of the mouse Ahr revealed that this protein contains 805 amino acid residues. The structural organisation of Ahr is representative of proteins belonging to bHLH-PAS (Per/Arnt/Sim) family of transcription factors (Burbach et al., 1992). In human, AHR consists of 848 amino acid residues (Uniprot). The bHLH domain, located

in the N-terminal region of AHR, is responsible for binding DNA (Figure 2) (Pandini et al., 2007). Additionally, the N-terminus functions as a mediator of AHR interaction with its nuclear partner – aryl hydrocarbon receptor nuclear translocator (ARNT).

The N-terminal region also contains the nuclear localisation signal (NLS), which is followed by PAS-A and PAS-B domains. These sequences have been found in PER, ARNT and SIM proteins. The latter is known as the *Drosophila* „single-minded" protein, involved in neuronal development. PAS domains are necessary for the binding of heat shock protein 90 (HSP90) and AHR-interacting protein (AIP). PAS domain is also responsible for ligand binding and AHR-ARNT interaction in the nucleus (Petrulis et al., 2000). The C-terminal end of AHR contains three separate transcription activation domains (TADs) (Ma et al., 1995). Between PAS-B and TAD resides a 81 peptides long inhibitory domain (ID), suggested to act as an inhibitor of the AHR TA domain without the presence of agonist (Figure 2) (LaPres et al., 2000).

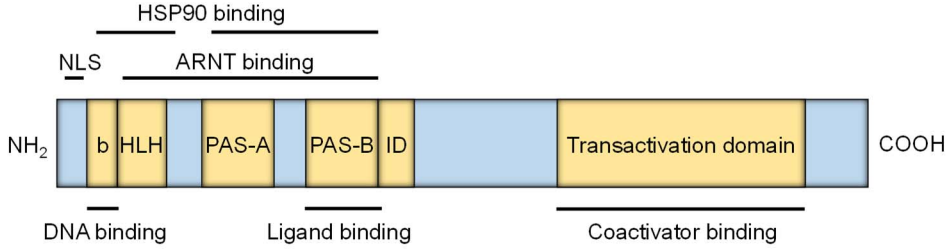

Figure 2. Schematic representation of AHR structure. bHLH – basic helix-loop-helix domain; PAS (Per-Arnt-Sim) domains (A and B); ID – inhibitory domain; TAD – transactivation domain; NLS – nuclear localisation signal; HSP90 – heat shock protein 90; ARNT – aryl hydrocarbon receptor nuclear translocator; the locations of functional binding domains are indicated by bars.

2.2.3. AHR signalling

In an inactive state, AHR resides in the cytoplasm and forms a complex with two HSP90 molecules, AIP and a co-chaperone p23 (Kazlauskas et al., 1999; Meyer & Perdew, 1999). After activation of AHR by its ligand, AHR translocates to the nucleus, where it is released from its cytoplasmic chaperone complex (Figure 3). AHR then heterodimerises with ARNT (Larigot et al., 2018). ARNT shares a structural similarity with AHR and the formation of the dimer in the nucleus is necessary for the AHR to bind to xenobiotic response elements (XREs, also known as dioxin response elements, DREs). The core sequence of this element and the main binding motif for AHR is 5'-GCGTG-3' (Dere et al., 2011).

Dimerization with ARNT is required for the transactivation of AHR target genes (including *CYP1A1*, the most well-known AHR target gene) (Nebert & Gonzalez, 1987). Additionally, AHR activation increases the expression of AHR

repressor (AHRR). Following an increase in AHRR concentration, a negative feedback loop is formed. AHRR starts binding to ARNT, thus inhibiting AHR-ARNT dimerization. Monomeric AHR is then transported to cytoplasm and subjected to proteosomal degradation (Figure 3) (Mimura & Fujii-Kuriyama, 2003).

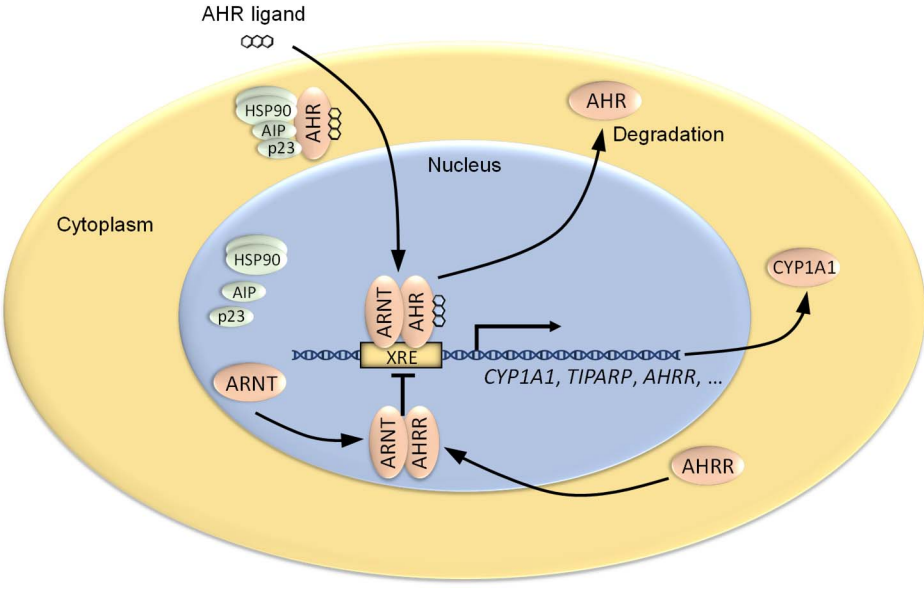

Figure 3. AHR signalling pathway. Mechanism of transcriptional activation of AHR target genes. Unliganded AHR resides in the cytoplasm, forming a complex with chaperone proteins. Following ligand binding, AHR translocates to the nucleus, is released from chaperones and heterodimerises with ARNT. The formed heterodimer binds XRE motif sequence in DNA and activates transcription of AHR target genes. Increasing concentrations of AHRR result in a negative feedback loop. AHRR binds ARNT, thus inhibiting AHR-ARNT dimerization and subjecting monomeric AHR to proteosomal degradation.

2.2.4. AHR ligands

AHR was initially discovered as a receptor for various xenobiotics. The most studied AHR ligand is TCDD, which is known to have the highest affinity to AHR (Denison & Nagy, 2003). The xenobiotic class of AHR ligands also include dibenzofurans, biphenyls, 3-methylcholantrene (3-MC), benzo[alpha]pyrene and benzanthracenes (Farrell et al., 1987; Gillner et al., 1985; Jensen et al., 2010; Kolasa et al., 2013).

More recent studies have presented knowledge about the existence of a plethora of endogenous ligands. Several flavonoids have been identified as AHR ligands, including quercetin and galangin (Ciolino et al., 1999; Zhang et al., 2003). AHR ligands can also be classified by their dietary origin. This class of AHR

ligands is most well-represented by indole-3-carbinol (I3C), which is derived from indole glucosinolates found in cruciferous vegetables (Bjeldanes et al., 1991; Gillner et al., 1985). This compound is further processed in the organisms by the acidic environment in the stomach and as a result several molecules are produced, capable to activate AHR (Murray et al., 2014). Of these ligands, indolo[3,2b]-carbazole has the highest affinity for the AHR (Bjeldanes et al., 1991). Interestingly, these dietary compounds have been shown to increase intestinal retention and function of group 3 innate lymphoid cells and intraepithelial lymphocytes through AHR (Li et al., 2011; Qiu et al., 2013). These findings have indicated that AHR and its endogenous ligands may serve barrier function roles.

Another class of endogenous AHR ligands are derivative products of tryptophan metabolism pathway. These include kynurenine, kynurenic acid, indoxyl sulfate, a putative endogenous ligand ITE and a UV photoproduct 6-formylindolo[3,2b]carbazole (FICZ) (DiNatale et al., 2010; Mezrich et al., 2010; Öberg et al., 2005; Schroeder et al., 2010). Studies investigating the functional properties of these ligands and subsequent AHR activation have revealed all of these tryptophan metabolism products to have important biological effects. Furthermore, recent studies have implied that the variety of AHR endogenous agonists also contain serotonin and dopamine (Manzella et al., 2018; Park et al., 2020).

The increasing number of discovered AHR ligands has raised discussion about the potential of using these ligands to manipulate AHR pathway with the aim to gain beneficial health outcomes. Indeed, based on this, various antagonists have been developed for this purpose, including e.g. CH-223191 and StemRegenin1 (Boitano et al., 2010; Kim et al., 2006). Interestingly, resveratrol, commonly present in red wine, has reported to be an antagonist of AHR (Casper et al., 1999).

2.2.5. AHR and cancer

The role of AHR in tumourigenesis and cancer progression has been demonstrated using *Ahr* knock-out (*Ahr*KO) mice. It has been shown that after diethylnitrosamine treatment these mice develop more liver adenomas and inflammatory marker gene expression (Fan et al., 2010). Studies have also reported the spontaneous formation of colon tumours in these mice, suggesting Ahr to have a tumour suppressor role (Metidji et al., 2018). Moreover, Ahr activation in wild type animals by exogenous ligands protected against chemically induced and genetic models of colon cancer (Díaz-Díaz et al., 2016; Furumatsu et al., 2011; Kawajiri et al., 2009; Villarroya-Beltri et al., 2013). The suppressive role of Ahr on cancer has also been reported in multiple tissues. Ahr is proposed to act as a tumour suppressor in melanoma, as its knock-down was shown to induce primary melanoma progression (Contador-Troca et al., 2013). *AHR* was also reported to be a tumour suppressor-like gene in glioblastoma (Jin et al., 2019). Additional studies have shown AHR to be a tumour suppressor in SHH medulloblastoma (Sarić et al., 2020) and in pituitary adenomas (Formosa et al., 2017; Sarić et al., 2020).

Notably, there is a lot of controversy related to the functions of AHR in regulation of tumourigenesis. Evidence derived from multiple studies, which in contrast to being a tumour suppressor, have shown AHR to function as an oncogenic protein. It was shown that AHR is highly expressed in several human tumours, including glioblastoma (Gramatzki et al., 2009). AHR antagonism or knock-down was shown to inhibit glioblastoma cell survival and migration in this study. A following study demonstrated that an endogenous AHR ligand kynurenine, produced by the metabolism of tryptophan by indoleamine-2,3-dioxygenase and tryptophan-2,3-dioxygenase, promoted AHR-dependent glioblastoma cell survival and motility (Opitz et al., 2011). In addition, the oncogenic role of AHR in medulloblastoma and adenocarcinoma has been reported, further showing the dual and controversial role of AHR in cancer (Dever & Opanashuk, 2012; Jaffrain-Rea et al., 2009). Tumour promoting role of AHR has additionally been shown in head and neck cancer (DiNatale et al., 2011). High AHR expression has also been reported in clear cell renal cell carcinoma and oesophageal tumours (Ishida et al., 2015; Zhang et al., 2012). Collectively, the controversy behind cell and tissue specific roles of AHR in cancer remains. The increased detailed knowledge about AHR expression in different tissues and ligand-specific conditions is needed to clarify the functions of AHR and its role in cancer formation and progression.

2.2.6. Therapeutic targeting of AHR

Increasing evidence today points to the fact that AHR and AHR activation by its ligands have an important part in several diseases, including cancers. This has led to the prospect of using AHR as a therapeutic target, which can be modulated by exogenous stimuli. Considering the controversial roles of the AHR in cancer, the main challenge behind this, however, is the selection of the right modulator, taking into consideration the type of cancer, but also the dose of the ligand. Nevertheless, the potential of targeting AHR in cancer treatment has gained immense attention in scientific community (Mulero-Navarro & Fernandez-Salguero, 2016; Paris et al., 2021). Additionally, there is increasing knowledge about the importance of tumour microenvironment (TME) in cancer progression. The presence of different immune cells in TME are considered to have key functions in regulation of the intricate signalling between cancer and immune system. Crucially, the role of AHR in TME has been shown by a number of studies (Liu et al., 2017; Platten et al., 2012; Sadik et al., 2020; Xue et al., 2018).

Several cancers are resistant to chemotherapy and this has demanded the development of new possibilities in cancer treatment. Cancer immunotherapy is now widely considered one of the most promising treatment opportunities. Survival of cancer cells is often mediated by checkpoint immunomodulation, resulting in disrupted balance between immune surveillance and cancer cell proliferation (Alsaab et al., 2017). Checkpoint inhibitors, such as those preventing the interaction between programmed cell death 1 (PD-1) and programmed cell death

ligand 1 (PD-L1), are a novel class of inhibitors and are now being widely used in medicine.

PD-1 is highly upregulated in T-cells in the tumour microenvironment (Mahoney et al., 2015). PD-L1 binding to PD-1 results in the dampening of T-cell activity, culminating in an exhausted phenotype and decreased proliferation of these immune cells (Alsaab et al., 2017). Despite PD-L1 being expressed in multiple non-cancer tissues or cells to control the degree of inflammation, cancer cells have hijacked this mechanism and upregulate PD-L1 expression to escape immune surveillance. The role of AHR in this process has been shown in liver cancer cells (Wang et al., 2017). Moreover, a recent study demonstrated that kynurenine was secreted from melanoma cancer cells to cytotoxic (CD8+) T-lymphocytes in TME via SLC7A8 and PAT4 transporters. This resulted in AHR binding to *PDCD1* gene (encoding for PD-1 protein) promoter and up-regulation of its expression (Liu et al., 2018). Although the use of checkpoint inhibitors has demonstrated enormous success, the positive outcome from the use of these inhibitors is limited to a subset of cancers, referring to the necessity of additional treatment options. Nowadays, inhibitors of IDO and TDO, enzymes responsible for the production of kynurenine, as well as AHR inhibitors combined with the use of immune checkpoint inhibitors are in the highlight of cancer immune therapies, showing huge potential in targeted treatment (Sadik et al., 2020).

2.2.7. AHR in stem cells

Nowadays, regenerative therapies by stem cell transplantation are being used in disease treatment. Therefore, there is a need to study the role and expression of AHR in stem cells. Previous studies have aimed to use AHR as a target in modulating different properties of adult stem cells. It was shown that StemRegenin1, an antagonist of AHR, increased the expansion and proliferative capacity of hematopoietic stem cells (HSCs) (Boitano et al., 2010). HSC self-renewal has been shown to be influenced by AHR modulation in another study. This study demonstrated the attenuating effect of RNA-binding protein Musashi-2 (MSI2) on AHR signalling through post-transcriptional regulation of canonical AHR signalling pathway components (Rentas et al., 2016).

The relevance of AHR in stem cells has also been investigated using known AHR ligands. In mouse, it has been established that embryonic stem cell differentiation into cardiomyocytes is disrupted by TCDD (Neri et al., 2011; Wang et al., 2016). In addition, human embryonic stem cell (hESC) differentiation into cardiomyocytes was recently shown to be impaired by TCDD (Fu et al., 2019). Studies aiming to characterise the role of AHR during neural differentiation of stem cells have revealed the suppressive effect of TCDD on neural precursor cell proliferation in mouse (Latchney et al., 2011). During hESC differentiation, TCDD treatment was shown to increase neural rosette formation and the number of cells positive for tyrosine-hydroxylase (TH), an enzyme involved in dopamine synthesis. The result of this study indicated that AHR activation in stem cells could

influence pluripotent stem cell differentiation (Sarma et al., 2019). In addition, TCDD has been shown to affect endodermal differentiation, by impairing hESC differentiation into pancreatic lineage. TCDD induced hypermethylation of *PRKAG1* in these cells, an important regulator of insulin secretion (Kubi et al., 2019).

Apart from having an effect on differentiation of stem cells, the role of AHR in the maintenance of pluripotency in human embryonic stem cells has been studied. It was shown recently that AHR is a key factor in the maintenance of hESC self-renewal. Importantly, the known endogenous ligand kynurenine was shown to be responsible for this effect (Yamamoto et al., 2019). These data altogether indicate fundamental roles of AHR in stem cells.

2.2.8. AHR in ovarian folliculogenesis

The evidence on the importance of AHR in reproductive system became apparent by studies investigating the effect of TCDD on oestrus cycle and ovulation in rats. It was found that TCDD caused irregularities in cycles, showing features of prolonged oestrus (Li et al., 1995a). Furthermore, it was shown that TCDD caused reduced ovulation rate and number of ovulated oocytes, although exact mechanisms remained elusive (Li et al., 1995a). Additionally, it was established that in female rats, TCDD reduced the ovarian weight gain induced by equine chorionic gonadotropin (eCG) as well as number of animals ovulating (Li et al., 1995b). Moreover, it was found that TCDD alters the level of hormones, as it increased serum oestradiol levels, but decreased peak levels of FSH and LH in gonadotropin-primed animals. A decrease in oestradiol levels, which normally occurs in preovulatory follicles after LH-surge, was diminished in TCDD-treated rats. Additionally, it was shown that TCDD influenced the number of ovulating rats as well as the number of recovered oocytes in hypophysectomised rats. These results suggested TCDD to have a bilateral effect on reproduction by alteration of the normal functioning of HPO axis, but also directly influencing the ovary (Li et al., 1995b). Although the involvement of AHR was not studied, at that time TCDD was known to be the highest-affinity ligand of the aryl hydrocarbon receptor.

Further evidence on the importance of Ahr in reproductive system has become apparent using *Ahr*KO mice. Studies have found these mice to display several reproductive defects, such as slower ovarian follicle growth and reduced ability to ovulate, reduced number of antral follicles and reduced number of corpora lutea (Barnett et al., 2007a; Barnett et al., 2007b; Benedict et al., 2000, 2003). Additionally, disturbed ovarian cyclicity has been reported (Baba et al., 2005). These mice have been reported to have difficulties in maintaining pregnancy. Although *Ahr*KO mice are fertile, they exhibit smaller litter size that WT mice (Abbott et al., 1999; Baba et al., 2005). Previous studies have also demonstrated that *Ahr*KO mice are characterised by decreased ability to produce oestrogen in the ovary. It was shown that Ahr is involved in direct regulation of *Cyp19a1*, a

gene encoding the enzyme aromatase, which is involved in oestradiol production (Baba et al., 2005). Additionally, a recent study demonstrated reduced intra-ovarian *Cyp19a1* expression and oestradiol synthesis in juvenile *Ahr*KO mice (Devillers et al., 2020). The *Ahr*KO mouse model has also revealed that these mice have reduced responsiveness of antral follicles to gonadotropins FSH and LH. This reduction was shown to be caused by reduced number of FSH and LH receptors on follicular granulosa cells (Baba et al., 2005; Barnett et al., 2007a). The importance of Ahr in the ovary has further been suggested by studies showing that Ahr regulates the *Fshr* gene expression by binding to its promoter via E-box (Barnett et al., 2007a; Teino et al., 2012).

2.2.9. Regulation of AHR

Researchers have thoroughly investigated the role of AHR in multiple tissues by modulating its activity using various agonists and antagonists. However, there is less data on how *Ahr* expression is regulated. Thus far, studies investigating the expression of *Ahr* in different tissues and organs have implied that the regulation of *Ahr* is highly dependent on species, tissue and cellular context (Esser et al., 2018).

Studies investigating *Ahr* expression in ovarian granulosa cells have shown that in macaque, *Ahr* is significantly upregulated in response to ovulatory LH-surge (Chaffin et al., 1999). Contrarily, in mature rat granulosa cells, a reduction in *Ahr* mRNA levels has been shown during pro-oestrus, which represents the ovulatory stage in response to LH-surge (Chaffin et al., 2000). Additionally, studies using rat granulosa cells have presented evidence on the downregulation of *Ahr* in response to FSH treatment, although these experiments were done *in vitro* and may not represent relevant physiological conditions (Bussmann & Barañao, 2006).

Previous studies investigating AHR during the initial stages of development in human cells have shown that AHR is expressed at 1-cell stage, downregulated at 2-cell stage, followed by upregulation at later stages (Yamamoto et al., 2019). Contrarily, in mouse, *Ahr* expression is repressed in embryonic stem cells (ESCs), but increases after differentiation of these cells to embryoid bodies (EBs) (Peters & Wiley, 1995; Wu et al., 2002). In mouse ESCs, *Ahr* expression has also been shown to be repressed by pluripotency factors Oct4, Sox2 and Nanog by binding of these proteins to the regulatory regions of *Ahr* gene (Ko et al., 2014). The repression of *Ahr* by pluripotency factors was necessary for the maintenance of mitotic progression and pluripotency (Ko et al., 2016). Differences between species are also illustrated by the data showing that in human embryonic stem cells (hESCs), AHR expression has been detected in several cell lines (Yamamoto et al., 2019). Additionally, AHR was indicated as a key factor responsible for the maintenance of pluripotency and AHR activation by its endogenous ligand kynurenine was considered as a responsible mechanism (Yamamoto et al., 2019). This study also showed downregulation of *AHR* in response to ectodermal

differentiation, further suggesting the role of AHR in maintenance of pluripotency. Taken together, *AHR* regulation in cells with high developmental potential, including ovarian granulosa cells and pluripotent stem cells, seems to be under strict regulation of multiple factors and is dependent on species, tissues, but also the stage of development.

3. AIMS OF THE STUDY

The aims of this dissertation were to investigate the expression of aryl hydrocarbon receptor in cells with high developmental potential, i.e. oocyte supporting mouse granulosa cells and human embryonic stem cells. In particular, this study focused on elucidating the following topics:

1) Investigating the expression as well as the regulation mechanisms of Ahr in murine granulosa cells of preovulatory follicles after the LH-surge.

2) Studying the expression as well as the regulation mechanisms of Ahr in ovarian granulosa cells during the follicle maturation to preovulatory phase prior to LH-surge.

3) Clarifying the expression pattern and role of AHR in pluripotency and differentiation in human embryonic stem cells.

4. RESULTS AND DISCUSSION

4.1. Transcriptional repression of the *Ahr* gene by LHCGR signalling in preovulatory granulosa cells is controlled by chromatin accessibility (Ref. I)

4.1.1. hCG inhibits Ahr protein and mRNA expression in murine GCs

Previous studies have shown that *Ahr* expression changes in granulosa cells (GCs) of preovulatory follicles. An increase in *Ahr* mRNA levels in response to hCG treatment was shown in macaque preovulatory GCs (Chaffin et al., 1999). Contrarily, *Ahr* expression was shown to be repressed in rat GCs at this stage of folliculogenesis (Chaffin et al., 2000). In addition, studies have shown regulation of *Ahr* expression by FSH (Bussmann & Barañao, 2006; Teino et al., 2012). However, there is little data on the mechanisms, how gonadotropin hormones regulate *Ahr* expression. This study aimed to elucidate this matter, focusing on studying Lhcgr-dependent regulation of Ahr in murine preovulatory GCs.

Experiments in the current study were grounded on classical superovulation scheme. This method is based on exogenous hormonal stimulation of sexually immature prepubertal female mice lacking naturally occurring cyclic maturation of ovarian follicles. To study the expression of *Ahr* gene and Ahr protein level in preovulatory GCs, mice were initially primed with 5 IU of PMSG, which is an analogue of FSH. PMSG is widely used for induction of follicular maturation in the ovary. PMSG causes somatic GCs to proliferate and differentiate, which, concurrent with oocyte maturation, leads to formation of dominant follicles ready to ovulate. To induce ovulation, mice were 48 h later injected with 5 IU of hCG, which is an analogue of LH.

To measure the effect of hCG on Ahr protein level in preovulatory GCs, cells were isolated before or 12 h after the injection of ovulatory dose of hCG or vehicle (control), followed by Western blot analysis. The results from three independent experiments showed a 4-fold reduction in Ahr protein level (Fig. 1A in Ref. I). Next, we aimed to determine whether the change in protein level is paralleled with changes in gene expression. For this, mRNA levels in isolated GCs were measured by RT-qPCR before (0 h) or after (48 h) PMSG treatment and after hCG (PMSG 48 h + hCG 12 h) treatment. PMSG treatment for 48h resulted in significant increase of *Ahr* mRNA level and subsequent decrease after 12 h hCG treatment (Fig. 1B in Ref. I). In addition to GCs, lysates were made from whole ovaries with the aim to evaluate whether the change in *Ahr* expression is confined to GCs. Although 48 h PMSG treatment increased *Ahr* mRNA levels, 12 h hCG treatment had no significant effect on downregulation of *Ahr* transcript levels (Fig. 1B. in Ref. I), most likely because of the presence of other types of cells.

To further demonstrate the effect of hCG on *Ahr* repression, PMSG-primed mice were treated with 5 IU hCG or saline (vehicle control) for 12 h. RT-qPCR

analysis of *Ahr* mRNA revealed over 75% reduction in GCs isolated from mice treated with hCG compared to vehicle treated control (Fig. 1C in Ref. I). Considering the aim to get a more detailed understanding on the regulation of *Ahr* in further experiments, an *in vitro* system was set up aiming to emulate conditions present *in vivo*. For this, GCs isolated from PMSG-treated mice were cultured for 12 h with or without the presence of 5 IU/ml hCG. Measurement of *Ahr* mRNA levels showed similar expression pattern, implying that Lhcgr signalling on *Ahr* expression can be successfully studied *in vitro* (Fig. 1C in Ref. I). As with *in vivo* experiments, *Ahr* upregulation was seen after 12 h of GC culture in non-treated (control) conditions (Fig. 1C in Ref. I). The residual effect of increased *Ahr* expression is also apparent in the results of *in vivo* experiments, which were performed to investigate temporal changes in *Ahr* expression during 12 h (Fig. 1D in Ref. I). Specifically, in non-treated mice (PMSG 48 h + NT 12 h) a continuous increase in *Ahr* expression was seen throughout 12 h. Conversely, profound hCG-dependent downregulation of *Ahr* occurs after 8 h when compared to PMSG-treated cells (0 h). Moreover, a significant decrease in *Ahr* mRNA can be observed after 4 h when compared to vehicle treated control (Fig. 1D in Ref. I). Thus, we conclude that the start of *Ahr* repression is set to happen within this time-frame.

4.1.2. hCG-dependent decrease in *Ahr* mRNA levels involves PKA activation but not ongoing protein synthesis

Our next goal was to evaluate possible signalling pathways taking part in hCG-dependent downregulation of *Ahr* expression. Previous studies have shown that Lhcgr signalling results in elevation of intracellular cAMP levels, which in turn activates PKA (Marsh & Savard, 1966). Downstream effects of PKA signalling in GCs have further been demonstrated to include activation of ERK1/2 pathway, but also modification of histones. To study whether PKA pathway participates in hCG-dependent downregulation of *Ahr*, GCs from PMSG-primed mice were cultured and treated with forskolin (Fsk, 10 μM), which is a known PKA activator. Similarly to hCG, 12 h Fsk treatment resulted in nearly 85% downregulation of *Ahr*, suggestive of the involvement of PKA pathway in hCG-dependent repression of *Ahr* (Fig. 2A in Ref. I). Indeed, addition of PKA inhibitor H89 (10 μM) eliminated this effect, when the cells were treated with either hCG or Fsk, further demonstrating that PKA activation is necessary in suppressing *Ahr* expression by hCG (Fig. 2A in Ref. I). The involvement of ERK1/2 signalling in this process was evaluated by adding ERK1/2 inhibitor U0126 (10 μM) to the culture medium of hCG or Fsk treated GCs. hCG and Fsk repressed *Ahr* expression in the presence of ERK1/2 inhibitor, indicating that this pathway is not required for *Ahr* repression (Fig. 2A in Ref. I).

To gain further insight into the mechanisms participating in *Ahr* regulation by hCG, we investigated if *Ahr* repression requires new protein synthesis. For this, GCs were treated for 1 h with cycloheximide (CHX, 10 μg/ml), which inhibits translation in cells. After this, either hCG or Fsk was added to the medium. CHX

treatment without additional stimulus resulted in 5-fold increase of *Ahr* transcript levels (in Fig. 2B in Ref. I). This suggests that new protein synthesis is required for the repression of *Ahr* at the basal level in preovulatory GCs. However, inhibition of translation did not abolish the repressive effect of hCG or Fsk on *Ahr* mRNA levels, implying that hCG-dependent downregulation of *Ahr* by PKA is likely to result from a more direct signalling cascade, which does not require ongoing protein synthesis (Fig. 2B in Ref. I).

4.1.3. The expression of *Ahr* is controlled by transcription rate but not mRNA stability

The abundance of mRNA in cells can be controlled transcriptionally by rate of transcription or post-transcriptionally by mRNA degradation. Considering these two levels of gene regulation mechanisms, the next aim was to clarify, how the amount of *Ahr* mRNA is controlled in preovulatory GCs in response to hCG. To measure transcriptional activity of *Ahr*, we quantified *Ahr* hnRNA levels by RT-qPCR. Being a predecessor of mRNA, hnRNA is unspliced and due to its very short half-life the amount of hnRNA in cells can be considered a good indicator of transcriptional activity of a gene (Bentley, 1999; Elferink & Reiners, 1996). To measure *Ahr* hnRNA levels, we used primers covering the boundaries of exon 2 and intron 2 (Fig. 3A in Ref. I). The results of *in vivo* experiments showed a significant 40% reduction in *Ahr* hnRNA levels after 4 h of hCG vs vehicle treatment and nearly 70% reduction at 12 h (Fig. 3B in Ref. I). A decrease in hnRNA level is a clear indicator of reduced transcriptional activity. To confirm this, we performed experiments investigating the effect of hCG on *Ahr* mRNA stability in the presence of actinomycin D (ActD, 1 μg/ml), which blocks transcription in cells. For this, GCs isolated from PMSG-primed mice were pre-incubated with or without 5 IU/ml hCG for 4 h *in vitro* and *Ahr* mRNA levels were analysed by RT-qPCR at 0, 1.5, 3 and 4.5 h after addition of ActD. There were no significant differences in the degradation rate of *Ahr* mRNA between hCG and vehicle treated GCs (Fig. 3C in Ref. I). These results verify that hCG-dependent repression of *Ahr* gene in preovulatory GCs is an outcome of decreased transcriptional activity.

Studies using reporter gene assays have shown that *Ahr* expression can be controlled by various cis-elements or trans-factors (FitzGerald et al., 1996; Garrison & Denison, 2000). Thus, we aimed to measure, if hCG has a repressive effect on *Ahr* promoter activity. We transfected GCs with a vector containing *Ahr* promoter region from –1425 to +367 bp relative to transcription start site (TSS). After transfection, GCs were incubated for 9 h before adding 5 IU/ml hCG or vehicle to the medium followed by reporter gene activity analysis 12 h later. After normalisation to β-galactosidase transfection control, no effect of hCG was seen on *Ahr* promoter activity, when compared to vehicle treated cells (Fig. 3D in Ref. I). Simultaneously, *Ahr* mRNA levels were measured to evaluate if the unseen effect may have been caused by the long pre-incubation period (9 h). The

repressive effect of hCG on endogenous expression of *Ahr* was still present (data not shown).

However, transiently transfected reporter genes, as opposed to endogenous genes, are not necessarily assembled into similar structure of chromatin, the latter of which may be crucial for gene regulation (Hebbar & Archer, 2008; Smith & Hager, 1997). To address this issue, we evaluated if chromatin remodelling may have a role in *Ahr* regulation. Chromatin proteins histones can be modified in various ways to alter the state of chromatin and thus the accessibility of regulatory factors influencing transcription. Common histone modifications – acetylation and deacetylation – are descriptive of active and inactive regions in genome, respectively. To investigate if repression of *Ahr* expression by hCG is influenced by chromatin remodelling, we used a selective HDAC inhibitor Trichostatin A (TSA, 100 ng/ml). GCs from PMSG-primed mice were cultured and pre-treated with TSA for 1 h. Then, hCG or Fsk was added to the medium for 12 h, followed by RT-qPCR analysis. Results of this experiment showed that the repressive effect of hCG or Fsk was abolished in the presence of TSA (Fig. 3E in Ref. I), confirming that chromatin remodelling events are necessary for *Ahr* down-regulation.

4.1.4. hCG-dependent repression of Ahr transcription is caused by chromatin remodelling

Our next aim was to confirm in a more detailed manner if hCG-dependent down-regulation of *Ahr* is a result of reduced chromatin accessibility by using the *in vivo* chromatin accessibility assay (Chromatin Accessibility by Real-Time PCR, CHART-PCR). Nuclei were isolated from hCG or vehicle treated GCs and subjected to DNase I digestion. The accessibility of chromatin was indirectly evaluated by measuring the level of recovered DNA by qPCR in regions of interest, in this case *Ahr* promoter region from –176 to –77 bp and *Ahr* intron region +5859 to +5929 bp from TSS (Fig. 4A in Ref. I). The results, showing fold change of recovered DNA from GCs of hCG-treated over GCs of vehicle treated mice, demonstrate a significant increase (4-fold and 7-fold) in qPCR amplified DNA from *Ahr* promoter region after 10 or 20 U of DNase I digestion relative to undigested nuclei (Fig. 4B in Ref. I). Furthermore, the amount of recovered DNA in *Ahr* intron region +5859 to +5929 bp was not altered by hCG treatment compared to vehicle controls (Fig. 4B in Ref. I). Together, these results show that Lhcgr-dependent downregulation of *Ahr* expression occurs via decreased chromatin accessibility at the promoter region of *Ahr* gene.

Collectively, this study showed that *Ahr* expression in murine preovulatory GCs is repressed by Lhcgr signalling. Repression of *Ahr* was shown to be dependent on PKA activation, whereas ERK signalling is not involved. Additional elucidation of regulation mechanisms revealed that Lhcgr-dependent *Ahr* repression occurs by decreased transcriptional activity, not by mRNA degradation. Several studies have shown that rapid epigenetic changes take place in ovarian

cells during folliculogenesis (Christenson et al., 2001; LaVoie, 2005). Several genes important for folliculogenesis have been shown to be regulated by epigenetic mechanisms, e.g. histone modifications resulting in changed chromatin structure (Nimz et al., 2010). In this study, we showed that Lhcgr-dependent *Ahr* repression is diminished in the presence of histone deacetylase inhibitor, suggesting that epigenetic mechanisms are involved. Additionally, this study demonstrated decreased chromatin accessibility at *Ahr* promoter in response to hCG.

4.2. FSH/LH-dependent upregulation of Ahr in murine granulosa cells is controlled by PKA signalling and involves epigenetic regulation (Ref. II)

4.2.1. The upregulation of Ahr in GCs during follicular maturation requires both FSH and LH activity

It has been showed that *Ahr* mRNA levels increase during follicle maturation in adult cycling rats (Chaffin et al., 2000). Previous studies have also reported *Ahr* upregulation in response to PMSG (Baba et al., 2005; Teino et al., 2014). This hormone has an intrinsic FSH activity, but also a residual capacity to activate LH receptors (Combarnous et al., 1984). To study *Ahr* expression in murine ovarian GCs during follicle maturation, mice were injected once with 5 IU of PMSG, 5 IU of FSH or vehicle. GC isolation and Western blot analysis were performed 48 h later to measure the amount of Ahr protein. Data from three independent experiments showed an average of 6-fold increase in Ahr protein level after PMSG treatment (Fig. 1A and B in Ref. II). FSH alone had no effect on the abundance of Ahr protein (Fig. 1A and B in Ref. II). Similar results were obtained in *Ahr* mRNA analysis from PMSG-treated GCs, although to a smaller extent (3.5-fold) (Fig. 1C in Ref. II). Notably, the extent of the increase is comparable to those reported by Chaffin et al. (2000) in cycling rats. Interestingly, a minor, but statistically significant upregulation of *Ahr* mRNA was measured in response to FSH treatment (Fig. 1C in Ref. II). Treatment of mice with 5 IU of LH or 5 IU of hCG alone had no effect on *Ahr* expression (Suppl. Fig. 1A in Ref. II).

The difference in FSH action on the change of Ahr protein and mRNA level may be caused by the short half-life of FSH compared to PMSG. On the other hand, additional LH activity may be required to elevate Ahr protein level. To clarify this issue, mice were injected four times every 12 h either with 1.5 IU FSH or with a combination of 1.5 IU FSH and 1.25 IU LH. GCs were isolated 48 h after the initial injection. Western blot analysis of Ahr protein showed that FSH alone is insufficient to increase Ahr protein (Fig. 1D and E in Ref. II). However, its level was significantly increased, when mice received a combined treatment of FSH and LH (Fig. 1D and E in Ref. II). These data demonstrate that both gonadotropins are necessary to elevate Ahr protein in ovarian GCs and corroborate that the previously seen positive action of PMSG on Ahr likely

comprises LH-dependent signalling. Additionally, measurement of *Ahr* mRNA levels in these cells confirmed the necessity of both FSH and LH to upregulate *Ahr* expression (Fig. 1F in Ref. II). FSH treatment alone, following this experimental protocol, again elevated *Ahr* mRNA level while having no effect on Ahr protein. In addition, *Ahr* hnRNA levels – a good surrogate of transcriptional activity due to its short half-life – were measured, and similar results to mRNA were seen (Suppl. Fig. 1B in Ref. II). To demonstrate the efficiency of hormonal stimulation, we also measured the expression of follicle maturation marker genes, which were upregulated in response to combined (FSH + LH) treatment (Suppl. Fig. 1C in Ref. II).

Our data show that both gonadotropins are crucially important in elevating Ahr levels. On the other hand, neither of the gonadotropins alone were able to increase Ahr. While FSH is an initiator of follicle maturation, the inability to augment Ahr levels could be explained by its incapacity to induce proper follicular maturation. Indeed, it has been shown that FSH treatment alone results in a smaller number of antral follicles compared to FSH + LH or PMSG treatment (Ruman et al., 2005). The lack of proper maturation was further reflected by our results showing no changes in follicle maturation marker genes' expression in response to FSH treatment (Suppl. Fig. 1C in Ref. II), again emphasizing the importance of additional LH signalling. Although LH has a fundamental role in follicle maturation, it is distinct from FSH and its importance becomes more predominant in the second stage of follicle maturation, as the expression of LH receptors starts to occur in GCs of antral follicles (Menon et al., 2018). Therefore, the action of FSH on *Ahr* transcription, but not on protein level, may be a simultaneous result of improper follicle maturation and lack of sufficient LH signalling.

Alternatively, the possibility of post-transcriptional regulation of *Ahr* transcript cannot be ruled out. Recent studies have suggested that LH may control the expression of a large set of genes by regulating their protein levels post-transcriptionally (Bahrami et al., 2017; Khan et al., 2015; Li et al., 2015). LH holding a cohort of genes at steady-state levels by miRNAs has also been shown in GCs (Carletti & Christenson, 2009). Thus, it is a promising idea that the expression of *Ahr* may also be allocated to LH-dependent post-transcriptional control, e.g. via regulating *Ahr*-targeting miRNA(s). This, however, needs to be confirmed in further studies. Collectively, these data show that FSH and LH are both required for the upregulation of Ahr in ovarian GCs during follicle maturation, but also suggest there may be post-transcriptional regulatory processes involved, most likely dependent on LH action.

4.2.2. The effect of PMSG on the expression dynamics of Ahr and follicle maturation marker genes

To start gaining insight into the mechanistic side of Ahr regulation, we first aimed to investigate the temporal pattern of Ahr expression in response to PMSG. For this, mice were injected with 5 IU of PMSG or vehicle (NT) and GCs were

isolated before (0h) or every 12 h up to 48 h post-injection. Ahr protein levels were analysed by Western blot. The results showed that compared to vehicle treated GCs, Ahr levels are significantly increased 24 h after hormone injection (1.6-fold) and increased continuously up to five times 48 h later (Fig. 2A and B in Ref. II). Analysis of *Ahr* transcript levels followed this pattern, although statistically significant upregulation was detected 36 h after injection (Fig. 2C in Ref. II). The temporal pattern herein is also concordant with formerly suggested notion that the rise in Ahr levels requires LH signalling, which is predominant in the second stage of follicle maturation.

Follicle maturation and GC differentiation is accompanied by an increase in several marker genes, most notably *Fshr*, *Cyp19a1* and *Lhcgr* (Chakraborty & Roy, 2015; Findlay & Drummond, 1999; Lapolt et al., 1992; Peng et al., 1991; Richards, 1994; Richards et al., 1995; Sites et al., 1994). To validate the efficiency of hormonal stimulation, we measured the expression of these three marker genes in GCs isolated at aforementioned time points. The results show the upregulation of all three genes compared to NT control (Fig. 2D, E and F in Ref. II). PMSG increased *Fshr* mRNA 5.3-fold at 24 h reaching to 7.3-fold increase 48 h after injection (Fig. 2D in Ref. II). *Cyp19a1* mRNA levels were elevated 20-fold already after 12 h and culminated to a 40-fold increase at 48 h (Fig. 2E in Ref. II), whereas *Lhcgr* expression was increased 25-fold after 12 h and 95-fold at 48 h after PMSG (Fig. 2F in Ref. II). Maximal points in the expression of the latter two were detected at 36 h – 50-fold and 130-fold increase for *Cyp19a1* and *Lhcgr*, respectively. No changes in non-treated controls were detected.

Studies have shown that Ahr may participate in oestradiol production in the ovary by regulating the expression of aromatase – a product of *Cyp19a1* gene (Baba et al., 2005). In addition, *Fshr* has been reported to be regulated directly by Ahr in the ovary (Barnett et al., 2007a; Teino et al., 2012). Considering this, another focus of determining the dynamic pattern of the expression of these selected genes was the comparison between Ahr and its possible target genes in ovarian GCs. However, the increase in Ahr expression occurred relatively late when compared to *Fshr* and *Cyp19a1* (Fig. 2A-E in Ref. II). There is a possibility that relatively high dose of PMSG is sufficient to upregulate these two genes via different routes and diminishes the importance of Ahr. Still, since *Fshr* and *Cyp19a1* are crucial for follicle development, the importance of Ahr in regulation of *Fshr* and *Cyp19a1* at basal level cannot be excluded.

4.2.3. Ahr is upregulated in large antral follicles in response to PMSG

To analyse Ahr spatial distribution in ovarian follicles, ovaries were collected from 5IU PMSG or non-treated (NT) mice and cryosections were subjected to immunofluorescence analysis. Results indicated that Ahr, to a small extent, seems to be present in follicles of all sizes (Fig. 3A in Ref. II). PMSG treatment results in ovarian maturation and is illustrated by the presence of large antral follicles (Fig. 3B in Ref. II). Furthermore, Ahr was highly expressed in these follicles,

particularly in mural GCs, whereas cumulus GCs seemed to lack Ahr protein (Fig. 3B in Ref. II). This finding is supported by a study showing higher *Ahr* mRNA levels in mural GCs compared to cumulus cells (Wigglesworth et al., 2015). Moreover, this correlation was also evident in the case of *Lhcgr* mRNA, which supports our previous suggestion of the importance of Lhcgr signalling on Ahr expression.

Collectively, these results show that Ahr is upregulated in large antral follicles. Additionally, it was demonstrated that PMSG induces follicular maturation in our experiments, as evidenced by the previously seen increased maturation marker gene expression (Fig. 2D in Ref. II) and formation of antral follicles (Fig. 3B in Ref. II).

4.2.4. Ahr is downregulated by protein kinase A signalling pathway

Ovarian follicle maturation to preovulatory stage requires FSH signalling (McGee & Hsueh, 2000). It is known that most of the actions of FSH are mediated by elevation of intracellular cAMP levels, which activates PKA (Puri et al., 2016; Salvador et al., 2001). The next aim of this study was to investigate, if and how is PKA signalling involved in *Ahr* expression regulation. Follicle maturation was induced by injecting mice with 5 IU PMSG and GCs were isolated 24 h later, since this is the time when Ahr upregulation begins, as demonstrated earlier (Fig. 2A, B and C in Ref. II). GCs were then cultured *in vitro*. To monitor the activity of PKA we measured the levels of phosphorylated cAMP response element (p-CREB). CREB is a well-known target protein of PKA (Gonzalez-Robayna et al., 1999; Mukherjee et al., 1996). After 4 h cell culture, Western blot analysis showed a profound decrease (up to 83%) in the amount of p-CREB, indicative of diminished PKA activity. Simultaneously, an increase in the amount of Ahr protein (3.9-fold increase) was seen, compared to 0 h (Fig. 4A and B in Ref. II). This suggests that PKA activity may be necessary to repress Ahr due to the inverse correlation between the two. Indeed, treatment of GCs with 10 μM Fsk – a compound that activates adenylyl cyclase and thus activates PKA signalling pathway – resulted in a significant decrease in Ahr protein level (38%), while p-CREB levels were measured to be 2.8-fold higher (Fig. 4A and B in Ref. II).

The decline in p-CREB levels (and PKA activity), which was observed after 4 h cell culture, may be caused by the loss of gonadotropin stimulus present in *in vivo* conditions. To test this, PKA activity was further monitored by measuring the expression of *Fshr*, *Cyp19a1* and *Lhcgr*. The expression of these genes have been shown to be dependent on PKA signalling (Hunzicker-Dunn & Maizels, 2006; Minegishi et al., 1997). RT-qPCR analysis of mRNA levels of these genes showed that rapid downregulation occurs after GC cell culture, suggestive of the diminished PKA activity (Suppl. Fig. 3A, B and C in Ref. II). Forskolin, on the other hand, reversed this effect by elevating the expression of these genes at 12 h and 24 h time points (Suppl. Fig. 3 A, B and C in Ref. II).

The effect of forskolin on *Ahr* repression was also evident after measuring *Ahr* mRNA levels, which decreased by 44% after 2 h followed by 58% decrease after 4 h Fsk treatment (Fig. 4D in Ref. II). To verify that PKA signalling was responsible for the decrease, GCs were treated with known PKA inhibitor H89 in combination with Fsk or vehicle (NT). Addition of H89 abolished the repressive effect of Fsk on *Ahr*, confirming that PKA signalling is required in *Ahr* down-regulation. Moreover, after further cell culture for up to 24 h we saw a consistent increase in *Ahr* mRNA (Suppl. Fig. 4A in Ref. II) and also *Ahr* hnRNA (Suppl. Fig. 4B in Ref. II), concordant with the previously made assumption of the loss of PKA activity in cell culture without external stimuli.

To assess PKA activity in ovarian GCs during follicle maturation *in vivo*, mice were primed with 5 IU PMSG or vehicle (NT) and GCs were isolated before (0h) or every 12 h during 48 of treatment. Western blot analysis was performed to measure p-CREB levels. The results showed the presence of p-CREB in non-treated cells, indicating basal levels of PKA activity in GCs (Fig. 4E and F in Ref. II). When mice received PMSG, decrease in p-CREB levels was observed 24 h after the injection (Fig. 4E and F in Ref. II), similarly to the results shown previously (Maizels et al., 2001). Although this effect was reversed at 36 h and 48 h, the opposite relationship between Ahr and p-CREB levels in the mid-phase of ovarian follicle maturation process suggests that decrease in PKA activity is required for elevating Ahr in the ovary.

4.2.5. The increased transcription of *Ahr* in response to PMSG is regulated by chromatin accessibility

Our next aim was to elucidate, whether *Ahr* transcript levels were augmented by increased transcriptional activity of the *Ahr* gene or was the increase controlled by changes in mRNA stability. To study the PMSG effect on the rate of transcription, we initially measured *Ahr* hnRNA levels in GCs of PMSG and vehicle treated mice. Cells were isolated every 12 h at various time points (0 to 48 h during gonadotropin treatment). RT-qPCR measurements show that PMSG induced a 4.7-fold upregulation of *Ahr* hnRNA at 36 h and 5.6-fold increase at 48 h after the injection, respectively (Fig. 5A in Ref. II). To evaluate the influence of PMSG on *Ahr* mRNA stability, GCs from PMSG-primed mice (48 h) were cultured and the effect of PMSG on *Ahr* mRNA levels were measured with or without the presence of the transcription inhibitor actinomycin D (ActD). Cells were collected at this point in time due to abundance of Ahr protein, mRNA and hnRNA levels, the latter being indicative on active transcription. *Ahr* mRNA levels increased in cell culture 4 h after the addition of 5 IU/ml PMSG to the medium, but were significantly reduced after abolishing transcription with ActD (Fig. 5B in Ref. II). Additionally, there were no differences in *Ahr* transcript levels between NT and PMSG-treated cells in the presence of ActD (Fig. 5B in Ref. II), showing that PMSG does not augment *Ahr* mRNA stability. Together,

these data show that PMSG-dependent Ahr upregulation in ovarian GCs is regulated via increased mRNA synthesis.

It has been reported that activation of genes by FSH signalling involves multiple cis-elements and trans-acting factors (Fitzgerald et al., 1998; Richards, 1994). In addition, *Ahr* promoter contains several regulatory elements that can be targeted by gonadotropin signalling (Fitzgerald et al., 1998; Garrison & Denison, 2000). To study if PMSG influences *Ahr* promoter activity, GCs from immature mice were cultured and transfected with a reporter vector containing *Ahr* promoter. The 1792 bp reporter vector (–1425 to +367 bp relative to TSS) was constructed previously (Teino et al., 2014). This sequence has been reported to contain all the necessary elements for constitutive promoter activity (Fitzgerald et al., 1998; Garrison & Denison, 2000). The effect of PMSG on luciferase activity was measured 48 later. Although promoter activity was detected in non-treated cells, no additional effect was observed in response to PMSG treatment (Fig. 5C in Ref. II). The undetected effect might be explained by the now widely accepted view that regulatory elements of a gene can be located far from TSS, but can also be explained by the presence of other regulatory mechanisms in PMSG-dependent upregulation of *Ahr*, e.g. chromatin remodelling.

Several studies have shown that the *Ahr* gene expression can be controlled epigenetically (Englert et al., 2012; Garrison et al., 2000; Zhang et al., 1996). We showed previously that in murine preovulatory GCs *Ahr* is downregulated by condensation at its promoter region in response to hCG (Teino et al., 2014). Considering the bivalent characteristic of *Ahr* promoter, as illustrated previously, our goal was to investigate if the upregulation of *Ahr* during follicle maturation (before the LH-surge) is controlled by changes in chromatin structure (Ko et al., 2014). For this, immature mice were treated with 5 IU of PMSG or vehicle for 48 h, after which GCs were isolated and subjected to CHART-PCR analysis. The state of chromatin structure was assessed by analysing DNase I accessibility to the region –176 to –77 bp of *Ahr* promoter. CHART-PCR results indicated that PMSG increased the accessibility of DNA at *Ahr* promoter (Fig. 5D in Ref. II). For positive control of this experiment, we used *Cyp19a1* due to the evidence that this gene is downregulated by chromatin remodelling in preovulatory GCs in response to hCG and due to our experimental data showing *Cyp19a1* to be upregulated during follicle maturation (Fig. 2E in Ref. II) (Lee et al., 2013). We demonstrated increased accessibility of DNase I to the promoter region of *Cyp19a1* (Fig. 5D in Ref. II). No effect of PMSG on DNase I accessibility to the promoter region of *Pax7*, used as negative control, was detected compared to non-treated control (Suppl. Fig. 5 in Ref. II).

A couple of studies have shown that FSH can modify the structure of chromatin through direct phosphorylation of histones by PKA. There are studies showing this process to cause a reduction in chromatin condensation, but also that PKA activity can be necessary to keep the chromatin in a closed state (Collas et al., 1999; DeManno et al., 1999; Salvador et al., 2001). Although FSH and LH exert their effect largely via PKA pathway, it is also established that low and high levels of PKA activity can have differential effects on target gene expression in granulosa

cells (Gonzalez-Robayna et al., 1999; Richards et al., 1995). Considering the temporal decrease in PKA activity in mid-phase follicle maturation (Fig. 4E and F in Ref. II), coupled with *Ahr* upregulation, it is reasonable to suggest that PKA may modulate *Ahr* expression through epigenetic changes this way. The exact mechanisms, however, remain unknown. Considering the repressive effect of PKA on *Ahr* expression in preovulatory follicles and during the initial phase of follicle maturation, it is an interesting subject for further studies to explain, how Ahr is upregulated in large antral follicles in the presence of PKA activity (Teino et al., 2014).

4.3. Impact of AHR ligand TCDD on human embryonic stem cells and early differentiation (Ref. III)

Recent studies have underscored the important role of AHR in cells with high developmental potential. Numerous studies have shown that modulating AHR activity by various agonists or antagonists can have profound effects on multiple biological processes, including cell proliferation and differentiation. Inhibition of AHR activity by StemRegenin 1 has been shown to induce the proliferation and expansion of hematopoietic stem cells (HSCs) (Boitano et al., 2010). In addition to adult stem cells, emerging evidence has also implied AHR to interfere with differentiation of embryonic stem cells (ESCs). It was recently shown that modulating AHR activity by TCDD profoundly impacted ESC differentiation into cardiomyocytes (Fu et al., 2019; Wang et al., 2016). The impact of TCDD has also been shown to affect neural and endodermal differentiation of ESCs (Kubi et al., 2019; Sarma et al., 2019). Several studies have determined that AHR is expressed in stem cells, although its expression level is dependent on developmental stage of the cells. Additionally, differences in mouse and human ESCs have been reported (Peters & Wiley, 1995; Wu et al., 2002; Yamamoto et al., 2019). Still, there is little data on AHR expression and functions in human ESCs and during the initial stages of hESC differentiation. Considering the future perspectives in regenerative therapies and the possibilities of using AHR as a therapeutic target, it is of interest to characterise AHR expression and study the role of this protein in pluripotent stem cells and during early differentiation.

4.3.1. AHR is downregulated during non-directed differentiation into embryoid bodies (EBs)

In order to gain first insight into the role of AHR during early differentiation, we studied its expression during hESC differentiation into embryoid bodies (EBs). Mimicking *in vivo* conditions, EBs resemble 3D structures containing cells from all three lineages. Previously, Ahr upregulation in mouse ESC differentiation into EBs has been shown (Ko et al., 2014). We aimed to investigate, how AHR is regulated in human ESCs.

Western blot analysis of AHR protein showed a significant decrease (77%) after 5 days of hESC differentiation into EBs (Fig. 1A in Ref. III). Downregulation was further confirmed by RT-qPCR analysis of *AHR* mRNA, which decreased by 66% in EBs (Fig. 1B in Ref. III). In addition to H9 hESC line, similar reduction in mRNA was determined in H1 cell line (Fig. 1B in Ref. III). To confirm the differentiation of hESCs, we analysed the expression level of core pluripotency marker genes – *OCT4*, *SOX2* and *NANOG*. Significant reduction in the expression of *OCT4* and *NANOG* validate the differentiation (Fig. 1C in Ref. III). The expression of *SOX2* remained unchanged, indicating that neural direction is the preferred lineage of spontaneous differentiation, as reported before (Fig. 1C in Ref. III) (Fong et al., 2008). Taken together, our data reveal that when studying AHR expression in stem cells, the species-dependent differences must be taken into account. In addition, a similar regulation in different hESC lines suggests a broader biological, rather than single cell line-specific role of this protein in the early stages of pluripotent stem cell differentiation.

4.3.2. AHR expression shows distinct patterns during directed differentiation into three lineages

Our next goal was to characterise *AHR* expression in a more detailed manner. Specifically, we aimed to determine the temporal pattern of *AHR* transcription during directed differentiation into all three lineages. H9 hESCs were differentiated into neural progenitors, definitive endoderm and early mesoderm cells using commercial differentiation kits. Accordingly, neural differentiation was carried out for 7 days, whereas endodermal and mesodermal differentiation was performed for 5 days. In neural progenitors, Western blot analysis showed a significant decrease (45%) in AHR protein levels after three days of differentiation (Fig. 2A in Ref. III). Starting from day 5, AHR levels showed an increasing trend, reaching to significantly higher levels at day 7, compared to day 4 (4.13-fold increase) or hESCs (2.27-fold increase) (Fig. 2A in Ref. III). Analysis of *AHR* mRNA levels revealed a similar pattern with minor differences. Particularly, AHR downregulation was seen already after 2 days of differentiation and remained low until day 5. Compared to day 5, higher AHR transcript levels were measured at day 6 and 7. However, when compared to hESCs, no statistically significant increase was detected (Fig. 2B in Ref. III). Similar expression pattern in hESCs after neural differentiation has been reported previously in a study, which showed that AHR levels decreased after 3 and 6 days of differentiation (Yamamoto et al., 2019). This study proposed that AHR and its endogenous ligand kynurenine are necessary to maintain the hESCs in a state of self-renewal.

For validation of differentiation, we measured the expression of pluripotency- and lineage-specific marker genes. Reduction of *OCT4* and *NANOG* mRNA was seen from day 1 (Fig. 2C in Ref. III). Additionally, *SOX2* levels remained unchanged, indicative of neural differentiation (Fig. 2C in Ref. III). Neural differentiation was further confirmed by measured increase in *PAX6* and *OTX2*

mRNA levels (Suppl. Fig. 1A and B in Ref. III). Differentiation of hESCs into definitive endoderm resulted in downregulation of AHR protein from day 3 and remained at low levels (20% of the initial level) at day 5 (Fig. 2D in Ref. III). RT-qPCR analysis of *AHR* mRNA showed comparable results, although a significant decrease was detected from day 2 (Fig. 2E in Ref. III). Differentiation was confirmed by downregulation of pluripotency marker genes *OCT4*, *SOX2* and *NANOG* (Fig. 2F in Ref. III) and upregulation of endodermal marker genes *SOX17* and *GATA4* (Suppl. Fig. 1C and D in Ref. III). AHR protein levels also decreased during mesodermal differentiation, starting from day 2 (41% decrease) (Fig. 2G in Ref. III). At day 5, AHR protein was essentially undetectable (Fig. 2G in Ref. III). Notably, from day 2, a second molecular weight band was detected by Western blot analysis. This may be the result of the used AHR antibody recognising an unknown protein. On the other hand, the inverse correlation between the AHR protein could indicate post-translational cleavage. *AHR* mRNA levels were downregulated by 49% at day 2, followed by nearly undetectable levels from day 3 (Fig. 2H in Ref. III). Pluripotency marker genes were down-regulated upon mesodermal differentiation (Fig. 2I in Ref. III) with concurrent upregulation of mesoderm-specific marker genes *T* and *HAND1* (Suppl. Fig. 1E and F in Ref. III). Overall, the downregulation of AHR after hESC differentiation is supportive of its role in pluripotency maintenance. Furthermore, distinct temporal pattern between different lineages indicates its lineage-specific roles.

4.3.3. TCDD does not affect pluripotency- or differentiation-specific marker gene expression

Considering the possibility of AHR in the maintenance of stem cell self-renewal, our next goal was to study the effect of TCDD, the most potent AHR agonist, on pluripotency of hESCs. Firstly, flow cytometry analysis was performed to determine the proportion of $OCT4^{+}SOX2^{+}NANOG^{+}$ cells in non-treated, DMSO or 10 nM TCDD-treated cells after 3 days. No effect of TCDD was seen, compared to non-treated (NT) or DMSO-treated cells (Suppl. Fig. 2A in Ref. III). TCDD did not alter the expression of *OCT4*, *SOX2* and *NANOG* compared to NT or DMSO-treated cells, suggesting no effect on pluripotency (Suppl. Fig. 2B in Ref. III). AHR activation and functionality of AHR pathway was validated by measuring the expression of well-known AHR target gene *CYP1A1*, which was upregulated 7-fold upon TCDD treatment (Suppl. Fig. 2C in Ref. III). Taken together, our results show that TCDD has no influence on hESC pluripotency.

Since TCDD-dependent derailment of differentiation in various cell types with high developmental potential has been reported, we aimed to study if TCDD has an effect on early differentiation of hESCs. For this, we analysed the effect of TCDD on lineage-specific marker genes in 10 nM TCDD or DMSO-treated cells. Previous studies have shown that the effects elicited by TCDD on early differentiation depend on the stage of treatment (Fu et al., 2019; Sarma et al., 2019). Particularly, pre-treatment of pluripotent cells before differentiation has

been shown to have the most profound influence (Fu et al., 2019). Accordingly, in this study hESCs were pre-treated with DMSO or 10 nM TCDD for 3 days prior to differentiation with commercial media containing DMSO or 10 nM TCDD.

Neural differentiation resulted in upregulation of lineage-specific marker genes *PAX6* and *OTX2* in the presence of DMSO (Fig. 3A and B in Ref III). However, TCDD did not alter the expression of these genes. Additionally, no effect of TCDD was observed on mRNA levels of *AHR*, *OCT4*, *SOX2* and *NANOG* (Suppl. Fig. 3A, B, C and D in Ref. III). TCDD upregulated *CYP1A1* expression in these cells, indicative of active AHR pathway (Suppl. Fig. 3E in Ref. III). Endodermal differentiation in the presence of DMSO resulted in upregulation of endodermal marker genes *SOX17* and *GATA4* (Fig. 3C and D in Ref. III). The expression of these genes was not influenced by TCDD (Fig. 3C and D in Ref. III). The lack of TCDD effect was also seen on the expression of *AHR*, *OCT4*, *SOX2* and *NANOG* in these cells (Suppl. Fig. 4A, B, C and D in Ref. III). In early mesoderm cells, upregulation of lineage-specific marker genes *T*, *HAND1* and *GSC* was observed in the presence of DMSO (Fig. 3E, F and G in Ref. III). Additional effect of TCDD on the expression of these genes was not observed (Fig. 3E, F and G in Ref. III). As with neural and endodermal differentiation, mesodermal differentiation resulted in downregulation of *AHR*, *OCT4*, *SOX2* and *NANOG* expression. Compared to DMSO treatment, TCDD appeared to have no significant influence of the expression of these genes (Suppl. Fig. 5A, B, C and D in Ref. III).

It has been previously shown that TCDD impairs differentiation of hESCs by inhibiting the expression of lineage-specific marker genes *T* (mesoderm) and *SOX17* (endoderm) (Fu et al., 2019; Kubi et al., 2019). However, our data demonstrate that TCDD does not affect early differentiation, at least in the context of this experimental setting and analysed marker genes. In addition, TCDD did not alter the expression of pluripotency marker genes. Previous studies, however, have demonstrated the binding of AHR to regulatory regions of *OCT4*, *SOX2* and *NANOG* (Cheng et al., 2015; Stanford et al., 2016; Yamamoto et al., 2019). It is imperative to consider that these results may be explained by different protocols used or the proposed ligand and cell specific roles of AHR.

4.3.4. Impact of TCDD on hESC and early differentiation

To gain a more detailed understanding on the effects of TCDD, we analysed its impact on global gene expression in hESCs and differentiated cells by high-throughput mRNA sequencing (RNA-seq). In hESCs, gene expression profile was analysed after 3 days of treatment with DMSO or 10 nM TCDD. Additionally, DMSO or 10 nM TCDD pre-treated (days) hESCs were differentiated into neural progenitor (7 days), definitive endoderm (5 days) or early mesoderm cells (5 days) in the presence of DMSO/TCDD followed by RNA-seq analysis (Fig. 4A in Ref. III). The results showed that TCDD altered gene expression

profile in hESCs and differentiated cells. The most prominent changes were detected in mesodermal lineage (Suppl. Fig. 6 in Ref. III). More specifically, 86 genes ($p < 0.05$) were upregulated in hESCs in response to TCDD treatment. 55 genes were upregulated in definitive endoderm cells, 263 in early mesoderm cells and 114 genes in neural progenitors (Fig. 4B in Ref. III). 32 genes were found to be downregulated in response to TCDD in hESCs. Also, TCDD downregulated 73 genes in endodermal cells, 501 in mesodermal cells and 59 in neural cells (Fig. 4C in Ref. III). The complete expression profile in pluripotent cells, but also differentiated cells was in line with the gene expression analysis presented above.

Gene ontology (GO) analysis was carried out with differentially expressed genes ($p < 0.05$) to find relevant biological pathways affected by TCDD treatment. In hESCs, top pathways related to genes that were influenced by TCDD were associated to pattern specification, embryonic organ development and organ morphogenesis (Fig. 4D in Ref. III). In definitive endoderm cells, cell proliferation and regulation of membrane potential were among top biological pathways associated with TCDD-regulated genes. In early mesoderm cells, differentially expressed genes were mostly related to intracellular signalling, whereas GO analysis after neural differentiation showed extracellular matrix and structure organisation among eminent pathways (Fig. 4C in Ref. III). There were TCDD-affected pathways that were common to different cell types, including cellular response to cyclic compounds in hESCs and neural progenitors. The overall results, however, reveal a distinct pattern between lineages, which suggests AHR to have lineage-specific roles during early differentiation. This assumption is supported by our finding that only a small set of genes were found to be regulated by TCDD commonly in multiple cell types (Fig. 4E in Ref. III). Among these were classical AHR target genes such as *TIPARP*, *CYP1A1*, *CYP1B1* and *AHRR*, but also several less-characterised genes or non-coding RNA genes were detected (Fig. 4E in Ref. III).

In order to get insight into potential direct AHR target genes, we performed ChIP-seq analysis with hESCs, definitive endoderm cells and neural progenitors after 100 nM TCDD treatment for 1.5 h. Early mesoderm cells were excluded from this experiment due to extremely low levels of AHR protein (Fig. 2G in Ref. III). For initial quality control, ChIP-qPCR with known AHR target genes was carried out before and after library preparation (Suppl. Fig. 7–9 in Ref. III). AHR binding to nearby regions of known target genes *CYP1A1* (Fig. 4F in Ref. III), *AHRR*, *TIPARP* and *CYP1B1* were evaluated as additional proof of the quality of the experimental procedure (Suppl. Fig. 10 in Ref. III). DNA motif analysis of combined enriched regions including all three cell types reveal previously known AHR consensus motif 5'-GCGTG-3' as top enriched motif, being present in 57% of target sequences (Fig. 4G in Ref. III). Analysis of ChIP-seq results revealed 199 AHR-bound (+/– TCDD) regions in hESCs (Fig. 4H in Ref. III). 93 AHR-bound regions were detected in neural progenitors and 49 AHR-bound regions in definitive endoderm cells (Fig. 4H in Ref. III). In addition, several genes were bound by AHR (within 200 kbp +/– from TSS) in different

cell types (Suppl. Table 1 in Ref. III). GO analysis of AHR-bound genes in hESCs revealed top biological pathways to be regulation of transcription and positive regulation of RNA metabolic processes (Fig. 4I in Ref. III). Top biological pathways related to AHR-bound genes after endodermal differentiation are cell-cell and WNT signalling, while AHR-bound regions in neural progenitor cells are associated to neurogenesis (Fig. 4H in Ref. III).

For further analysis with the aim to determine AHR target genes we sorted out differentially regulated genes that were also associated with AHR binding. In pluripotent hESCs, we discovered 28 upregulated genes which had TCDD-induced binding of AHR within +/– 200 bp from TSS, suggesting that AHR may be directly involved in activating the expression of these genes (Table 1 in Ref. III). Increase in mRNA levels and associated binding of AHR was common to several known AHR target genes, e.g. *AHRR*, *TIPARP*, *CYP1A1* and *CYP1B1* (Table 1 in Ref. III). In addition, these data suggest the role of AHR in regulating several genes known for their role in early embryonic development. *SIX3* and *SIX6* are involved in regulation of multipotent neuroretinal progenitor cells, *LRAT* in regulating retinoid homeostasis in early development and *RORA* has been shown to regulate differentiation and survival of Purkinje cells (Cook et al., 2015; Diacou et al., 2018; Sears & Palczewski, 2016). Also, the role of *LHX4* in the control and development of pituitary gland has also been established (Mullen et al., 2007). Interaction between AHR and these developmentally important genes has not been described before. However, *LHX4* and *SIX3* have been reported to be associated with cleft palate, a hallmark phenotype of TCDD toxicity (Mimura et al., 1997; Ozturk et al., 2013; Rochette et al., 2015).

TCDD exposure during foetal development has been associated with several health risks pointing to neurological defects. Sarma et al., (2019) showed that TCDD increased the number of neuronal rosettes and the amount of TH-positive neuronal cells during early hESC differentiation, although the exact mechanisms remained elusive. Our result may possibly enlighten this matter, as we found several TCDD-regulated possible target genes of AHR, which are shown to play a role in neural differentiation. For example, *RORA* is expressed and developmentally regulated in several regions of the brain. These include thalamus and Purkinje cells (Cook et al., 2015). *RORA* has been shown to be critical for survival and differentiation of Purkinje cells, but also for genes related to dendritic differentiation and glutamatergic pathway (Hamilton et al., 1996). Additionally, TCDD effects on AHR binding and upregulation of mRNA were common to *LRAT* and *EXOC2* (Table 1 in Ref. III). Furthermore, regulatory regions of these genes were bound by AHR not only in TCDD-treated cells, but also in non-treated cells. This suggests that AHR participates in endogenous regulation of these genes. The importance of *EXOC2* in human brain development has been demonstrated previously, as mutations in this gene can cause severe developmental defects (Bergen et al., 2020). Pathogenic variants of this gene have been associated with abnormalities in brain such as severe developmental delay, dysmorphism, poor motor skills and microcephaly. Moreover, a recent study showed that activation

of AHR by its endogenous ligand kynurenine was an essential link between Zika-virus induced microcephaly (Giovannoni et al., 2020).

Of the likely AHR target genes, found in this study, one of the most highly upregulated was *LRAT*. LRAT is a key protein in retinoid/visual cycle and is highly expressed in eye (Sears & Palczewski, 2016). Dysregulation of this gene has been related to early onset retinal dystrophy (Thompson et al., 2001). Intriguingly, GO analysis of TCDD-affected genes showed visual perception among the top biological pathways (Fig. 4D in Ref. III). Additionally, oculomotor defects have been reported in *Ahr* knockout mice (Chevallier et al., 2013). Moreover, *SIX3* and *SIX6*, possible AHR target genes in hESCs, are known to be expressed during development of the early stages of visual system and are required for the maintenance of multipotent retinal progenitor cells (Diacou et al., 2018; Jean et al., 1999; Oliver et al., 1995; Toy & Sundin, 1999). Taken together, the findings gathered in this study regarding AHR target genes in hESCs shed light on the roles of this protein in stem cells and early development, with additional suggestion on the important role in eye development.

Combined analysis of RNA-seq and ChIP-seq data in endodermal cells showed none of the genes associated with nearby AHR binding were differentially expressed. However, differentially expressed four genes in endodermal cells had AHR binding in hESCs. TCDD upregulated the expression of *CDCA7L* and *S1PR1*, whereas substantial downregulation of *MYADML2* and *CUZD1* was seen in these cells (Table 2 in Ref. III). Interestingly, *CUZD1* was upregulated in hESCs, illustrative of the cell-specific role of AHR in gene regulation during development. Mesodermal differentiation resulted in 16 differentially expressed genes upon TCDD treatment, which were associated with AHR binding in hESCs, definitive endoderm or neural progenitor cells (Table 3 in Ref. III). Intriguingly, in early mesoderm cells TCDD downregulated 10 of these genes. Considering essentially undetectable levels of AHR in these cells (Fig. 2G in Ref. III), this result hints that the influence of TCDD is more profound on pluripotent stem cells, rather than on differentiated cells. Thus, the observed results are possible secondary effects elicited by TCDD-AHR signalling in hESCs. This suggestion is supported by Fu et al., (2019), which emphasised the importance of TCDD pre-treatment of hESCs in this kind of experiment.

Gene expression analysis of genes associated with AHR binding after neural differentiation showed upregulation of known AHR target genes. The most noticeable change was in the expression of *CYP1A1* having a 35-fold increase (Table 4 in Ref. III). Most of the genes, presented in Table 4 in Ref. III, had TCDD-induced binding of AHR in hESCs and neural progenitor cells (including *LRAT* and *LINC00886*). The expression of *GRB7*, *IKZF3*, *CYP27A1*, *CCDC60* and *TSC22D1* were specific only to neural cells. Intriguingly, TCDD toxicity on early development can also be presumed by the seen upregulation of known trophectoderm marker genes *CCKBR* and *CDX2*.

As previous studies have reported TCDD to influence gene expression by epigenetic changes, our next aim was to analyse TCDD treatment on chromatin accessibility in hESCs on a global level by ATAC-seq (Assay for Transposase

Accessible Chromatin followed by high-throughput sequencing). ATAC-seq was performed using DMSO or 10 nM TCDD pre-treated hESCs, due to this window being most susceptible to TCDD effects (Fu et al., 2019; Sarma et al., 2019). We detected 157 overall genomic regions with differentially accessible chromatin caused by TCDD. Interestingly, results showed eight regions having reduced accessibility in response to TCDD compared to DMSO-treated cells. The expression of six genes that were associated with these regions (*CRYM*, *GFOD1*, *GLRX5*, *NPIPB3*, *SIRT5*, *TCLB1*), however, did not change in these cells. Contrarily, TCDD induced increase in chromatin accessibility within 149 regions that were associated with 222 genes. Among these, AHR binding to the proximity of TSS of 11 genes (e.g. *ITGA6*, *RCC2*, *ZNF532*, *DLX2*, *MALT1*, *PADI4*) was also determined. In several cases, AHR binding and increased chromatin accessibility was also accompanied by the increased expression of these genes, suggesting that AHR may be directly involved in transcriptional activation of these genes in human embryonic stem cells. In fact, *TIPARP*, a well-documented AHR target gene was among these few.

This study did not focus on studying endogenous roles of AHR in stem cells. Still, our data from ChIP-seq analysis revealed that a number of regions in DMSO-treated hESCs or differentiated cells were bound by AHR. Sequencing analysis of AHR binding sites revealed numerous genes with strong binding effect common only to hESC cells (e.g. *NDUFAF7*, *NEUROG3*, *OPA1*, *BICD1*, *EIF4G2*, *FAM218A*, *HS3ST5*, *METTL9*, *CCDC34*, *ALDH1A1*, *PRICKLE1*, *ROR1*, *SMAD7*, *SPN*, *ETAA1*, *KCTD1*, *PRDM2*, *DUSP6*, *ACACA*, *ASAP1*, *RBPMS*, *SLC8B1*, *TCPN1*). Contrarily, we found AHR binding within 1 kbp of TSS of a number of genes commonly in hESCs, endodermal and neural cells (e.g. *BAD*, *NUDT3*, *DAGLA*, *DICER1*, *CBX3*, *HNRNPA2B1*, *STRBP*, *SLC39A10*, *DDX17*, *TPRA1*, *SEPTIN7*, *EPC2*, *TIPARP*, *LINC00886*). Among these, strong AHR binding was seen in promoters of *DICER1*, *HNRNPA2B1*, *NUDT3* and *DDX17*, which have been shown to be involved in RNA processing (Suppl. Fig. 12 in Ref. III). Regulation of RNA metabolic and biosynthesis processes were also determined by GO analysis of AHR-bound genes to be one of the main biological pathways (Fig. 4I in Ref. III).

DICER1 is known to be a vital component in the miRNA bioprocessing. Importantly, DICER1 has been shown to be essential for stem cell renewal (Teijeiro et al., 2018). HNRNPA2B1 is an RNA-binding protein having a role in miRNA processing and alternative splicing, but also involved in miRNA sorting into exosomes (Alarcón et al., 2015; Villarroya-Beltri et al., 2013). NUDT3 and DDX17 are involved in mRNA decapping and miRNA biogenesis (Grudzien-Nogalska et al., 2016; Ngo et al., 2019). In addition, AHR binding was seen in the promoter of miR-302-367 cluster, which has been shown to regulate pluripotency (Balzano et al., 2018; Lipchina et al., 2012). Further studies are needed to clarify the role of AHR in regulation of these genes and thereby its functions in controlling stem cell pluripotency and early differentiation.

Research in the field of AHR biology has focused largely on studying the role of this protein in cancer. Numerous studies have reported that AHR is highly

expressed in several cancer tissues, including glioblastoma and hepatocellular carcinoma (Opitz et al., 2011; Wang et al., 2017). In these cancers, biomarkers have been described to predict therapeutic outcomes, including e.g. CDCA7L, BICD1, DDX17, S1PR1 and SEPT7 (Hou et al., 2016; Huang et al., 2017; Ji et al., 2019; Rostami et al., 2019; Xue et al., 2019; Zhang et al., 2016). This study provides evidence that AHR might be directly involved in regulation of these genes. Collectively, the identification of novel potential AHR targets opens up interesting avenues in discovering the role of AHR is cellular homeostasis and disease.

SUMMARY

AHR has been in the focus of scientific research over four decades. Although initially discovered as a mediator of toxicity for environmental pollutants, substantial evidence has been gathered showing that AHR has a fundamental role in normal physiology. This has been implied by studies with knock-out animal models as well as the gained knowledge about the vast number of endogenous ligands for this receptor. The explicit biological function of AHR in regulating cellular homeostasis has been shown in various organs and tissues, most prominently in the immune system, liver, skin, lung, fat tissue, brain and cancer. Additionally, AHR has been discovered to be important in reproductive system, as well as in stem cells. The complex nature of this protein has urged to study its expression and functions in different cells. The current thesis focused on characterising the expression of the *Ahr* gene in murine ovarian granulosa cells and human embryonic stem cells. A particular focus was placed on elucidating the regulatory mechanisms controlling the expression of *Ahr*. In addition, this thesis aimed to expand the knowledge on the role of AHR in cellular homeostasis by identification of AHR target genes.

The importance of Ahr in the ovary was first suggested by studies showing TCDD to act as an endocrine disruptor, inhibiting steroidogenesis. Further studies using Ahr knockout mice have shown its significant role in maintaining pregnancy and regulating development of ovarian follicles and ovulation. Mice lacking Ahr display disturbances in steroidogenesis, have slower ovarian follicle growth and less antral follicles. Moreover, studies have demonstrated reduced responsiveness to gonadotropin stimuli by decreased expression of FSH and LH receptors on GCs, leading to perturbed ovulation. These data have suggested that Ahr itself is regulated by gonadotropins.

In this study, the reduction of Ahr expression at mRNA and protein levels were shown in GCs of preovulatory follicles in response to LH signalling. The repressive effect of LH on *Ahr* expression was shown to be mediated by PKA, but not ERK pathway. Additionally, relatively rapid downregulation of *Ahr* in these cells resulted from decreased transcription rate and involved epigenetic remodelling at *Ahr* promoter. The second part of this study investigated the expression of Ahr during ovarian follicle maturation, prior to LH-surge. Conversely, *Ahr* expression was increased during this phase and was demonstrated to be dependent on both FSH and LH. The upregulation of Ahr was suggested to involve post-transcriptional regulation mechanisms likely dependent on LH signalling. This study showed that the increased amount of Ahr protein is confined to mural GCs of large antral follicles. The upregulation of Ahr resulted from increased transcription rate and was regulated by changes in chromatin condensation. Additionally, PKA repressed Ahr in these cells. This suggested that a decrease in FSH mediated PKA activity seen in the mid-phase of follicle maturation period may be an activation barrier for the upregulation of Ahr *in vivo*.

Collectively, the fluctuation of Ahr in differentiating murine granulosa cells indicates Ahr to have specific time- and tissue-dependent physiological role.

The fluctuation of AHR expression has also been previously described in cells with higher developmental potential than granulosa cells. With this in mind, the last part of this thesis aimed to describe the expression of AHR in pluripotent stem cells. Using hESCs, AHR expression was shown to decrease during the initial stages of differentiation. Additionally, directed differentiation into neural progenitors, early mesoderm and definitive endoderm cells showed distinct expression patterns. This study provided evidence that AHR ligand TCDD has a profound impact on gene expression during hESC differentiation. This was evidenced by genome-wide analysis of its effect on AHR binding to DNA as well as measured changes in the expression profile. Finally, the knowledge on AHR signalling was expanded by identification of novel AHR target genes, using combined analysis of high-throughput sequencing from ChIP-seq, RNA-seq and ATAC-seq. Taken together, the results presented in this dissertation broaden our understanding on the fundamental biological role of aryl hydrocarbon receptor. Notably, gaining this knowledge is a crucially important step in further studies aspiring to exploit its value as a therapeutic target in treatment of various diseases.

REFERENCES

Abbott, B. D., Schmid, J. E., Pitt, J. A., Buckalew, A. R., Wood, C. R., Held, G. A., & Diliberto, J. J. (1999). Adverse reproductive outcomes in the transgenic Ah receptor-deficient mouse. *Toxicology and Applied Pharmacology*. https://doi:10.1006/taap.1998.8601

Alarcón, C. R., Goodarzi, H., Lee, H., Liu, X., Tavazoie, S., & Tavazoie, S. F. (2015). HNRNPA2B1 Is a Mediator of m6A-Dependent Nuclear RNA Processing Events. *Cell*. https://doi.org/10.1016/j.cell.2015.08.011

Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. In *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2017.00561

Baba, T., Mimura, J., Nakamura, N., Harada, N., Yamamoto, M., Morohashi, K., & Fujii-Kuriyama, Y. (2005). Intrinsic Function of the Aryl Hydrocarbon (Dioxin) Receptor as a Key Factor in Female Reproduction. *Molecular and Cellular Biology*. https://doi.org/10.1128/mcb.25.22.10040-10051.2005

Bahrami, A., Miraie-Ashtiani, S. R., Sadeghi, M., & Najafi, A. (2017). MiRNA-mRNA network involved in folliculogenesis interactome: Systems biology approach. *Reproduction*. https://doi.org/10.1530/REP-17-0049

Bai, Q., Assou, S., Haouzi, D., Ramirez, J. M., Monzo, C., Becker, F., Gerbal-Chaloin, S., Hamamah, S., & de Vos, J. (2012). Dissecting the First Transcriptional Divergence During Human Embryonic Development. *Stem Cell Reviews and Reports*. https://doi.org/10.1007/s12015-011-9301-3

Balzano, F., Cruciani, S., Basoli, V., Santaniello, S., Facchin, F., Ventura, C., & Maioli, M. (2018). MiR200 and MiR302: Two big families influencing stem cell behavior. In *Molecules*. https://doi.org/10.3390/molecules23020282

Bao, B., & Garverick, H. A. (1998). Expression of Steroidogenic Enzyme and Gonadotropin Receptor Genes in Bovine Follicles during Ovarian Follicular Waves: A Review. In *Journal of Animal Science*. https://doi.org/10.2527/1998.7671903x

Barnett, K. R., Tomic, D., Gupta, R. K., Babus, J. K., Roby, K. F., Terranova, P. F., & Flaws, J. A. (2007). The aryl hydrocarbon receptor is required for normal gonadotropin responsiveness in the mouse ovary. *Toxicology and Applied Pharmacology*. https://doi.org/10.1016/j.taap.2007.05.014

Barnett, K. R., Tomic, D., Gupta, R. K., Miller, K. P., Meachum, S., Paulose, T., & Flaws, J. A. (2007). The aryl hydrocarbon receptor affects mouse ovarian follicle growth via mechanisms involving estradiol regulation and responsiveness. *Biology of Reproduction*. https://doi.org/10.1095/biolreprod.106.057687

Benedict, J. C., Lin, T. M., Loeffler, I. K., Peterson, R. E., & Flaws, J. A. (2000). Physiological role of the aryl hydrocarbon receptor in mouse ovary development. *Toxicological Sciences*. https://doi.org/10.1093/toxsci/56.2.382

Benedict, J. C., Miller, K. P., Lin, T. M., Greenfeld, C., Babus, J. K., Peterson, R. E., & Flaws, J. A. (2003). Aryl hydrocarbon receptor regulates growth, but not atresia, of mouse preantral and antral follicles. *Biology of Reproduction*. https://doi.org/10.1095/biolreprod.102.007492

Bentley, D. (1999). Coupling RNA polymerase II transcription with pre-mRNA processing. In *Current Opinion in Cell Biology*. https://doi.org/10.1016/S0955-0674(99)80048-9

Bergen, N. J. V., Ahmed, S. M., Collins, F., Cowley, M., Vetro, A., Dale, R. C., Hock, D. H., De Caestecker, C., Minal, M., Massey, S., Gladys, H., Pisano, T., Glover, S., Gusman, J., Stroud, D. A., Dinger, M., Guerrini, R., MacAra, I. G., & Christodoulou, J. (2020). Mutations in the exocyst component EXOC2 cause severe defects in human brain development. *Journal of Experimental Medicine*. https://doi.org/10.1084/JEM.20192040

Bjeldanes, L. F., Kim, J. Y., Grose, K. R., Bartholomew, J. C., & Bradfield, C. A. (1991). Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: Comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.88.21.9543

Boitano, A. E., Wang, J., Romeo, R., Bouchez, L. C., Parker, A. E., Sutton, S. E., Walker, J. R., Flaveny, C. A., Perdew, G. H., Denison, M. S., Schultz, P. G., & Cooke, M. P. (2010). Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. https://doi.org/10.1126/science.1191536

Boyer, L. A., Tong, I. L., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G., Gifford, D. K., Melton, D. A., Jaenisch, R., & Young, R. A. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*. https://doi.org/10.1016/j.cell.2005.08.020

Burbach, K. M., Poland, A., & Bradfield, C. A. (1992). Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.89.17.8185

Bussmann, U. A., & Barañao, J. L. (2006). Regulation of aryl hydrocarbon receptor expression in rat granulosa cells. *Biology of Reproduction*. https://doi.org/10.1095/biolreprod.106.053017

Carletti, M. Z., & Christenson, L. K. (2009). Rapid effects of LH on gene expression in the mural granulosa cells of mouse periovulatory follicles. *Reproduction*. https://doi.org/10.1530/REP-08-0457

Carlone, D. L., & Richards, J. A. S. (1997). Functional interactions, phosphorylation, and levels of 3',5'-cyclic adenosine monophosphate-regulatory element binding protein and steroidogenic factor-1 mediate hormone-regulated and constitutive expression of aromatase in gonadal cells. *Molecular Endocrinology*. https://doi.org/10.1210/me.11.3.292

Casarini, L., Lispi, M., Longobardi, S., Milosa, F., la Marca, A., Tagliasacchi, D., Pignatti, E., & Simoni, M. (2012). LH and hCG Action on the Same Receptor Results in Quantitatively and Qualitatively Different Intracellular Signalling. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0046682

Casper, R. F., Quesne, M., Rogers, I. M., Shirota, T., Jolivet, A., Milgrom, E., & Savouret, J. F. (1999). Resveratrol has antagonist activity on the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. *Molecular Pharmacology*, *56*(4), 784–790. https://www.ncbi.nlm.nih.gov/pubmed/10496962

Chaffin, C. L., Stouffer, R. L., & Duffy, D. M. (1999). Gonadotropin and steroid regulation of steroid receptor and aryl hydrocarbon receptor messenger ribonucleic acid in macaque granulosa cells during the periovulatory interval. *Endocrinology*. https://doi.org/10.1210/endo.140.10.7056

Chaffin, C. L., Trewin, A. L., & Hutz, R. J. (2000). Estrous cycle-dependent changes in the expression of aromatic hydrocarbon receptor (AHR) and AHR-nuclear translocator (ARNT) mRNAs in the rat ovary and liver. *Chemico-Biological Interactions*. https://doi.org/10.1016/S0009-2797(99)00157-X

Chakraborty, P., & Roy, S. K. (2015). Expression of FSH receptor in the hamster ovary during perinatal development. *Molecular and Cellular Endocrinology*. https://doi.org/10.1016/j.mce.2014.11.014

Cheng, J., Li, W., Kang, B., Zhou, Y., Song, J., Dan, S., Yang, Y., Zhang, X., Li, J., Yin, S., Cao, H., Yao, H., Zhu, C., Yi, W., Zhao, Q., Xu, X., Zheng, M., Zheng, S., Li, L., … Wang, Y. J. (2015). Tryptophan derivatives regulate the transcription of Oct4 in stem-like cancer cells. *Nature Communications*. https://doi.org/10.1038/ncomms8209

Chevallier, A., Mialot, A., Petit, J. M., Fernandez-Salguero, P., Barouki, R., Coumoul, X., & Beraneck, M. (2013). Oculomotor Deficits in Aryl Hydrocarbon Receptor Null Mouse. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0053520

Christenson, L. K., Stouffer, R. L., & Strauss, J. F. (2001). Quantitative Analysis of the Hormone-induced Hyperacetylation of Histone H3 Associated with the Steroidogenic Acute Regulatory Protein Gene Promoter. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M101650200

Ciolino, H. P., Daschner, P. J., & Yeh, G. C. (1999). Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochemical Journal*. https://doi.org/10.1042/0264-6021:3400715

Collas, P., Le Guellec, K., & Taskén, K. (1999). The A-kinase-anchoring protein AKAP95 is a multivalent protein with a key role in chromatin condensation at mitosis. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.147.6.1167

Combarnous, Y., Guillou, F., & Martinat, N. (1984). Comparison of in Vitro follicle-stimulating hormone (FSH) activity of equine gonadotropins (luteinizing hormone, FSH, and chorionic gonadotropin) in male and female rats. *Endocrinology*. https://doi.org/10.1210/endo-115-5-1821

Contador-Troca, M., Alvarez-Barrientos, A., Barrasa, E., Rico-Leo, E. M., Catalina-Fernández, I., Menacho-Márquez, M., Bustelo, X. R., García-Borrón, J. C., Gómez-Durán, A., Sáenz-Santamaría, J., & Fernández-Salguero, P. M. (2013). The dioxin receptor has tumor suppressor activity in melanoma growth and metastasis. *Carcinogenesis*. https://doi.org/10.1093/carcin/bgt248

Cook, D. N., Kang, H. S., & Jetten, A. M. (2015). Retinoic Acid-Related Orphan Receptors (RORs): Regulatory Functions in Immunity, Development, Circadian Rhythm, and Metabolism. *Nuclear Receptor Research*. https://doi.org/10.11131/2015/101185

DeManno, D. A., Cottom, J. E., Kline, M. P., Peters, C. A., Maizels, E. T., & Hunzicker-Dunn, M. (1999). Follicle-stimulating hormone promotes histone H3 phosphorylation on serine-10. *Molecular Endocrinology*. https://doi.org/10.1210/mend.13.1.0222

Denison, M. S., & Nagy, S. R. (2003). Activation of the Aryl Hydrocarbon Receptor by Structurally Diverse Exogenous and Endogenous Chemicals. In *Annual Review of Pharmacology and Toxicology*. https://doi.org/10.1146/annurev.pharmtox.43.100901.135828

Dere, E., Lo, R., Celius, T., Matthews, J., & Zacharewski, T. R. (2011). Integration of Genome-Wide Computation DRE Search, AhR ChIP-chip and Gene Expression Analyses of TCDD-Elicited Responses in the Mouse Liver. *BMC Genomics*. https://doi.org/10.1186/1471-2164-12-365

Dever, D. P., & Opanashuk, L. A. (2012). The aryl hydrocarbon receptor contributes to the proliferation of human medulloblastoma cells. *Molecular Pharmacology*. https://doi.org/10.1124/mol.111.077305

Devillers, M. M., Petit, F., Giton, F., François, C. M., Juricek, L., Coumoul, X., Magre, S., Cohen-Tannoudji, J., & Guigon, C. J. (2020). Age-dependent vulnerability of the ovary to AhR-mediated TCDD action before puberty: Evidence from mouse models. *Chemosphere*. https://doi.org/10.1016/j.chemosphere.2020.127361

Diacou, R., Zhao, Y., Zheng, D., Cvekl, A., & Liu, W. (2018). Six3 and Six6 Are Jointly Required for the Maintenance of Multipotent Retinal Progenitors through Both Positive and Negative Regulation. *Cell Reports*. https://doi.org/10.1016/j.celrep.2018.10.106

Díaz-Díaz, C. J., Ronnekleiv-Kelly, S. M., Nukaya, M., Geiger, P. G., Balbo, S., Dator, R., Megna, B. W., Carney, P. R., Bradfield, C. A., & Kennedy, G. D. (2016). The aryl hydrocarbon receptor is a repressor of inflammation-associated colorectal tumorigenesis in mouse. *Annals of Surgery*. https://doi.org/10.1097/SLA.0000000000001874

DiNatale, B. C., Murray, I. A., Schroeder, J. C., Flaveny, C. A., Lahoti, T. S., Laurenzana, E. M., Omiecinski, C. J., & Perdew, G. H. (2010). Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicological Sciences*. https://doi.org/10.1093/toxsci/kfq024

DiNatale, B. C., Schroeder, J. C., & Perdew, G. H. (2011). Ah receptor antagonism inhibits constitutive and cytokine inducible IL6 production in head and neck tumor cell lines. *Molecular Carcinogenesis*. https://doi.org/10.1002/mc.20702

Edson, M. A., Nagaraja, A. K., & Matzuk, M. M. (2009). The mammalian ovary from genesis to revelation. In *Endocrine Reviews*. https://doi.org/10.1210/er.2009-0012

Elferink, C. J., & Reiners, J. J. (1996). Quantitative RT-PCR on CYP1A1 heterogeneous nuclear RNA: A surrogate for the in vitro transcription run-on assay. *BioTechniques*. https://doi.org/10.2144/19962003470

Englert, N. A., Turesky, R. J., Han, W., Bessette, E. E., Spivack, S. D., Caggana, M., Spink, D. C., & Spink, B. C. (2012). Genetic and epigenetic regulation of AHR gene expression in MCF-7 breast cancer cells: Role of the proximal promoter GC-rich region. *Biochemical Pharmacology*. https://doi.org/10.1016/j.bcp.2012.06.013

Esser, C., Lawrence, B. P., Sherr, D. H., Perdew, G. H., Puga, A., Barouki, R., & Coumoul, X. (2018). Old receptor, new tricks—The ever-expanding universe of aryl hydrocarbon receptor functions. Report from the 4th AHR meeting, 29–31 August 2018 in Paris, France. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms19113603

Fan, Y., Boivin, G. P., Knudsen, E. S., Nebert, D. W., Xia, Y., & Puga, A. (2010). The aryl hydrocarbon receptor functions as a tumor suppressor of liver carcinogenesis. *Cancer Research*. https://doi.org/10.1158/0008-5472.CAN-09-3090

Farrell, K., Safe, L., & Safe, S. (1987). Synthesis and aryl hydrocarbon receptor binding properties of radiolabeled polychlorinated dibenzofuran congeners. *Archives of Biochemistry and Biophysics*. https://doi.org/10.1016/0003-9861(87)90485-1

Fernandez-Salguero, P., Pineau, T., Hilbert, D. M., McPhail, T., Lee, S. S. T., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., & Gonzalez, F. J. (1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science*. https://doi.org/10.1126/science.7732381

Findlay, J. K., & Drummond, A. E. (1999). Regulation of the FSH receptor in the ovary. In *Trends in Endocrinology and Metabolism*. https://doi.org/10.1016/S1043-2760(98)00144-1

FitzGerald, C. T., Fernandez-Salguero, P., Gonzalez, F. J., Nebert, D. W., & Puga, A. (1996). Differential regulation of mouse Ah receptor gene expression in cell lines of different tissue origins. *Archives of Biochemistry and Biophysics*. https://doi.org/10.1006/abbi.1996.0378

Fitzgerald, C. T., Nebert, D. W., & Puga, A. (1998). Regulation of mouse Ah receptor (Ahr) gene basal expression by members of the Sp family of transcription factors. *DNA and Cell Biology*. https://doi.org/10.1089/dna.1998.17.811

Fong, H., Hohenstein, K. A., & Donovan, P. J. (2008). Regulation of Self-Renewal and Pluripotency by Sox2 in Human Embryonic Stem Cells. *Stem Cells*. https://doi.org/10.1634/stemcells.2007-1002

Formosa, R., Borg, J., & Vassallo, J. (2017). Aryl hydrocarbon receptor (AHR) is a potential tumour suppressor in pituitary adenomas. *Endocrine-Related Cancer*. https://doi.org/10.1530/ERC-17-0112

Fortune, J. E., Hilbert, J. L., Fortune, J. E., & Hilbert, J. L. (1986). Estradiol secretion by granulosa cells from rats with four- or five-day estrous cycles: The development of responses to follicle-stimulating hormone versus luteinizing hormone. *Endocrinology*. https://doi.org/10.1210/endo-118-6-2395

Freeman, M. E. (2006). Neuroendocrine control of the ovarian cycle of the rat. In *Knobil and Neill's Physiology of Reproduction*. https://doi.org/10.1016/B978-012515400-0/50048-8

Fu, H., Wang, L., Wang, J., Bennett, B. D., Li, J. L., Zhao, B., & Hu, G. (2019). Dioxin and AHR impairs mesoderm gene expression and cardiac differentiation in human embryonic stem cells. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2018.09.247

Furumatsu, K., Nishiumi, S., Kawano, Y., Ooi, M., Yoshie, T., Shiomi, Y., Kutsumi, H., Ashida, H., Fujii-Kuriyama, Y., Azuma, T., & Yoshida, M. (2011). A role of the aryl hydrocarbon receptor in attenuation of colitis. *Digestive Diseases and Sciences*. https://doi.org/10.1007/s10620-011-1643-9

Garrison, P. M., & Denison, M. S. (2000). Analysis of the murine AhR gene promoter. *Journal of Biochemical and Molecular Toxicology*. https://doi.org/10.1002/(SICI)1099-0461(2000)14:1<1::AID-JBT1>3.0.CO;2-4

Garrison, P. M., Rogers, J. M., Brackney, W. R., & Denison, M. S. (2000). Effects of histone deacetylase inhibitors on the Ah receptor gene promoter. *Archives of Biochemistry and Biophysics*. https://doi.org/10.1006/abbi.1999.1620

Ghafari, F., Gutierrez, C. G., & Hartshorne, G. M. (2007). Apoptosis in mouse fetal and neonatal oocytes during meiotic prophase one. *BMC Developmental Biology*. https://doi.org/10.1186/1471-213X-7-87

Gillner, M., Bergman, J., Cambillau, C., Fernström, B., & Gustafsson, J. A. (1985). Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorobenzo-p-dioxin in rat liver. *Molecular Pharmacology*. 28 (4) 357–363

Giovannoni, F., Bosch, I., Polonio, C. M., Torti, M. F., Wheeler, M. A., Li, Z., Romorini, L., Rodriguez Varela, M. S., Rothhammer, V., Barroso, A., Tjon, E. C., Sanmarco, L. M., Takenaka, M. C., Modaresi, S. M. S., Gutiérrez-Vázquez, C., Zanluqui, N. G., dos Santos, N. B., Munhoz, C. D., Wang, Z., … Quintana, F. J. (2020). AHR is a Zika virus host factor and a candidate target for antiviral therapy. *Nature Neuroscience*. https://doi.org/10.1038/s41593-020-0664-0

Gonzalez-Robayna, I. J., Alliston, T. N., Buse, P., Firestone, G. L., & Richards, J. S. (1999). Functional and subcellular changes in the A-kinase-signaling pathway: Relation to aromatase and Sgk expression during the transition of granulosa cells to luteal cells. *Molecular Endocrinology*. https://doi.org/10.1210/mend.13.8.0334

Gramatzki, D., Pantazis, G., Schittenhelm, J., Tabatabai, G., Köhle, C., Wick, W., Schwarz, M., Weller, M., & Tritschler, I. (2009). Aryl hydrocarbon receptor inhibition downregulates the TGF-B/Smad pathway in human glioblastoma cells. *Oncogene*. https://doi.org/10.1038/onc.2009.104

Grudzien-Nogalska, E., Jiao, X., Song, M. G., Hart, R. P., & Kiledjian, M. (2016). Nudt3 is an mRNA decapping enzyme that modulates cell migration. *RNA*. https://doi.org/10.1261/rna.055699.115

Guigon, C. J., & Magre, S. (2006). Contribution of germ cells to the differentiation and maturation of the ovary: Insights from models of germ cell depletion. In *Biology of Reproduction*. https://doi.org/10.1095/biolreprod.105.047134

Guraya, S. S., & Guraya, S. S. (1985). Follicular Atresia. In *Biology of Ovarian Follicles in Mammals*. https://doi.org/10.1007/978-3-642-70154-2_7

Hahn, M. E. (2002). Aryl hydrocarbon receptors: Diversity and evolution. *Chemico-Biological Interactions*. https://doi.org/10.1016/S0009-2797(02)00070-4

Hamilton, B. A., Frankel, W. N., Kerrebrock, A. W., Hawkins, T. L., FitzHugh, W., Kusumi, K., Russell, L. B., Mueller, K. L., Van Berkel, V., Birren, B. W., Kruglyak, L., & Lander, E. S. (1996). Disruption of the nuclear hormone receptor RORα in staggerer mice. *Nature*. https://doi.org/10.1038/379736a0

Hebbar, P. B., & Archer, T. K. (2008). Altered histone H1 stoichiometry and an absence of nucleosome positioning on transfected DNA. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M709121200

Hirshfield, A. N., & DeSanti, A. M. (1995). Patterns of ovarian cell proliferation in rats during the embryonic period and the first three weeks postpartum. *Biology of Reproduction*. https://doi.org/10.1095/biolreprod53.5.1208

Hirshfield, Anne Newman. (1991). Development of Follicles in the Mammalian Ovary. *International Review of Cytology*. https://doi.org/10.1016/S0074-7696(08)61524-7

Hou, M., Liu, X., Cao, J., & Chen, B. (2016). SEPT7 overexpression inhibits glioma cell migration by targeting the actin cytoskeleton pathway. *Oncology Reports*. https://doi.org/10.3892/or.2016.4609

Howard, R. B., Pucell, A. G., Bumpus, F. M., & Husain, A. (1988). Rat ovarian renin: Characterization and changes during the estrous cycle. *Endocrinology*. https://doi.org/10.1210/endo-123-5-2331

Huang, S. P., Chang, Y. C., Low, Q. H., Wu, A. T. H., Chen, C. L., Lin, Y. F., & Hsiao, M. (2017). BICD1 expression, as a potential biomarker for prognosis and predicting response to therapy in patients with glioblastomas. *Oncotarget*. https://doi.org/10.18632/oncotarget.22667

Hunzicker-Dunn, M., & Maizels, E. T. (2006). FSH signaling pathways in immature granulosa cells that regulate target gene expression: Branching out from protein kinase A. In *Cellular Signalling*. https://doi.org/10.1016/j.cellsig.2006.02.011

Ishida, M., Mikami, S., Shinojima, T., Kosaka, T., Mizuno, R., Kikuchi, E., Miyajima, A., Okada, Y., & Oya, M. (2015). Activation of aryl hydrocarbon receptor promotes invasion of clear cell renal cell carcinoma and is associated with poor prognosis and cigarette smoke. *International Journal of Cancer*. https://doi.org/10.1002/ijc.29398

Jaffrain-Rea, M. L., Angelini, M., Gargano, D., Tichomirowa, M. A., Daly, A. F., Vanbellinghen, J. F., D'Innocenzo, E., Barlier, A., Giangaspero, F., Esposito, V., Ventura, L.,

Arcella, A., Theodoropoulou, M., Naves, L. A., Fajardo, C., Zacharieva, S., Rohmer, V., Brue, T., Gulino, A., … Beckers, A. (2009). Expression of aryl hydrocarbon receptor (AHR) and AHR-interacting protein in pituitary adenomas: Pathological and clinical implications. *Endocrine-Related Cancer*. https://doi.org/10.1677/ERC-09-0094

Jameson, J. L., Kay, T. W. H., Drust, D. S., Madison, L. D., & Albanese, C. (1991). Hormonal Regulation of Gonadotropin Gene Expression. In *Signaling Mechanisms and Gene Expression in the Ovary*. https://doi.org/10.1007/978-1-4612-3200-1_2

Jean, D., Bernier, G., & Gruss, P. (1999). Six6 (Optx2) is a novel murine Six3-related homeobox gene that demarcates the presumptive pituitary/hypothalamic axis and the ventral optic stalk. *Mechanisms of Development*. https://doi.org/10.1016/S0925-4773(99)00068-4

Jensen, B. A., Reddy, C. M., Nelson, R. K., & Hahn, M. E. (2010). Developing tools for risk assessment in protected species: Relative potencies inferred from competitive binding of halogenated aromatic hydrocarbons to aryl hydrocarbon receptors from beluga (Delphinapterus leucas) and mouse. *Aquatic Toxicology*. https://doi.org/10.1016/j.aquatox.2010.07.021

Ji, Q. K., Ma, J. W., Liu, R. H., Li, X. S., Shen, F. Z., Huang, L. Y., Hui, L., Ma, Y. J., & Jin, B. Z. (2019). CDCA7L promotes glioma proliferation by targeting CCND1 and predicts an unfavorable prognosis. *Molecular Medicine Reports*. https://doi.org/10.3892/mmr.2019.10349

Jin, U. H., Karki, K., Cheng, Y., Michelhaugh, S. K., Mittal, S., & Safe, S. (2019). The aryl hydrocarbon receptor is a tumor suppressor-like gene in glioblastoma. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.RA119.008882

Kawajiri, K., Kobayashi, Y., Ohtake, F., Ikuta, T., Matsushima, Y., Mimura, J., Pettersson, S., Pollenz, R. S., Sakaki, T., Hirokawa, T., Akiyama, T., Kurosumi, M., Poellinger, L., Kato, S., & Fujii-Kuriyama, Y. (2009). Aryl hydrocarbon receptor suppresses intestinal carcinogenesis in Apc Min/+ mice with natural ligands. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0902132106

Kazlauskas, A., Poellinger, L., & Pongratz, I. (1999). Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (aryl hydrocarbon) receptor. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.274.19.13519

Khan, H. A., Zhao, Y., Wang, L., Li, Q., Du, Y. A., Dan, Y., & Huo, L. J. (2015). Identification of miRNAs during mouse postnatal ovarian development and superovulation. *Journal of Ovarian Research*. https://doi.org/10.1186/s13048-015-0170-2

Kim, S. H., Henry, E. C., Kim, D. K., Kim, Y. H., Kum, J. S., Myoung, S. H., Lee, T. G., Kang, J. K., Gasiewicz, T. A., Sung, H. R., & Suh, P. G. (2006). Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o- tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-induced toxicity by antagonizing the aryl hydrocarbon receptor. *Molecular Pharmacology*. https://doi.org/10.1124/mol.105.021832

Ko, C. I., Fan, Y., de Gannes, M., Wang, Q., Xia, Y., & Puga, A. (2016). Repression of the Aryl Hydrocarbon Receptor Is Required to Maintain Mitotic Progression and Prevent Loss of Pluripotency of Embryonic Stem Cells. *Stem Cells*. https://doi.org/10.1002/stem.2456

Ko, C. I., Wang, Q., Fan, Y., Xia, Y., & Puga, A. (2014). Pluripotency factors and Polycomb Group proteins repress aryl hydrocarbon receptor expression in murine embryonic stem cells. *Stem Cell Research*. https://doi.org/10.1016/j.scr.2013.11.007

Kolasa, E., Houlbert, N., Balaguer, P., & Fardel, O. (2013). AhR- and NF-κB-dependent induction of interleukin-6 by co-exposure to the environmental contaminant benzanthracene and the cytokine tumor necrosis factor-α in human mammary MCF-7 cells. *Chemico-Biological Interactions*. https://doi.org/10.1016/j.cbi.2013.01.005

Kubi, J. A., Chen, A. C. H., Fong, S. W., Lai, K. P., Wong, C. K. C., Yeung, W. S. B., Lee, K. F., & Lee, Y. L. (2019). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the differentiation of embryonic stem cells towards pancreatic lineage and pancreatic beta cell function. *Environment International*. https://doi.org/10.1016/j.envint.2019.05.079

Lapolt, P. S., Tilly, J. L., Aihara, T., Nishimori, K., & Hsueh, A. J. W. (1992). Gonadotropin-induced up- and down-regulation of ovarian follicle-stimulating hormone (Fsh) receptor gene expression in immature rats: Effects of pregnant mare's serum gonadotropin, human chorionic gonadotropin, and recombinant fsh. *Endocrinology*. https://doi.org/10.1210/endo.130.3.1537292

LaPres, J. J., Glover, E., Dunham, E. E., Bunger, M. K., & Bradfield, C. A. (2000). ARA9 modifies agonist signaling through an increase in cytosolic aryl hydrocarbon receptor. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.275.9.6153

Larigot, L., Juricek, L., Dairou, J., & Coumoul, X. (2018). AhR signaling pathways and regulatory functions. In *Biochimie Open*. https://doi.org/10.1016/j.biopen.2018.05.001

Latchney, S. E., Lioy, D. T., Henry, E. C., Gasiewicz, T. A., Strathmann, F. G., Mayer-Pröschel, M., & Opanashuk, L. A. (2011). Neural precursor cell proliferation is disrupted through activation of the aryl hydrocarbon receptor by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Stem Cells and Development*. https://doi.org/10.1089/scd.2009.0529

LaVoie, H. A. (2005). Epigenetic control of ovarian function: The emerging role of histone modifications. In *Molecular and Cellular Endocrinology*. https://doi.org/10.1016/j.mce.2005.09.005

Lee, L., Asada, H., Kizuka, F., Tamura, I., Maekawa, R., Taketani, T., Sato, S., Yamagata, Y., Tamura, H., & Sugino, N. (2013). Changes in histone modification and DNA methylation of the StAR and Cyp19a1 promoter regions in granulosa cells undergoing luteinization during ovulation in rats. *Endocrinology*. https://doi.org/10.1210/en.2012-1610

Li, X., Johnson, D. C., & Rozman, K. K. (1995a). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on estrous cyclicity and ovulation in female Sprague-Dawley rats. *Toxicology Letters*. https://doi.org/10.1016/0378-4274(95)03252-G

Li, X., Johnson, D. C., & Rozman, K. K. (1995b). Reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (tcdd) in female rats: Ovulation, hormonal regulation, and possible mechanism(s). *Toxicology and Applied Pharmacology*. https://doi.org/10.1006/taap.1995.1157

Li, Y., Fang, Y., Liu, Y., & Yang, X. (2015). MicroRNAs in ovarian function and disorders. *Journal of Ovarian Research*. https://doi.org/10.1186/s13048-015-0162-2

Li, Y., Innocentin, S., Withers, D. R., Roberts, N. A., Gallagher, A. R., Grigorieva, E. F., Wilhelm, C., & Veldhoen, M. (2011). Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell*. https://doi.org/10.1016/j.cell.2011.09.025

Lipchina, I., Studer, L., & Betel, D. (2012). The expanding role of miR-302-367 in pluripotency and reprogramming. In *Cell Cycle*. https://doi.org/10.4161/cc.19846

Liu, Y., Liang, X., Dong, W., Fang, Y., Lv, J., Zhang, T., Fiskesund, R., Xie, J., Liu, J., Yin, X., Jin, X., Chen, D., Tang, K., Ma, J., Zhang, H., Yu, J., Yan, J., Liang, H., Mo, S., … Huang, B. (2018). Tumor-Repopulating Cells Induce PD-1 Expression in CD8+ T Cells by Transferring Kynurenine and AhR Activation. *Cancer Cell*. https://doi.org/10.1016/j.ccell.2018.02.005

Liu, Y., Liang, X., Yin, X., Lv, J., Tang, K., Ma, J., Ji, T., Zhang, H., Dong, W., Jin, X., Chen, D., Li, Y., Zhang, S., Xie, H. Q., Zhao, B., Zhao, T., Lu, J., Hu, Z. W., Cao, X., … Huang, B. (2017). Blockade of IDO-kynurenine-AhR metabolic circuitry abrogates IFN-γ-induced immunologic dormancy of tumor-repopulating cells. *Nature Communications*. https://doi.org/10.1038/ncomms15207

Loh, Y. H., Wu, Q., Chew, J. L., Vega, V. B., Zhang, W., Chen, X., Bourque, G., George, J., Leong, B., Liu, J., Wong, K. Y., Sung, K. W., Lee, C. W. H., Zhao, X. D., Chiu, K. P., Lipovich, L., Kuznetsov, V. A., Robson, P., Stanton, L. W., … Ng, H. H. (2006). The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genetics*. https://doi.org/10.1038/ng1760

Loumaye, E., Dreano, M., Galazka, A., Howles, C., Ham, L., Munafo, A., Eshkol, A., Giudice, E., De Luca, E., Sirna, A., Antonetti, F., Giartosio, C. E., Scaglia, L., Kelton, C., Campbell, R., Chappel, S., Duthu, B., Cymbalista, S., & Lepage, P. (1998). Recombinant follicle stimulating hormone: Development of the first biotechnology product for the treatment of infertility. *Human Reproduction Update*. https://doi.org/10.1093/humupd/4.6.862

Luderer, U., & Schwartz, N. B. (1994). Acute changes in pulsatile LH and FSH secretion after ovariectomy in rats: Treatment with oestradiol for 24 h suppresses LH, but not FSH for at least 48 h. *Journal of Reproduction and Fertility*. https://doi.org/10.1530/jrf.0.1000613

Ma, Q., Dong, L., & Whitlock, J. P. (1995). Transcriptional activation by the mouse Ah receptor. Interplay between multiple stimulatory and inhibitory functions. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.270.21.12697

Mahoney, K. M., Rennert, P. D., & Freeman, G. J. (2015). Combination cancer immunotherapy and new immunomodulatory targets. In *Nature Reviews Drug Discovery*. https://doi.org/10.1038/nrd4591

Maizels, E. T., Mukherjee, A., Sithanandam, G., Peters, C. A., Cottom, J., Mayo, K. E., & Hunzicker-Dunn, M. (2001). Developmental regulation of mitogen-activated protein kinase-activated kinases-2 and -3 (MAPKAPK-2/-3) in vivo during corpus luteum formation in the rat. *Molecular Endocrinology*. https://doi.org/10.1210/mend.15.5.0634

Manzella, C., Singhal, M., Alrefai, W. A., Saksena, S., Dudeja, P. K., & Gill, R. K. (2018). Serotonin is an endogenous regulator of intestinal CYP1A1 via AhR. *Scientific Reports*. https://doi.org/10.1038/s41598-018-24213-5

Marsh, J. M., & Savard, K. (1966). The stimulation of progesterone synthesis in bovine corpora lutea by adenosine 3', 5'-monophosphate (1). *Steroids*. https://doi.org/10.1016/0039-128X(66)90088-2

McClamrock, H. D. (2003). Recombinant gonadotropins. In *Clinical Obstetrics and Gynecology*. https://doi.org/10.1097/00003081-200306000-00009

McClellan, K. A., Gosden, R., & Taketo, T. (2003). Continuous loss of oocytes throughout meiotic prophase in the normal mouse ovary. *Developmental Biology*. https://doi.org/10.1016/S0012-1606(03)00132-5

McGee, E. A., & Hsueh, A. J. W. (2000). Initial and cyclic recruitment of ovarian follicles. In *Endocrine Reviews*. https://doi.org/10.1210/er.21.2.200

Menon, K. M. J., Menon, B., & Gulappa, T. (2018). Regulation of Luteinizing Hormone Receptor mRNA Expression in the Ovary: The Role of miR-122. In *Vitamins and Hormones*. https://doi.org/10.1016/bs.vh.2018.01.010

Merchant, H., & Zamboni, L. (1972). Presence of connections between follicles in juvenile mouse ovaries. *American Journal of Anatomy*. https://doi.org/10.1002/aja.1001340110

Metidji, A., Omenetti, S., Crotta, S., Li, Y., Nye, E., Ross, E., Li, V., Maradana, M. R., Schiering, C., & Stockinger, B. (2018). The Environmental Sensor AHR Protects from Inflammatory Damage by Maintaining Intestinal Stem Cell Homeostasis and Barrier Integrity. *Immunity*. https://doi.org/10.1016/j.immuni.2018.07.010

Meyer, B. K., & Perdew, G. H. (1999). Characterization of the AhR-hsp90-XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization. *Biochemistry*. https://doi.org/10.1021/bi982223w

Mezrich, J. D., Fechner, J. H., Zhang, X., Johnson, B. P., Burlingham, W. J., & Bradfield, C. A. (2010). An Interaction between Kynurenine and the Aryl Hydrocarbon Receptor Can Generate Regulatory T Cells. *The Journal of Immunology*. https://doi.org/10.4049/jimmunol.0903670

Milgrom, E., De Roux, N., Ghinea, N., Beau, I., Loosfelt, H., Vannier, B., Savouret, J. F., & Misrahi, M. (1997). Gonadotrophin and thyrotrophin receptors. *Hormone Research in Paediatrics*. https://doi.org/10.1159/000191310

Mimura, J., & Fujii-Kuriyama, Y. (2003). Functional role of AhR in the expression of toxic effects by TCDD. *Biochimica et Biophysica Acta – General Subjects*. https://doi.org/10.1016/S0304-4165(02)00485-3

Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T. N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., & Fujii-Kuriyama, Y. (1997). Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes to Cells*. https://doi.org/10.1046/j.1365-2443.1997.1490345.x

Minegishi, T., Tano, M., Kishi, H., Kameda, T., & Miyamoto, K. (1997). Follicle-stimulating hormone regulation on its receptor messenger ribonucleic acid levels in cultured rat granulosa cells. *Biochimica et Biophysica Acta – Molecular Cell Research*. https://doi.org/10.1016/S0167-4889(97)00095-5

Mukherjee, A., Park-Sarge, O. K., & Mayo, K. E. (1996). Gonadotropins induce rapid phosphorylation of the 3',5'-cyclic adenosine monophosphate response element binding protein in ovarian granulosa cells. *Endocrinology*. https://doi.org/10.1210/endo.137.8.8754745

Mulero-Navarro, S., & Fernandez-Salguero, P. M. (2016). New trends in Aryl hydrocarbon receptor biology. In *Frontiers in Cell and Developmental Biology*. https://doi.org/10.3389/fcell.2016.00045

Mullen, R. D., Colvin, S. C., Hunter, C. S., Savage, J. J., Walvoord, E. C., Bhangoo, A. P. S., Ten, S., Weigel, J., Pfäffle, R. W., & Rhodes, S. J. (2007). Roles of the LHX3 and LHX4 LIM-homeodomain factors in pituitary development. In *Molecular and Cellular Endocrinology*. https://doi.org/10.1016/j.mce.2006.12.019

Murray, I. A., Patterson, A. D., & Perdew, G. H. (2014). Aryl hydrocarbon receptor ligands in cancer: Friend and foe. In *Nature Reviews Cancer*. https://doi.org/10.1038/nrc3846

Nebert, D. W., & Gonzalez, F. J. (1987). P450 genes: structure, evolution, and regulation. In *Annual review of biochemistry*. https://doi.org/10.1146/annurev.bi.56.070187.004501

Neill, J. D., Plant, T. M., Pfaff, D. W., Challis, J. R. G., De Kretser, D. M., Richards, J. A. S., & Wassarman, P. M. (2006). Knobil and neill's physiology of reproduction. In *Knobil and Neill's Physiology of Reproduction*. https://dx.doi.org/10.1016/B978-0-12-397175-3.00026-0

Neri, T., Merico, V., Fiordaliso, F., Salio, M., Rebuzzini, P., Sacchi, L., Bellazzi, R., Redi, C. A., Zuccotti, M., & Garagna, S. (2011). The differentiation of cardiomyocytes from mouse embryonic stem cells is altered by dioxin. *Toxicology Letters*. https://doi.org/10.1016/j.toxlet.2011.02.008

Ngo, T. D., Partin, A. C., & Nam, Y. (2019). RNA Specificity and Autoregulation of DDX17, a Modulator of MicroRNA Biogenesis. *Cell Reports*. https://doi.org/10.1016/j.celrep.2019.11.059

Nimrod, A., Erickson, G. F., & Ryan, K. J. (1976). A specific FSH receptor in rat granulosa cells: Properties of binding in vitro. *Endocrinology*. https://doi.org/10.1210/endo-98-1-56

Nimz, M., Spitschak, M., Fürbass, R., & Vanselow, J. (2010). The pre-ovulatory luteinizing hormone surge is followed by down-regulation of CYP19A1, HSD3B1, and CYP17A1 and chromatin condensation of the corresponding promoters in bovine follicles. *Molecular Reproduction and Development*. https://doi.org/10.1002/mrd.21257

Nwabuobi, C., Arlier, S., Schatz, F., Guzeloglu-Kayisli, O., Lockwood, C. J., & Kayisli, U. A. (2017). hCG: Biological functions and clinical applications. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms18102037

O'Shea, J. D. (1970). An ultrastructural study of smooth muscle-like cells in the theca externa of ovarian follicles in the rat. *The Anatomical Record*. https://doi.org/10.1002/ar.1091670202

O'Shea, J. D., Cran, D. G., Hay, M. F., & Moor, R. M. (1978). Ultrastructure of the theca interna of ovarian follicles in sheep. *Cell and Tissue Research*. https://doi.org/10.1007/BF00229610

Öberg, M., Bergander, L., Håkansson, H., Rannug, U., & Rannug, A. (2005). Identification of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole, in cell culture medium, as a factor that controls the background aryl hydrocarbon receptor activity. *Toxicological Sciences*. https://doi.org/10.1093/toxsci/kfi154

Oliver, G., Mailhos, A., Wehr, R., Copeland, N. G., Jenkins, N. A., & Gruss, P. (1995). Six3, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. *Development*. 121: 4045-4055

Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G. J., Miller, C. L., Lutz, C., Radlwimmer, B., Lehmann, I., Von Deimling, A., Wick, W., & Platten, M. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*. https://doi.org/10.1038/nature10491

Ozturk, F., Li, Y., Zhu, X., Guda, C., & Nawshad, A. (2013). Systematic analysis of palatal transcriptome to identify cleft palate genes within TGFβ3-knockout mice alleles: RNA-Seq analysis of TGFβ3 Mice. *BMC Genomics*. https://doi.org/10.1186/1471-2164-14-113

Pandini, A., Denison, M. S., Song, Y., Soshilov, A. A., & Bonati, L. (2007). Structural and functional characterization of the aryl hydrocarbon receptor ligand binding domain by homology modeling and mutational analysis. *Biochemistry*. https://doi.org/10.1021/bi061460t

Paris, A., Tardif, N., Galibert, M. D., & Corre, S. (2021). AhR and cancer: From gene profiling to targeted therapy. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms22020752

Park, H., Jin, U. H., Karki, K., Jayaraman, A., Allred, C., Michelhaugh, S. K., Mittal, S., Chapkin, R. S., & Safe, S. (2020). Dopamine is an aryl hydrocarbon receptor agonist. *Biochemical Journal*. https://doi.org/10.1042/BCJ20200440

Peng, X. R., Hsueh, A. J. W., Lapolt, P. S., Bjersing, L., & Ny, T. (1991). Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. *Endocrinology*. https://doi.org/10.1210/endo-129-6-3200

Pepling, M. E. (2006). From primordial germ cell to primordial follicle: Mammalian female germ cell development. In *Genesis*. https://doi.org/10.1002/dvg.20258

Pepling, M. E., & Spradling, A. C. (1998). Female mouse germ cells form synchronously dividing cysts. *Development*. 125: 3323-3328

Peters, H. (1969). The development of the mouse ovary from birth to maturity. *Acta Endocrinologica*. https://doi.org/10.1530/acta.0.0620098

Peters, J. M., & Wiley, L. M. (1995). Evidence that murine preimplantation embryos express aryl hydrocarbon receptor. *Toxicology and Applied Pharmacology*. https://doi.org/10.1006/taap.1995.1186

Petrulis, J. R., Hord, N. G., & Perdew, G. H. (2000). Subcellular localization of the aryl hydrocarbon receptor is modulated by the immunophilin homolog hepatitis B virus X-associated protein 2. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M006873200

Pierce, J. G., & Parsons, T. F. (1981). Glycoprotein hormones: Structure and function. *Annual Review of Biochemistry*. https://doi.org/10.1146/annurev.bi.50.070181.002341

Platten, M., Litzenburger, U., & Wick, W. (2012). The aryl hydrocarbon receptor in tumor immunity. *OncoImmunology*. https://doi.org/10.4161/onci.19071

Poland, A., Glover, E. & Kende, A. S. (1976). Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. *Journal of Biological Chemistry*. 251(16), 4936-46

Puri, P., Little-Ihrig, L., Chandran, U., Law, N. C., Hunzicker-Dunn, M., & Zeleznik, A. J. (2016). Protein Kinase A: A Master Kinase of Granulosa Cell Differentiation. *Scientific Reports*. https://doi.org/10.1038/srep28132

Qiu, J., Guo, X., Chen, Z. ming E., He, L., Sonnenberg, G. F., Artis, D., Fu, Y. X., & Zhou, L. (2013). Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. *Immunity*. https://doi.org/10.1016/j.immuni.2013.08.002

Quiñones-Jenab, V., Jenab, S., Ogawa, S., Funabashi, T., Weesner, G. D., & Pfaff, D. W. (1996). Estrogen regulation of gonadotropin-releasing hormone receptor messenger RNA in female rat pituitary tissue. *Molecular Brain Research*. https://doi.org/10.1016/0169-328X(95)00322-J

Rentas, S., Holzapfel, N. T., Belew, M. S., Pratt, G. A., Voisin, V., Wilhelm, B. T., Bader, G. D., Yeo, G. W., & Hope, K. J. (2016). Musashi-2 attenuates AHR signalling to expand human haematopoietic stem cells. *Nature*. https://doi.org/10.1038/nature17665

Richards, J. S. (1994). Hormonal Control of Gene Expression in the Ovary. *Endocrine Reviews*. https://doi.org/10.1210/edrv-15-6-725

Richards, J. S., Fitzpatrick, S. L., Clemens, J. W., Morris, J. K., Alliston, T., & Sirois, J. (1995). Ovarian cell differentiation: A cascade of multiple hormones, cellular signals, and regulated genes. *Recent Progress in Hormone Research*. https://doi.org/10.1016/b978-0-12-571150-0.50014-7

Robker, R. L., & Richards, J. S. (1998). Hormone-induced proliferation and differentiation of granulosa cells: A coordinated balance of the cell cycle regulators cyclin D2 and p27(kip1). *Molecular Endocrinology*. https://doi.org/10.1210/mend.12.7.0138

Rochette, C., Jullien, N., Saveanu, A., Caldagues, E., Bergada, I., Braslavsky, D., Pfeifer, M., Reynaud, R., Herman, J. P., Barlier, A., Brue, T., Enjalbert, A., & Castinetti, F. (2015). Identifying the deleterious effect of rare LHX4 allelic variants, a challenging issue. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0126648

Rostami, N., Nikkhoo, A., Ajjoolabady, A., Azizi, G., Hojjat-Farsangi, M., Ghalamfarsa, G., Yousefi, B., Yousefi, M., & Jadidi-Niaragh, F. (2019). S1PR1 as a Novel Promising Therapeutic Target in Cancer Therapy. In *Molecular Diagnosis and Therapy*. https://doi.org/10.1007/s40291-019-00401-5

Ruman, J. I., Pollak, S., Trousdale, R. K., Klein, J., & Lustbader, J. W. (2005). Effects of long-acting recombinant human follicle-stimulating hormone analogs containing N-linked glycosylation on murine folliculogenesis. *Fertility and Sterility*. https://doi.org/10.1016/j.fertnstert.2004.12.027

Sadik, A., Somarribas Patterson, L. F., Öztürk, S., Mohapatra, S. R., Panitz, V., Secker, P. F., Pfänder, P., Loth, S., Salem, H., Prentzell, M. T., Berdel, B., Iskar, M., Faessler, E., Reuter, F., Kirst, I., Kalter, V., Foerster, K. I., Jäger, E., Guevara, C. R., … Opitz, C. A. (2020). IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. *Cell*. https://doi.org/10.1016/j.cell.2020.07.038

Safe, S., Jin, U. H., Park, H., Chapkin, R. S., & Jayaraman, A. (2020). Aryl hydrocarbon receptor (AHR) ligands as selective ahr modulators (SAHRMS). In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms21186654

Salvador, L. M., Park, Y., Cottom, J., Maizels, E. T., Jones, J. C. R., Schillace, R. V., Carr, D. W., Cheung, P., Allis, C. D., Jameson, J. L., & Hunzicker-Dunn, M. (2001). Follicle-stimulating Hormone Stimulates Protein Kinase A-mediated Histone H3 Phosphorylation and Acetylation Leading to Select Gene Activation in Ovarian Granulosa Cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.M106710200

Sarić, N., Selby, M., Ramaswamy, V., Kool, M., Stockinger, B., Hogstrand, C., Williamson, D., Marino, S., Taylor, M. D., Clifford, S. C., & Basson, M. A. (2020). The AHR pathway represses TGFβ-SMAD3 signalling and has a potent tumour suppressive role in SHH medulloblastoma. *Scientific Reports*. https://doi.org/10.1038/s41598-019-56876-z

Sarma, S. N., Nagano, R., & Ohsako, S. (2019). Tyroxine hydroxylase-positive neuronal cell population is increased by temporal dioxin exposure at early stage of differentiation from human embryonic stem cells. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms20112687

Schmidt, J. V., Su, G. H. T., Reddy, J. K., Simon, M. C., & Bradfield, C. A. (1996). Characterization of a murine Ahr null allele: Involvement of the Ah receptor in hepatic growth and development. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.93.13.6731

Schroeder, J. C., DiNatale, B. C., Murray, I. A., Flaveny, C. A., Liu, Q., Laurenzana, E. M., Lin, J. M., Strom, S. C., Omiecinski, C. J., Amin, S., & Perdew, G. H. (2010). The

uremic toxin 3-indoxyl sulfate is a potent endogenous agonist for the human aryl hydrocarbon receptor. *Biochemistry*. https://doi.org/10.1021/bi901786x

Sears, A. E., & Palczewski, K. (2016). Lecithin:Retinol Acyltransferase: A Key Enzyme Involved in the Retinoid (visual) Cycle. *Biochemistry*. https://doi.org/10.1021/acs.biochem.6b00319

Shoham, Z., & Insler, V. (1996). Recombinant technique and gonadotropins production: New era in reproductive medicine. In *Fertility and Sterility*. https://doi.org/10.1016/s0015-0282(16)58437-4

Sites, C. K., Patterson, K., Jamison, C. S., Friezner Degen, S. J., & LaBarbera, A. R. (1994). Follicle-stimulating hormone (FSH) increases FSH receptor messenger ribonucleic acid while decreasing FSH binding in cultured porcine granulosa cells. *Endocrinology*. https://doi.org/10.1210/endo.134.1.8275955

Smith, C. L., & Hager, G. L. (1997). Transcriptional regulation of mammalian genes in vivo. A tale of two templates. In *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.272.44.27493

Stanford, E. A., Wang, Z., Novikov, O., Mulas, F., Landesman-Bollag, E., Monti, S., Smith, B. W., Seldin, D. C., Murphy, G. J., & Sherr, D. H. (2016). The role of the aryl hydrocarbon receptor in the development of cells with the molecular and functional characteristics of cancer stem-like cells. *BMC Biology*. https://doi.org/10.1186/s12915-016-0240-y

Teijeiro, V., Yang, D., Majumdar, S., González, F., Rickert, R. W., Xu, C., Koche, R., Verma, N., Lai, E. C., & Huangfu, D. (2018). DICER1 Is Essential for Self-Renewal of Human Embryonic Stem Cells. *Stem Cell Reports*. https://doi.org/10.1016/j.stemcr.2018.07.013

Teino, I., Kuuse, S., Ingerpuu, S., Maimets, T., & Tiido, T. (2012). The Aryl hydrocarbon receptor regulates mouse Fshr promoter activity through an E-Box binding site. *Biology of Reproduction*. https://doi.org/10.1095/biolreprod.111.095596

Teino, I., Matvere, A., Kuuse, S., Ingerpuu, S., Maimets, T., Kristjuhan, A., & Tiido, T. (2014). Transcriptional repression of the Ahr gene by LHCGR signaling in pre-ovulatory granulosa cells is controlled by chromatin accessibility. *Molecular and Cellular Endocrinology*. https://doi.org/10.1016/j.mce.2013.10.011

Thompson, D. A., Li, Y., McHenry, C. L., Carlson, T. J., Ding, X., Sieving, P. A., Apfelstedt-Sylla, E., & Gal, A. (2001). Mutations in the gene encoding lecithin retinol acyltransferase are associated with early-onset severe retinal dystrophy. *Nature Genetics*. https://doi.org/10.1038/88828

Tilly, J. L. (1996). The molecular basis of ovarian cell death during germ cell attrition, follicular atresia, and luteolysis. In *Frontiers in bioscience : a journal and virtual library*. https://doi.org/10.2741/a111

Tingen, C., Kim, A., & Woodruff, T. K. (2009). The primordial pool of follicles and nest breakdown in mammalian ovaries. *Molecular Human Reproduction*. https://doi.org/10.1093/molehr/gap073

Toy, J., & Sundin, O. H. (1999). Expression of the Optx2 homeobox gene during mouse development. *Mechanisms of Development*. https://doi.org/10.1016/S0925-4773(99)00049-0

Villarroya-Beltri, C., Gutiérrez-Vázquez, C., Sánchez-Cabo, F., Pérez-Hernández, D., Vázquez, J., Martin-Cofreces, N., Martinez-Herrera, D. J., Pascual-Montano, A., Mittelbrunn, M., & Sánchez-Madrid, F. (2013). Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nature Communications*. https://doi.org/10.1038/ncomms3980

Wang, L. T., Chiou, S. S., Chai, C. Y., Hsi, E., Yokoyama, K. K., Wang, S. N., Huang, S. K., & Hsu, S. H. (2017). Intestine-specific homeobox gene ISX integrates IL6 signaling, tryptophan catabolism, and immune suppression. *Cancer Research*. https://doi.org/10.1158/0008-5472.CAN-17-0090

Wang, Q., Hisaka, K., Carreira, V., Ko, C. I., Fan, Y., Zhang, X., Biesiada, J., Medvedovic, M., & Puga, A. (2016). Ah receptor activation by dioxin disrupts activin, BMP, and WNT signals during the early differentiation of mouse embryonic stem cells and inhibits cardiomyocyte functions. *Toxicological Sciences*. https://doi.org/10.1093/toxsci/kfv246

Wang, Z., Snyder, M., Kenison, J. E., Yang, K., Lara, B., Lydell, E., Bennani, K., Novikov, O., Federico, A., Monti, S., & Sherr, D. H. (2021). How the AHR became important in cancer: The role of chronically active AHR in cancer aggression. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms22010387

Wigglesworth, K., Lee, K. B., Emori, C., Sugiura, K., & Eppig, J. J. (2015). Transcriptomic diversification of developing cumulus and mural granulosa cells in mouse ovarian follicles. *Biology of Reproduction*. https://doi.org/10.1095/biolreprod.114.121756

Wu, Q., Ohsako, S., Baba, T., Miyamoto, K., & Tohyama, C. (2002). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on preimplantation mouse embryos. *Toxicology*. https://doi.org/10.1016/S0300-483X(02)00047-1

Xue, P., Fu, J., & Zhou, Y. (2018). The aryl hydrocarbon receptor and tumor immunity. In *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2018.00286

Xue, Y., Jia, X., Li, C., Zhang, K., Li, L., Wu, J., Yuan, J., & Li, Q. (2019). DDX17 promotes hepatocellular carcinoma progression via inhibiting Klf4 transcriptional activity. *Cell Death and Disease*. https://doi.org/10.1038/s41419-019-2044-9

Yamamoto, T., Hatabayashi, K., Arita, M., Yajima, N., Takenaka, C., Suzuki, T., Takahashi, M., Oshima, Y., Hara, K., Kagawa, K., & Kawamata, S. (2019). Kynurenine signaling through the aryl hydrocarbon receptor maintains the undifferentiated state of human embryonic stem cells. *Science Signaling*. https://doi.org/10.1126/scisignal.aaw3306

Zeleznik, A. J., Schuler, H. M., & Reichert, L. E. (1981). Gonadotropin-binding sites in the rhesus monkey ovary: Role of the vasculature in the selective distribution of human chorionic gonadotropin to the preovulatory follicle. *Endocrinology*. https://doi.org/10.1210/endo-109-2-356

Zhang, Jianhao, Zong, H., Li, S., Zhang, D., Zhang, L., & Xia, Q. (2012). Activation of aryl hydrocarbon receptor suppresses invasion of esophageal squamous cell carcinoma cell lines. *Tumori*. https://doi.org/10.1700/1053.11514

Zhang, Jianzhong, Watson, A. J., Probst, M. R., Minehart, E., & Hankinson, O. (1996). Basis for the loss of aryl hydrocarbon receptor gene expression in clones of a mouse hepatoma cell line. *Molecular Pharmacology*. 50 (6), 1454-1462

Zhang, N., Liu, L., Fan, N., Zhang, Q., Wang, W., Zheng, M., Ma, L., Li, Y., & Shi, L. (2016). The requirement of SEPT2 and SEPT7 for migration and invasion in human breast cancer via MEK/ERK activation. *Oncotarget*. https://doi.org/10.18632/oncotarget.11402

Zhang, S., Qin, C., & Safe, S. H. (2003). Flavonoids as aryl hydrocarbon receptor agonists/antagonists: Effects of structure and cell context. *Environmental Health Perspectives*. https://doi.org/10.1289/ehp.6322

Zoller, L. C., & Weisz, J. (1979). A quantitative cytochemical study of glucose-6-phosphate dehydrogenase and Δ5-3 β-hydroxysteroid dehydrogenase activity in the

Membrana granulosa of the ovulable type of follicle of the rat. *Histochemistry*. https://doi.org/10.1007/BF00493314

Zoller, Lawrence C., & Weisz, J. (1980). A demonstration of regional differences in lysosome membrane permeability in the membrana granulosa of graafian follicles in cryostat sections of the rat ovary: A quantitative cytochemical study. *Endocrinology*. https://doi.org/10.1210/endo-106-3-871

https://www.uniprot.org/uniprot/P35869

SUMMARY IN ESTONIAN

Arüülsüsivesinike retseptori uurimine hiire munasarja granuloosarakkudes ja inimese embrüonaalsetes tüvirakkudes

Arüülsüsivesinike retseptor (AHR) on transkriptsioonifaktor, mida on tänaseks uuritud üle neljakümne aasta. Kuigi AHR avastati esialgu kui erinevate mürgiste keskkonnakemikaalide toime vahendaja, on nüüdseks kogunenud hulgaliselt tõendeid selle valgu füsioloogilise tähtsuse kohta. Sellele järeldusele on aidanud jõuda katsed AHR-i nokaut katseloomadega. Ühtlasi on praeguseks avastatud suur hulk endogeenseid ühendeid, millel on võime moduleerida rakkudes AHR-i aktiivsust. AHR-i bioloogilist tähtsust on näidatud paljudes organites ja kudedes. Enim on tähelepanu pälvinud selle retseptori osatähtsus immuunsüsteemis, maksas, nahas, kopsudes, rasvkoes, ajus ning vähkkoes. Andmed on samuti viidanud AHR-i olulisusele reproduktiivsüsteemis, aga ka arenevates kudedes ning tüvirakkudes. Tulenevalt selle valgu komplekssest iseloomust, on AHR-i uuringutes peetud vajalikuks pidada silmas asjaolu, et AHR-il võivad sageli olla raku- ja koespetsiifilised ülesanded. Käesolev töö keskendus arüülsüsivesinike retseptori uurimisele hiire munasarja granuloosarakkudes ning inimese embrüonaalsetes tüvirakkudes. Täpsemalt uuriti ka seda, milliste mehhanismide kaudu reguleeritakse *AHR*-i geeni avaldumist. Lisaks avardati teadmisi AHR-i füsioloogilisest tähtsusest, tehes kindlaks, milliste geenide avaldumist uuritav transkriptsioonifaktor otseselt mõjutab.

Ahr-i tähtsus munasarjas sai algselt ilmsiks katsete käigus, mis näitasid, et keskkonnas levinud saasteainel ja Ahr-i ligandil TCDD-l on negatiivne mõju endokriinsüsteemile, mõjutades oluliselt steroidhormoonide tootmist. Edasised katsed *Ahr*-i nokaut hiirtega näitasid, et Ahr on vajalik tiinuse säilitamises, munasarja folliikulite kasvus ning ovulatsioonis. Neil hiirtel täheldati häireid steroidhormoonide sünteesis, aeglasemat folliikulite kasvu ning väiksemat antraalsete folliikulite arvu. Veelgi enam, Ahr-i puudumine väljendus munasarja folliikulite vähenenud reageerimisvõimekuses gonadotropiinidele. Viimane oli põhjustatud vähenenud folliikuleid stimuleeriva hormooni (FSH) ning luteiniseeriva hormooni (LH) retseptorite arvust granuloosarakkude pinnal, millega kaasnevad häired ovulatsiooni toimumisel. Gonadotropiinide (FSH ja LH) rangelt reguleeritud tase organismis ning Ahr-i tähtsus munasarjas on andnud põhjust uurida, milline on gonadotropiinide mõju Ahr-ile.

Käesolevas töös näidati hiire munasarja granuloosarakkude näitel, et LH stimulatsioon, mis kutsub esile ovulatsiooni, pärssis neis rakkudes *Ahr*-i geeni avaldumist. LH represseeriv mõju Ahr-ile toimus läbi proteiin kinaas A (PKA) signaaliraja. Lisaks, LH suhteliselt kiire inhibeeriv mõju *Ahr*-ile toimus läbi transkriptsioonilise aktiivsuse vähenemise ning hõlmas epigeneetilist remodelleerimist *Ahr*-i promootoralal. Töö teises osas uuriti, kuidas toimub *Ahr*-i geeni avaldumine munasarjas küpseva folliikuli granuloosarakkudes, enne ovulatoorset

LH doosi. Töö tulemustest selgus, et folliikulite küpsemise käigus kuni preovulatoorse staadiumini suurenes nii Ahr-i mRNA kui ka valgu tase. Näidati, et valgu taseme suurenemiseks on vajalik mõlema gonadotropiini, nii FSH kui ka LH koosmõju. Katsete tulemused, mis näitasid FSH mõju Ahr-i mRNA, aga mitte valgu taseme suurenemisele, viitasid post-transkriptsioonilisele regulatsioonimehhanismile, milleks on tõenäoliselt vajalik LH signaaliraja aktivatsioon. Leiti, et Ahr-i valgu tase tõuseb esmajoones suurte, antraalsete folliikulite muraalsetes granuloosarakkudes. Mehhanismide uurimise tulemusena selgus, et *Ahr*-i mRNA taseme tõus oli põhjustatud geeni suurenenud transkriptsioonilisest aktiivsusest ning kontrollitud kromatiini pakkimise kaudu *Ahr*-i promootoralal. Leiti, et PKA signaalirada mõjus pärssivalt *Ahr*-i avaldumisele ka küpsevate folliikulite granuloosarakkudes *in vitro*. Hinnates lähemalt PKA aktiivsust granuloosarakkudes *in vivo*, järeldus, et hetkeline vähenemine PKA aktiivsuses folliikulite küpsemise keskstaadiumi võib olla vajalik selleks, et *Ahr*-i geen saaks avalduda. Muuhulgas, muutuv *Ahr*-i avaldumise tase diferentseeruvates hiire munasarja granuloosarakkudes viitab Ahr-i spetsiifilistele, ajast ning koest sõltuvatele füsioloogilistele ülesannetele.

Varasemad uuringud on täheldanud *Ahr*-i avaldumise taseme muutusi ka suurema arengupotentsiaaliga rakkudes kui granuloosarakud. Seda silmas pidades keskenduti käesoleva töö viimases osas AHR-i uurimisele embrüonaalsetes tüvirakkudes. Inimese embrüonaalsetes tüvirakkudes näidati AHR-i taseme vähenemist rakkude spontaansel diferentseerumisel. *AHR*-i avaldumise vähenemist nähti ka tüvirakkude suunatud diferentseerimisel neuraalseteks eellasrakkudesks, varajase mesodermi ja definitiivse endodermi rakkudeks, kuigi erinevate arengusuundade vahel ilmnesid märgatavad erinevused. Lisaks sellele näidati antud töös, et TCDD-l on oluline mõju tüvirakkude diferentseerumisele. Sellele viitas ülegenoomne sekveneerimisanalüüs, mille käigus hinnati antud kemikaali mõju AHR-i seondumisele DNA-ga, aga ka geenide avaldumise mustri muutusele. Viimaks, kombineerides ülegenoomse sekveneerimise andmeid AHR-i seondumiskohtadest genoomis, avaldunud geenide tasemest ning kromatiini ligipääsetavusest, tuvastati mitmed uudsed AHR-i märklaudgeenid neis rakkudes. Kokkuvõtvalt avardavad töös esitatud tulemused teadmisi AHR-i füsioloogilisest tähtsusest. Saadud teadmised *AHR*-i avaldumise ning ülesannete kohta on olulised selleks, et kasutada AHR-i moduleerimist mitmete haiguste ravi eesmärgil.

AKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisors Toivo Maimets and Arnold Kristjuhan. Thank You, Toivo, for the opportunity to work in Your lab. I am really grateful for all the lessons You have given me. I highly value the guidance and motivation I have received from You! Thank You, Arnold, for always finding time to give good advice, when it was needed.

I am thankful to Tarmo Tiido, whose laboratory I joined during my Bachelor studies. Tarmo introduced me to laboratory work and taught me many necessary skills. I am really grateful for You being very thorough and having a high standard of quality for work and I appreciate that You expected the same from me. Looking back on having those long conversations on various topics always brings a smile on my face. They were educational in so many ways and shaped me as a person. I am extremely thankful to Indrek Teino for passing on the skills and techniques for laboratory work. I am also grateful for your lessons on things that matter the most. I am thankful for You being so helpful and outgoing, whenever there were problems to be solved. I appreciate Your activity on organizing all the events for the Chair of Cell Biology, which have given so many great memories.

Thank You, Sulev Kuuse, for always being helpful in planning the experiments, providing the means necessary and keeping the animal facility up and running. This work could not have been done without You. I am thankful to Tõnis Org for great suggestions regarding laboratory work and the assistance on analysing experimental data.

I am indebted to Lilian Kadaja-Saarepuu for all the help and support, for always being so considerate and finding solutions to problems. Thank You, Tiiu, Teele and Janika, for the help with the administrative side.

I am really thankful to all the other members of the Chair of Cell Biology, who I have had the pleasure to encounter during the time I spent here. I am grateful to all of You for contributing to the creation of such a nice atmosphere to be in. Thank You, Martin, Allan, Artjom and Valmar for interesting conversations. Thank You, Annika, for good words. Your work on keeping things organized in the lab is highly appreciated. Thank You, Kersti Kristjuhan, Viljar Jaks, Sulev Ingerpuu, Dmitri Lubenets and Kristiina Mäemets-Allas for being inspirational to younger generations. I have really enjoyed all the common events with fellow cell biologists. Let's hope the studies on uncovering the secrets of cellular mechanics will continue on for a long time among with the preservation of the nice educational environment.

Last, but not least, I could not have reached to where I am without the support of my friends and family. I am especially thankful to Gerly for being on this journey together with me. I am thankful to my parents for the support and for pushing me forward. I acknowledge the valuable life lessons I have gained from my family and be sure to take them into account during the voyage into the unknown.

PUBLICATIONS

CURRICULUM VITAE

Name: Antti Matvere
Date of birth: September 7, 1990
Nationality: Estonian
Contact: Chair of Cell Biology,
Institute of Molecular and Cell Biology,
University of Tartu,
Riia Str. 23, 51010, Tartu, Estonia
E-mail: antti.matvere@gmail.com
Researcher ID: K-2096-2014
ORCID: 0000-0001-5999-9865

Education

2015–2021	University of Tartu, PhD studies in Molecular and Cell Biology
2012–2015	University of Tartu, MSc in Natural Sciences (Gene Technology)
2009–2012	University of Tartu, BSc in Natural Sciences (Gene Technology)
2006–2009	Tartu Hugo Treffner Gymnasium, Department of Maths and Physics
1997–2006	Võru Kesklinna Kool

Professional employment

01.09.2020–30.04.2021
University of Tartu, Faculty of Science and Technology,
Institute of Molecular and Cell Biology, Specialist (1.00)
01.03.2019–31.01.2020
University of Tartu, Faculty of Science and Technology,
Institute of Molecular and Cell Biology, Laboratory Assistant (0.50)
15.05.2018–28.02.2019
University of Tartu, Faculty of Science and Technology,
Institute of Molecular and Cell Biology, Laboratory Assistant (0.40)

Scientific work

Scientific research mainly focused of studying Ahr expression and functions in murine ovarian granulosa cells and human embryonic stem cells. Supervision of one bachelor student and co-supervision of one bachelor and one master student.

Fellowships

2018 Olev and Talvi Maimets Scholarship, University of Tartu Foundation

Special courses

2017 Competence course in laboratory animal science focusing on rats and mice for scientists and persons taking care of animals. Estonian University of Life Sciences, Tartu, Estonia
2018 AHR meeting, University Paris Descartes, Paris, France
2018 Workshop: Mutated neo-antigens in hepatocellular carcinoma, "HEPAMUT", ERA-NET TRANSCAN-2, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Naples, Italy

Language skills

Estonian Mother tongue
English C1

Additional information

Hobbies and interests:

chess, hiking, astronomy, playing guitar, jogging, soccer, badminton, snowboarding

List of publications:

Teino, Indrek; **Matvere, Antti**; Kuuse, Sulev; Ingerpuu, Sulev; Maimets, Toivo; Kristjuhan, Arnold; Tiido, Tarmo (2014). Transcriptional repression of the Ahr gene by LHCGR signalling in preovulatory granulosa cells is controlled by chromatin accessibility. Molecular and Cellular Endocrinology, 382 (1), 292–301. **https://doi.org/10.1016/j.mce.2013.10.011.**

Matvere, Antti; Teino, Indrek; Varik, Inge; Kuuse, Sulev; Tiido, Tarmo; Kristjuhan, Arnold; Maimets, Toivo (2019). FSH/LH-Dependent Upregulation of Ahr in Murine Granulosa Cells Is Controlled by PKA Signalling and Involves Epigenetic Regulation. International Journal of Molecular Sciences, 20 (12), 3068. **https://doi.org/10.3390/ijms20123068.**

Teino, Indrek; **Matvere, Antti**; Pook, Martin; Varik, Inge; Pajusaar, Laura; Uudeküll, Keyt; Vaher, Helen; Trei, Annika; Kristjuhan, Arnold; Org, Tõnis; Maimets, Toivo (2020). Impact of AHR Ligand TCDD on Human Embryonic Stem Cells and Early Differentiation. International Journal of Molecular Sciences, 21 (23), 9052. **https://doi.org/10.3390/ijms21239052.**

ELULOOKIRJELDUS

Name: Antti Matvere
Sünniaeg: September 7, 1990
Rahvus: Eestlane
Kontakt: Rakubioloogia õppetool,
Molekulaar- ja rakubioloogia instituut,
Tartu Ülikool,
Riia tn. 23, 51010, Tartu, Eesti
E-post: antti.matvere@gmail.com
Researcher ID: K-2096-2014
ORCID: 0000-0001-5999-9865

Hariduskäik

2015–2021 Tartu Ülikool, doktorantuur (molekulaar ja rakubioloogia)
2012–2015 Tartu Ülikool, magistrantuur (geenitehnoloogia)
2009–2012 Tartu Ülikool, bakalaureuseõpe (geenitehnoloogia)
2006–2009 Tartu Hugo Treffneri Gümnaasium, reaalharu
1997–2006 Võru Kesklinna Kool

Teenistuskäik

01.09.2020–30.04.2021
Tartu Ülikool, Loodus- ja täppisteaduste valdkond,
molekulaar- ja rakubioloogia instituut, spetsialist (1,00)
01.03.2019–31.01.2020
Tartu Ülikool, Loodus- ja täppisteaduste valdkond,
molekulaar- ja rakubioloogia instituut, laborant (0,50)
15.05.2018–28.02.2019
Tartu Ülikool, Loodus- ja täppisteaduste valdkond,
molekulaar- ja rakubioloogia instituut, laborant (0,40)

Teadustöö

Teadustöö peamiselt keskendunud arüülsüsivesinike retseptori uurimisele hiire munasarja granuloosarakkudes ja inimese embrüonaalsetes tüvirakkudes. Ühe bakalaureuseõppe tudengi juhendamine ja ühe bakalaureuseõppe tudengi ja ühe magistriõppe tudengi kaasjuhendamine.

Stipendiumid

2018 Olev ja Talvi Maimetsa stipendium, Tartu Ülikooli sihtasutus

Erialane täiendus

2017 Katseloomakursus. Eesti Maaülikool, Tartu, Eesti
2018 AHR konverents, University Paris Descartes, Pariis, Prantsusmaa
2018 Töötuba: Muteerunud neoantigeenid hepatotsellulaarses kartsinoomis, "HEPAMUT", ERA-NET TRANSCAN-2, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Napoli, Itaalia

Keeled

Eesti keel	Emakeel
Inglise keel	Hea kõnes ja kirjas

Lisainformatsioon

Huvialane tegevus:

male, matkamine, astronoomia, kitarrimäng, jooksmine, jalgpall, sulgpall, lumelauasõit

Publikatsioonide nimekiri:

Teino, Indrek; **Matvere, Antti**; Kuuse, Sulev; Ingerpuu, Sulev; Maimets, Toivo; Kristjuhan, Arnold; Tiido, Tarmo (2014). Transcriptional repression of the Ahr gene by LHCGR signalling in preovulatory granulosa cells is controlled by chromatin accessibility. Molecular and Cellular Endocrinology, 382 (1), 292–301. **https://doi.org/10.1016/j.mce.2013.10.011.**

Matvere, Antti; Teino, Indrek; Varik, Inge; Kuuse, Sulev; Tiido, Tarmo; Kristjuhan, Arnold; Maimets, Toivo (2019). FSH/LH-Dependent Upregulation of Ahr in Murine Granulosa Cells Is Controlled by PKA Signalling and Involves Epigenetic Regulation. International Journal of Molecular Sciences, 20 (12), 3068. **https://doi.org/10.3390/ijms20123068.**

Teino, Indrek; **Matvere, Antti**; Pook, Martin; Varik, Inge; Pajusaar, Laura; Uudeküll, Keyt; Vaher, Helen; Trei, Annika; Kristjuhan, Arnold; Org, Tõnis; Maimets, Toivo (2020). Impact of AHR Ligand TCDD on Human Embryonic Stem Cells and Early Differentiation. International Journal of Molecular Sciences, 21 (23), 9052. **https://doi.org/10.3390/ijms21239052.**

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous grassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan**. Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu**. Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.

42. **Veljo Kisand**. Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa**. Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa**. Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik**. Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo**. Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo**. Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots**. Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero**. Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees**. Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks**. Cholecystokinin (CCK) – induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and serotonin. Tartu, 1999, 123 p.
52. **Ebe Sild**. Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva**. Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna**. Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro**. Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane**. Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm**. Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg**. Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild**. The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu**. Studies of the TOL plasmid transcription factor XylS. Tartu, 2000, 88 p.
61. **Dina Lepik**. Modulation of viral DNA replication by tumor suppressor protein p53. Tartu, 2000, 106 p.
62. **Kai Vellak**. Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu, 2000, 122 p.

63. **Jonne Kotta**. Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu, 2000, 160 p.
64. **Georg Martin**. Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000, 139 p.
65. **Silvia Sepp**. Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira**. On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000, 96 p.
67. **Priit Zingel**. The role of planktonic ciliates in lake ecosystems. Tartu, 2001, 111 p.
68. **Tiit Teder**. Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu, 2001, 122 p.
69. **Hannes Kollist**. Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu, 2001, 80 p.
70. **Reet Marits**. Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu, 2001, 112 p.
71. **Vallo Tilgar**. Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002, 126 p.
72. **Rita Hõrak**. Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002, 108 p.
73. **Liina Eek-Piirsoo**. The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002, 74 p.
74. **Krõõt Aasamaa**. Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002, 110 p.
75. **Nele Ingerpuu**. Bryophyte diversity and vascular plants. Tartu, 2002, 112 p.
76. **Neeme Tõnisson**. Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002, 124 p.
77. **Margus Pensa**. Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003, 110 p.
78. **Asko Lõhmus**. Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003, 168 p.
79. **Viljar Jaks**. p53 – a switch in cellular circuit. Tartu, 2003, 160 p.
80. **Jaana Männik**. Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003, 140 p.
81. **Marek Sammul**. Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003, 159 p
82. **Ivar Ilves**. Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003, 89 p.
83. **Andres Männik**. Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003, 109 p.

84. **Ivika Ostonen**. Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003, 158 p.
85. **Gudrun Veldre**. Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003, 199 p.
86. **Ülo Väli**. The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004, 159 p.
87. **Aare Abroi**. The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004, 135 p.
88. **Tiina Kahre**. Cystic fibrosis in Estonia. Tartu, 2004, 116 p.
89. **Helen Orav-Kotta**. Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004, 117 p.
90. **Maarja Öpik**. Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004, 175 p.
91. **Kadri Tali**. Species structure of *Neotinea ustulata*. Tartu, 2004, 109 p.
92. **Kristiina Tambets**. Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004, 163 p.
93. **Arvi Jõers**. Regulation of p53-dependent transcription. Tartu, 2004, 103 p.
94. **Lilian Kadaja**. Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004, 103 p.
95. **Jaak Truu**. Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004, 128 p.
96. **Maire Peters**. Natural horizontal transfer of the *pheBA* operon. Tartu, 2004, 105 p.
97. **Ülo Maiväli**. Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004, 130 p.
98. **Merit Otsus**. Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004, 103 p.
99. **Mikk Heidemaa**. Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004, 167 p.
100. **Ilmar Tõnno**. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004, 111 p.
101. **Lauri Saks**. Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004, 144 p.
102. **Siiri Rootsi**. Human Y-chromosomal variation in European populations. Tartu, 2004, 142 p.
103. **Eve Vedler**. Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.
104. **Andres Tover**. Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 126 p.
105. **Helen Udras**. Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005, 100 p.

106. **Ave Suija**. Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005, 162 p.
107. **Piret Lõhmus**. Forest lichens and their substrata in Estonia. Tartu, 2005, 162 p.
108. **Inga Lips**. Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005, 156 p.
109. **Krista Kaasik**. Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005, 121 p.
110. **Juhan Javoiš**. The effects of experience on host acceptance in ovipositing moths. Tartu, 2005, 112 p.
111. **Tiina Sedman**. Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005, 103 p.
112. **Ruth Aguraiuja**. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005, 112 p.
113. **Riho Teras**. Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 106 p.
114. **Mait Metspalu**. Through the course of prehistory in India: tracing the mtDNA trail. Tartu, 2005, 138 p.
115. **Elin Lõhmussaar**. The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006, 124 p.
116. **Priit Kupper**. Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006, 126 p.
117. **Heili Ilves**. Stress-induced transposition of Tn*4652* in *Pseudomonas Putida*. Tartu, 2006, 120 p.
118. **Silja Kuusk**. Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006, 126 p.
119. **Kersti Püssa**. Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006, 90 p.
120. **Lea Tummeleht**. Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006, 94 p.
121. **Toomas Esperk**. Larval instar as a key element of insect growth schedules. Tartu, 2006, 186 p.
122. **Harri Valdmann**. Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers**. Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli**. Gata3 and Gata2 in inner ear development. Tartu, 2007, 123 p.
125. **Kai Rünk**. Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007, 143 p.

126. **Aveliina Helm**. Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007, 89 p.
127. **Leho Tedersoo**. Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007, 233 p.
128. **Marko Mägi**. The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007, 135 p.
129. **Valeria Lulla**. Replication strategies and applications of Semliki Forest virus. Tartu, 2007, 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007, 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007, 171 p.
132. **Tatjana Krama**. Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007, 112 p.
133. **Signe Saumaa**. The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida.* Tartu, 2007, 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007, 96 p.
135. **Priit Kilgas**. Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007, 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007, 95 p.
137. **Kärt Padari**. Protein transduction mechanisms of transportans. Tartu, 2008, 128 p.
138. **Siiri-Lii Sandre**. Selective forces on larval colouration in a moth. Tartu, 2008, 125 p.
139. **Ülle Jõgar**. Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008, 99 p.
140. **Lauri Laanisto**. Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008, 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008, 105 p.
142. **Birgot Paavel**. Bio-optical properties of turbid lakes. Tartu, 2008, 175 p.
143. **Kaire Torn**. Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg**. Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd**. Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.
146. **Lauri Saag**. Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.

147. **Ulvi Karu**. Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm**. Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks**. Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu**. Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš**. Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko**. Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast**. Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats**. Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova**. The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida.* Tartu, 2009, 124 p.
157. **Tsipe Aavik**. Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2009, 112 p.
158. **Kaja Kiiver**. Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.
159. **Meelis Kadaja**. Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell. Tartu, 2009, 126 p.
160. **Pille Hallast**. Human and chimpanzee Luteinizing hormone/Chorionic Gonadotropin beta (*LHB/CGB*) gene clusters: diversity and divergence of young duplicated genes. Tartu, 2009, 168 p.
161. **Ain Vellak**. Spatial and temporal aspects of plant species conservation. Tartu, 2009, 86 p.
162. **Triinu Remmel**. Body size evolution in insects with different colouration strategies: the role of predation risk. Tartu, 2009, 168 p.
163. **Jaana Salujõe**. Zooplankton as the indicator of ecological quality and fish predation in lake ecosystems. Tartu, 2009, 129 p.
164. **Ele Vahtmäe**. Mapping benthic habitat with remote sensing in optically complex coastal environments. Tartu, 2009, 109 p.
165. **Liisa Metsamaa**. Model-based assessment to improve the use of remote sensing in recognition and quantitative mapping of cyanobacteria. Tartu, 2009, 114 p.

166. **Pille Säälik**. The role of endocytosis in the protein transduction by cell-penetrating peptides. Tartu, 2009, 155 p.
167. **Lauri Peil**. Ribosome assembly factors in *Escherichia coli.* Tartu, 2009, 147 p.
168. **Lea Hallik**. Generality and specificity in light harvesting, carbon gain capacity and shade tolerance among plant functional groups. Tartu, 2009, 99 p.
169. **Mariliis Tark**. Mutagenic potential of DNA damage repair and tolerance mechanisms under starvation stress. Tartu, 2009, 191 p.
170. **Riinu Rannap**. Impacts of habitat loss and restoration on amphibian populations. Tartu, 2009, 117 p.
171. **Maarja Adojaan**. Molecular variation of HIV-1 and the use of this knowledge in vaccine development. Tartu, 2009, 95 p.
172. **Signe Altmäe**. Genomics and transcriptomics of human induced ovarian folliculogenesis. Tartu, 2010, 179 p.
173. **Triin Suvi**. Mycorrhizal fungi of native and introduced trees in the Seychelles Islands. Tartu, 2010, 107 p.
174. **Velda Lauringson**. Role of suspension feeding in a brackish-water coastal sea. Tartu, 2010, 123 p.
175. **Eero Talts**. Photosynthetic cyclic electron transport – measurement and variably proton-coupled mechanism. Tartu, 2010, 121 p.
176. **Mari Nelis**. Genetic structure of the Estonian population and genetic distance from other populations of European descent. Tartu, 2010, 97 p.
177. **Kaarel Krjutškov**. Arrayed Primer Extension-2 as a multiplex PCR-based method for nucleic acid variation analysis: method and applications. Tartu, 2010, 129 p.
178. **Egle Köster**. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu, 2010, 101 p.
179. **Erki Õunap**. Systematic studies on the subfamily Sterrhinae (Lepidoptera: Geometridae). Tartu, 2010, 111 p.
180. **Merike Jõesaar**. Diversity of key catabolic genes at degradation of phenol and *p*-cresol in pseudomonads. Tartu, 2010, 125 p.
181. **Kristjan Herkül**. Effects of physical disturbance and habitat-modifying species on sediment properties and benthic communities in the northern Baltic Sea. Tartu, 2010, 123 p.
182. **Arto Pulk**. Studies on bacterial ribosomes by chemical modification approaches. Tartu, 2010, 161 p.
183. **Maria Põllupüü**. Ecological relations of cladocerans in a brackish-water ecosystem. Tartu, 2010, 126 p.
184. **Toomas Silla**. Study of the segregation mechanism of the Bovine Papillomavirus Type 1. Tartu, 2010, 188 p.
185. **Gyaneshwer Chaubey**. The demographic history of India: A perspective based on genetic evidence. Tartu, 2010, 184 p.
186. **Katrin Kepp**. Genes involved in cardiovascular traits: detection of genetic variation in Estonian and Czech populations. Tartu, 2010, 164 p.

187. **Virve Sõber**. The role of biotic interactions in plant reproductive performance. Tartu, 2010, 92 p.
188. **Kersti Kangro**. The response of phytoplankton community to the changes in nutrient loading. Tartu, 2010, 144 p.
189. **Joachim M. Gerhold**. Replication and Recombination of mitochondrial DNA in Yeast. Tartu, 2010, 120 p.
190. **Helen Tammert**. Ecological role of physiological and phylogenetic diversity in aquatic bacterial communities. Tartu, 2010, 140 p.
191. **Elle Rajandu**. Factors determining plant and lichen species diversity and composition in Estonian *Calamagrostis* and *Hepatica* site type forests. Tartu, 2010, 123 p.
192. **Paula Ann Kivistik**. ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida*. Tartu, 2010, 118 p.
193. **Siim Sõber**. Blood pressure genetics: from candidate genes to genome-wide association studies. Tartu, 2011, 120 p.
194. **Kalle Kipper**. Studies on the role of helix 69 of 23S rRNA in the factor-dependent stages of translation initiation, elongation, and termination. Tartu, 2011, 178 p.
195. **Triinu Siibak**. Effect of antibiotics on ribosome assembly is indirect. Tartu, 2011, 134 p.
196. **Tambet Tõnissoo**. Identification and molecular analysis of the role of guanine nucleotide exchange factor RIC-8 in mouse development and neural function. Tartu, 2011, 110 p.
197. **Helin Räägel**. Multiple faces of cell-penetrating peptides – their intracellular trafficking, stability and endosomal escape during protein transduction. Tartu, 2011, 161 p.
198. **Andres Jaanus**. Phytoplankton in Estonian coastal waters – variability, trends and response to environmental pressures. Tartu, 2011, 157 p.
199. **Tiit Nikopensius**. Genetic predisposition to nonsyndromic orofacial clefts. Tartu, 2011, 152 p.
200. **Signe Värv**. Studies on the mechanisms of RNA polymerase II-dependent transcription elongation. Tartu, 2011, 108 p.
201. **Kristjan Välk**. Gene expression profiling and genome-wide association studies of non-small cell lung cancer. Tartu, 2011, 98 p.
202. **Arno Põllumäe**. Spatio-temporal patterns of native and invasive zooplankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.
203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demographic processes and variations in diet in northern Eurasia. Tartu, 2011, 143 p.
205. **Teele Jairus**. Species composition and host preference among ectomycorrhizal fungi in Australian and African ecosystems. Tartu, 2011, 106 p.
206. **Kessy Abarenkov**. PlutoF – cloud database and computing services supporting biological research. Tartu, 2011, 125 p.

207. **Marina Grigorova**. Fine-scale genetic variation of follicle-stimulating hormone beta-subunit coding gene (*FSHB*) and its association with reproductive health. Tartu, 2011, 184 p.
208. **Anu Tiitsaar**. The effects of predation risk and habitat history on butterfly communities. Tartu, 2011, 97 p.
209. **Elin Sild**. Oxidative defences in immunoecological context: validation and application of assays for nitric oxide production and oxidative burst in a wild passerine. Tartu, 2011, 105 p.
210. **Irja Saar**. The taxonomy and phylogeny of the genera *Cystoderma* and *Cystodermella* (Agaricales, Fungi). Tartu, 2012, 167 p.
211. **Pauli Saag**. Natural variation in plumage bacterial assemblages in two wild breeding passerines. Tartu, 2012, 113 p.
212. **Aleksei Lulla**. Alphaviral nonstructural protease and its polyprotein substrate: arrangements for the perfect marriage. Tartu, 2012, 143 p.
213. **Mari Järve**. Different genetic perspectives on human history in Europe and the Caucasus: the stories told by uniparental and autosomal markers. Tartu, 2012, 119 p.
214. **Ott Scheler**. The application of tmRNA as a marker molecule in bacterial diagnostics using microarray and biosensor technology. Tartu, 2012, 93 p.
215. **Anna Balikova**. Studies on the functions of tumor-associated mucin-like leukosialin (CD43) in human cancer cells. Tartu, 2012, 129 p.
216. **Triinu Kõressaar**. Improvement of PCR primer design for detection of prokaryotic species. Tartu, 2012, 83 p.
217. **Tuul Sepp**. Hematological health state indices of greenfinches: sources of individual variation and responses to immune system manipulation. Tartu, 2012, 117 p.
218. **Rya Ero**. Modifier view of the bacterial ribosome. Tartu, 2012, 146 p.
219. **Mohammad Bahram**. Biogeography of ectomycorrhizal fungi across different spatial scales. Tartu, 2012, 165 p.
220. **Annely Lorents**. Overcoming the plasma membrane barrier: uptake of amphipathic cell-penetrating peptides induces influx of calcium ions and downstream responses. Tartu, 2012, 113 p.
221. **Katrin Männik**. Exploring the genomics of cognitive impairment: whole-genome SNP genotyping experience in Estonian patients and general population. Tartu, 2012, 171 p.
222. **Marko Prous**. Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae). Tartu, 2012, 192 p.
223. **Triinu Visnapuu**. Levansucrases encoded in the genome of *Pseudomonas syringae* pv. tomato DC3000: heterologous expression, biochemical characterization, mutational analysis and spectrum of polymerization products. Tartu, 2012, 160 p.
224. **Nele Tamberg**. Studies on Semliki Forest virus replication and pathogenesis. Tartu, 2012, 109 p.

225. **Tõnu Esko**. Novel applications of SNP array data in the analysis of the genetic structure of Europeans and in genetic association studies. Tartu, 2012, 149 p.
226. **Timo Arula**. Ecology of early life-history stages of herring *Clupea harengus membras* in the northeastern Baltic Sea. Tartu, 2012, 143 p.
227. **Inga Hiiesalu**. Belowground plant diversity and coexistence patterns in grassland ecosystems. Tartu, 2012, 130 p.
228. **Kadri Koorem**. The influence of abiotic and biotic factors on small-scale plant community patterns and regeneration in boreonemoral forest. Tartu, 2012, 114 p.
229. **Liis Andresen**. Regulation of virulence in plant-pathogenic pectobacteria. Tartu, 2012, 122 p.
230. **Kaupo Kohv**. The direct and indirect effects of management on boreal forest structure and field layer vegetation. Tartu, 2012, 124 p.
231. **Mart Jüssi**. Living on an edge: landlocked seals in changing climate. Tartu, 2012, 114 p.
232. **Riina Klais**. Phytoplankton trends in the Baltic Sea. Tartu, 2012, 136 p.
233. **Rauno Veeroja**. Effects of winter weather, population density and timing of reproduction on life-history traits and population dynamics of moose (*Alces alces*) in Estonia. Tartu, 2012, 92 p.
234. **Marju Keis**. Brown bear (*Ursus arctos*) phylogeography in northern Eurasia. Tartu, 2013, 142 p.
235. **Sergei Põlme**. Biogeography and ecology of *alnus*- associated ectomycorrhizal fungi – from regional to global scale. Tartu, 2013, 90 p.
236. **Liis Uusküla**. Placental gene expression in normal and complicated pregnancy. Tartu, 2013, 173 p.
237. **Marko Lõoke**. Studies on DNA replication initiation in *Saccharomyces cerevisiae.* Tartu, 2013, 112 p.
238. **Anne Aan**. Light- and nitrogen-use and biomass allocation along productivity gradients in multilayer plant communities. Tartu, 2013, 127 p.
239. **Heidi Tamm**. Comprehending phylogenetic diversity – case studies in three groups of ascomycetes. Tartu, 2013, 136 p.
240. **Liina Kangur**. High-Pressure Spectroscopy Study of Chromophore-Binding Hydrogen Bonds in Light-Harvesting Complexes of Photosynthetic Bacteria. Tartu, 2013, 150 p.
241. **Margus Leppik**. Substrate specificity of the multisite specific pseudouridine synthase RluD. Tartu, 2013, 111 p.
242. **Lauris Kaplinski**. The application of oligonucleotide hybridization model for PCR and microarray optimization. Tartu, 2013, 103 p.
243. **Merli Pärnoja**. Patterns of macrophyte distribution and productivity in coastal ecosystems: effect of abiotic and biotic forcing. Tartu, 2013, 155 p.
244. **Tõnu Margus**. Distribution and phylogeny of the bacterial translational GTPases and the Mqsr/YgiT regulatory system. Tartu, 2013, 126 p.
245. **Pille Mänd**. Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants. Tartu, 2013, 128 p.

246. **Mario Plaas**. Animal model of Wolfram Syndrome in mice: behavioural, biochemical and psychopharmacological characterization. Tartu, 2013, 144 p.
247. **Georgi Hudjašov**. Maps of mitochondrial DNA, Y-chromosome and tyrosinase variation in Eurasian and Oceanian populations. Tartu, 2013, 115 p.
248. **Mari Lepik**. Plasticity to light in herbaceous plants and its importance for community structure and diversity. Tartu, 2013, 102 p.
249. **Ede Leppik**. Diversity of lichens in semi-natural habitats of Estonia. Tartu, 2013, 151 p.
250. **Ülle Saks**. Arbuscular mycorrhizal fungal diversity patterns in boreo-nemoral forest ecosystems. Tartu, 2013, 151 p.
251. **Eneli Oitmaa**. Development of arrayed primer extension microarray assays for molecular diagnostic applications. Tartu, 2013, 147 p.
252. **Jekaterina Jutkina**. The horizontal gene pool for aromatics degradation: bacterial catabolic plasmids of the Baltic Sea aquatic system. Tartu, 2013, 121 p.
253. **Helen Vellau**. Reaction norms for size and age at maturity in insects: rules and exceptions. Tartu, 2014, 132 p.
254. **Randel Kreitsberg**. Using biomarkers in assessment of environmental contamination in fish – new perspectives. Tartu, 2014, 107 p.
255. **Krista Takkis**. Changes in plant species richness and population performance in response to habitat loss and fragmentation.Tartu, 2014, 141 p.
256. **Liina Nagirnaja**. Global and fine-scale genetic determinants of recurrent pregnancy loss. Tartu, 2014, 211 p.
257. **Triin Triisberg**. Factors influencing the re-vegetation of abandoned extracted peatlands in Estonia. Tartu, 2014, 133 p.
258. **Villu Soon**. A phylogenetic revision of the *Chrysis ignita* species group (Hymenoptera: Chrysididae) with emphasis on the northern European fauna. Tartu, 2014, 211 p.
259. **Andrei Nikonov**. RNA-Dependent RNA Polymerase Activity as a Basis for the Detection of Positive-Strand RNA Viruses by Vertebrate Host Cells. Tartu, 2014, 207 p.
260. **Eele Õunapuu-Pikas**. Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences. Tartu, 2014, 135 p.
261. **Marju Männiste**. Physiological ecology of greenfinches: information content of feathers in relation to immune function and behavior. Tartu, 2014, 121 p.
262. **Katre Kets**. Effects of elevated concentrations of CO_2 and O_3 on leaf photosynthetic parameters in *Populus tremuloides*: diurnal, seasonal and inter-annual patterns. Tartu, 2014, 115 p.
263. **Külli Lokko**. Seasonal and spatial variability of zoopsammon communities in relation to environmental parameters. Tartu, 2014, 129 p.
264. **Olga Žilina**. Chromosomal microarray analysis as diagnostic tool: Estonian experience. Tartu, 2014, 152 p.

265. **Kertu Lõhmus**. Colonisation ecology of forest-dwelling vascular plants and the conservation value of rural manor parks. Tartu, 2014, 111 p.
266. **Anu Aun**. Mitochondria as integral modulators of cellular signaling. Tartu, 2014, 167 p.
267. **Chandana Basu Mallick**. Genetics of adaptive traits and gender-specific demographic processes in South Asian populations. Tartu, 2014, 160 p.
268. **Riin Tamme**. The relationship between small-scale environmental heterogeneity and plant species diversity. Tartu, 2014, 130 p.
269. **Liina Remm**. Impacts of forest drainage on biodiversity and habitat quality: implications for sustainable management and conservation. Tartu, 2015, 126 p.
270. **Tiina Talve**. Genetic diversity and taxonomy within the genus *Rhinanthus*. Tartu, 2015, 106 p.
271. **Mehis Rohtla**. Otolith sclerochronological studies on migrations, spawning habitat preferences and age of freshwater fishes inhabiting the Baltic Sea. Tartu, 2015, 137 p.
272. **Alexey Reshchikov**. The world fauna of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae). Tartu, 2015, 247 p.
273. **Martin Pook**. Studies on artificial and extracellular matrix protein-rich surfaces as regulators of cell growth and differentiation. Tartu, 2015, 142 p.
274. **Mai Kukumägi**. Factors affecting soil respiration and its components in silver birch and Norway spruce stands. Tartu, 2015, 155 p.
275. **Helen Karu**. Development of ecosystems under human activity in the North-East Estonian industrial region: forests on post-mining sites and bogs. Tartu, 2015, 152 p.
276. **Hedi Peterson**. Exploiting high-throughput data for establishing relationships between genes. Tartu, 2015, 186 p.
277. **Priit Adler**. Analysis and visualisation of large scale microarray data, Tartu, 2015, 126 p.
278. **Aigar Niglas**. Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency. Tartu, 2015, 152 p.
279. **Silja Laht**. Classification and identification of conopeptides using profile hidden Markov models and position-specific scoring matrices. Tartu, 2015, 100 p.
280. **Martin Kesler**. Biological characteristics and restoration of Atlantic salmon *Salmo salar* populations in the Rivers of Northern Estonia. Tartu, 2015, 97 p.
281. **Pratyush Kumar Das**. Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling. Tartu, 2015, 205 p
282. **Priit Palta**. Computational methods for DNA copy number detection. Tartu, 2015, 130 p.
283. **Julia Sidorenko**. Combating DNA damage and maintenance of genome integrity in pseudomonads. Tartu, 2015, 174 p.
284. **Anastasiia Kovtun-Kante**. Charophytes of Estonian inland and coastal waters: distribution and environmental preferences. Tartu, 2015, 97 p.

285. **Ly Lindman**. The ecology of protected butterfly species in Estonia. Tartu, 2015, 171 p.
286. **Jaanis Lodjak**. Association of Insulin-like Growth Factor I and Corticosterone with Nestling Growth and Fledging Success in Wild Passerines. Tartu, 2016, 113 p.
287. **Ann Kraut**. Conservation of Wood-Inhabiting Biodiversity – Semi-Natural Forests as an Opportunity. Tartu, 2016, 141 p.
288. **Tiit Örd**. Functions and regulation of the mammalian pseudokinase TRIB3. Tartu, 2016, 182. p.
289. **Kairi Käiro**. Biological Quality According to Macroinvertebrates in Streams of Estonia (Baltic Ecoregion of Europe): Effects of Human-induced Hydromorphological Changes. Tartu, 2016, 126 p.
290. **Leidi Laurimaa**. *Echinococcus multilocularis* and other zoonotic parasites in Estonian canids. Tartu, 2016, 144 p.
291. **Helerin Margus**. Characterization of cell-penetrating peptide/nucleic acid nanocomplexes and their cell-entry mechanisms. Tartu, 2016, 173 p.
292. **Kadri Runnel**. Fungal targets and tools for forest conservation. Tartu, 2016, 157 p.
293. **Urmo Võsa**. MicroRNAs in disease and health: aberrant regulation in lung cancer and association with genomic variation. Tartu, 2016, 163 p.
294. **Kristina Mäemets-Allas**. Studies on cell growth promoting AKT signaling pathway – a promising anti-cancer drug target. Tartu, 2016, 146 p.
295. **Janeli Viil**. Studies on cellular and molecular mechanisms that drive normal and regenerative processes in the liver and pathological processes in Dupuytren's contracture. Tartu, 2016, 175 p.
296. **Ene Kook**. Genetic diversity and evolution of *Pulmonaria angustifolia* L. and *Myosotis laxa sensu lato* (Boraginaceae). Tartu, 2016, 106 p.
297. **Kadri Peil**. RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*. Tartu, 2016, 113 p.
298. **Katrin Ruisu**. The role of RIC8A in mouse development and its function in cell-matrix adhesion and actin cytoskeletal organisation. Tartu, 2016, 129 p.
299. **Janely Pae**. Translocation of cell-penetrating peptides across biological membranes and interactions with plasma membrane constituents. Tartu, 2016, 126 p.
300. **Argo Ronk**. Plant diversity patterns across Europe: observed and dark diversity. Tartu, 2016, 153 p.
301. **Kristiina Mark**. Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota). Tartu, 2016, 181 p.
302. **Jaak-Albert Metsoja**. Vegetation dynamics in floodplain meadows: influence of mowing and sediment application. Tartu, 2016, 140 p.
303. **Hedvig Tamman**. The GraTA toxin-antitoxin system of *Pseudomonas putida*: regulation and role in stress tolerance. Tartu, 2016, 154 p.

304. **Kadri Pärtel**. Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Tartu, 2016, 183 p.
305. **Maris Hindrikson**. Grey wolf (*Canis lupus*) populations in Estonia and Europe: genetic diversity, population structure and -processes, and hybridization between wolves and dogs. Tartu, 2016, 121 p.
306. **Polina Degtjarenko**. Impacts of alkaline dust pollution on biodiversity of plants and lichens: from communities to genetic diversity. Tartu, 2016, 126 p.
307. **Liina Pajusalu**. The effect of CO_2 enrichment on net photosynthesis of macrophytes in a brackish water environment. Tartu, 2016, 126 p.
308. **Stoyan Tankov**. Random walks in the stringent response. Tartu, 2016, 94 p.
309. **Liis Leitsalu**. Communicating genomic research results to population-based biobank participants. Tartu, 2016, 158 p.
310. **Richard Meitern**. Redox physiology of wild birds: validation and application of techniques for detecting oxidative stress. Tartu, 2016, 134 p.
311. **Kaie Lokk**. Comparative genome-wide DNA methylation studies of healthy human tissues and non-small cell lung cancer tissue. Tartu, 2016, 127 p.
312. **Mihhail Kurašin**. Processivity of cellulases and chitinases. Tartu, 2017, 132 p.
313. **Carmen Tali**. Scavenger receptors as a target for nucleic acid delivery with peptide vectors. Tartu, 2017, 155 p.
314. **Katarina Oganjan**. Distribution, feeding and habitat of benthic suspension feeders in a shallow coastal sea. Tartu, 2017, 132 p.
315. **Taavi Paal**. Immigration limitation of forest plants into wooded landscape corridors. Tartu, 2017, 145 p.
316. **Kadri Õunap**. The Williams-Beuren syndrome chromosome region protein WBSCR22 is a ribosome biogenesis factor. Tartu, 2017, 135 p.
317. **Riin Tamm**. In-depth analysis of factors affecting variability in thiopurine methyltransferase activity. Tartu, 2017, 170 p.
318. **Keiu Kask**. The role of RIC8A in the development and regulation of mouse nervous system. Tartu, 2017, 184 p.
319. **Tiia Möller**. Mapping and modelling of the spatial distribution of benthic macrovegetation in the NE Baltic Sea with a special focus on the eelgrass *Zostera marina* Linnaeus, 1753. Tartu, 2017, 162 p.
320. **Silva Kasela**. Genetic regulation of gene expression: detection of tissue- and cell type-specific effects. Tartu, 2017, 150 p.
321. **Karmen Süld**. Food habits, parasites and space use of the raccoon dog *Nyctereutes procyonoides*: the role of an alien species as a predator and vector of zoonotic diseases in Estonia. Tartu, 2017, p.
322. **Ragne Oja**. Consequences of supplementary feeding of wild boar – concern for ground-nesting birds and endoparasite infection. Tartu, 2017, 141 p.
323. **Riin Kont**. The acquisition of cellulose chain by a processive cellobiohydrolase. Tartu, 2017, 117 p.
324. **Liis Kasari**. Plant diversity of semi-natural grasslands: drivers, current status and conservation challenges. Tartu, 2017, 141 p.

325. **Sirgi Saar**. Belowground interactions: the roles of plant genetic relatedness, root exudation and soil legacies. Tartu, 2017, 113 p.
326. **Sten Anslan**. Molecular identification of Collembola and their fungal associates. Tartu, 2017, 125 p.
327. **Imre Taal**. Causes of variation in littoral fish communities of the Eastern Baltic Sea: from community structure to individual life histories. Tartu, 2017, 118 p.
328. **Jürgen Jalak**. Dissecting the Mechanism of Enzymatic Degradation of Cellulose Using Low Molecular Weight Model Substrates. Tartu, 2017, 137 p.
329. **Kairi Kiik**. Reproduction and behaviour of the endangered European mink (*Mustela lutreola*) in captivity. Tartu, 2018, 112 p.
330. **Ivan Kuprijanov**. Habitat use and trophic interactions of native and invasive predatory macroinvertebrates in the northern Baltic Sea. Tartu, 2018, 117 p.
331. **Hendrik Meister**. Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs. Tartu, 2018, 147 p.
332. **Ilja Gaidutšik**. Irc3 is a mitochondrial branch migration enzyme in *Saccharomyces cerevisiae.* Tartu, 2018, 161 p.
333. **Lena Neuenkamp**. The dynamics of plant and arbuscular mycorrhizal fungal communities in grasslands under changing land use. Tartu, 2018, 241 p.
334. **Laura Kasak**. Genome structural variation modulating the placenta and pregnancy maintenance. Tartu, 2018, 181 p.
335. **Kersti Riibak**. Importance of dispersal limitation in determining dark diversity of plants across spatial scales. Tartu, 2018, 133 p.
336. **Liina Saar**. Dynamics of grassland plant diversity in changing landscapes. Tartu, 2018, 206 p.
337. **Hanna Ainelo**. Fis regulates *Pseudomonas putida* biofilm formation by controlling the expression of *lapA*. Tartu, 2018, 143 p.
338. **Natalia Pervjakova**. Genomic imprinting in complex traits. Tartu, 2018, 176 p.
339. **Andrio Lahesaare**. The role of global regulator Fis in regulating the expression of *lapF* and the hydrophobicity of soil bacterium *Pseudomonas putida*. Tartu, 2018, 124 p.
340. **Märt Roosaare**. *K*-mer based methods for the identification of bacteria and plasmids. Tartu, 2018, 117 p.
341. **Maria Abakumova**. The relationship between competitive behaviour and the frequency and identity of neighbours in temperate grassland plants. Tartu, 2018, 104 p.
342. **Margus Vilbas**. Biotic interactions affecting habitat use of myrmecophilous butterflies in Northern Europe. Tartu, 2018, 142 p.
343. **Liina Kinkar**. Global patterns of genetic diversity and phylogeography of *Echinococcus granulosus* sensu stricto – a tapeworm species of significant public health concern. Tartu, 2018, 147 p.

344. **Teivi Laurimäe**. Taxonomy and genetic diversity of zoonotic tapeworms in the species complex of *Echinococcus granulosus* sensu lato. Tartu, 2018, 143 p.
345. **Tatjana Jatsenko**. Role of translesion DNA polymerases in mutagenesis and DNA damage tolerance in Pseudomonads. Tartu, 2018, 216 p.
346. **Katrin Viigand**. Utilization of α-glucosidic sugars by *Ogataea* (*Hansenula*) *polymorpha*. Tartu, 2018, 148 p.
347. **Andres Ainelo**. Physiological effects of the *Pseudomonas putida* toxin grat. Tartu, 2018, 146 p.
348. **Killu Timm**. Effects of two genes (DRD4 and SERT) on great tit (*Parus major*) behaviour and reproductive traits. Tartu, 2018, 117 p.
349. **Petr Kohout**. Ecology of ericoid mycorrhizal fungi. Tartu, 2018, 184 p.
350. **Gristin Rohula-Okunev**. Effects of endogenous and environmental factors on night-time water flux in deciduous woody tree species. Tartu, 2018, 184 p.
351. **Jane Oja**. Temporal and spatial patterns of orchid mycorrhizal fungi in forest and grassland ecosystems. Tartu, 2018, 102 p.
352. **Janek Urvik**. Multidimensionality of aging in a long-lived seabird. Tartu, 2018, 135 p.
353. **Lisanna Schmidt**. Phenotypic and genetic differentiation in the hybridizing species pair *Carex flava* and *C. viridula* in geographically different regions. Tartu, 2018, 133 p.
354. **Monika Karmin**. Perspectives from human Y chromosome – phylogeny, population dynamics and founder events. Tartu, 2018, 168 p.
355. **Maris Alver**. Value of genomics for atherosclerotic cardiovascular disease risk prediction. Tartu, 2019, 148 p.
356. **Lehti Saag**. The prehistory of Estonia from a genetic perspective: new insights from ancient DNA. Tartu, 2019, 171 p.
357. **Mari-Liis Viljur**. Local and landscape effects on butterfly assemblages in managed forests. Tartu, 2019, 115 p.
358. **Ivan Kisly**. The pleiotropic functions of ribosomal proteins eL19 and eL24 in the budding yeast ribosome. Tartu, 2019, 170 p.
359. **Mikk Puustusmaa**. On the origin of papillomavirus proteins. Tartu, 2019, 152 p.
360. **Anneliis Peterson**. Benthic biodiversity in the north-eastern Baltic Sea: mapping methods, spatial patterns, and relations to environmental gradients. Tartu, 2019, 159 p.
361. **Erwan Pennarun**. Meandering along the mtDNA phylogeny; causerie and digression about what it can tell us about human migrations. Tartu, 2019, 162 p.
362. **Karin Ernits**. Levansucrase Lsc3 and endo-levanase BT1760: characterization and application for the synthesis of novel prebiotics. Tartu, 2019, 217 p.
363. **Sille Holm**. Comparative ecology of geometrid moths: in search of contrasts between a temperate and a tropical forest. Tartu, 2019, 135 p.

364. **Anne-Mai Ilumäe**. Genetic history of the Uralic-speaking peoples as seen through the paternal haplogroup N and autosomal variation of northern Eurasians. Tartu, 2019, 172 p.
365. **Anu Lepik**. Plant competitive behaviour: relationships with functional traits and soil processes. Tartu, 2019, 152 p.
366. **Kunter Tätte**. Towards an integrated view of escape decisions in birds under variable levels of predation risk. Tartu, 2020, 172 p.
367. **Kaarin Parts**. The impact of climate change on fine roots and root-associated microbial communities in birch and spruce forests. Tartu, 2020, 143 p.
368. **Viktorija Kukuškina**. Understanding the mechanisms of endometrial receptivity through integration of 'omics' data layers. Tartu, 2020, 169 p.
369. **Martti Vasar**. Developing a bioinformatics pipeline gDAT to analyse arbuscular mycorrhizal fungal communities using sequence data from different marker regions. Tartu, 2020, 193 p.
370. **Ott Kangur**. Nocturnal water relations and predawn water potential disequilibrium in temperate deciduous tree species. Tartu, 2020, 126 p.
371. **Helen Post**. Overview of the phylogeny and phylogeography of the Y-chromosomal haplogroup N in northern Eurasia and case studies of two linguistically exceptional populations of Europe – Hungarians and Kalmyks. Tartu, 2020, 143 p.
372. **Kristi Krebs**. Exploring the genetics of adverse events in pharmacotherapy using Biobanks and Electronic Health Records. Tartu, 2020, 151 p.
373. **Kärt Ukkivi**. Mutagenic effect of transcription and transcription-coupled repair factors in *Pseudomonas putida*. Tartu, 2020, 154 p.
374. **Elin Soomets**. Focal species in wetland restoration. Tartu, 2020, 137 p.
375. **Kadi Tilk**. Signals and responses of ColRS two-component system in *Pseudomonas putida*. Tartu, 2020, 133 p.
376. **Indrek Teino**. Studies on aryl hydrocarbon receptor in the mouse granulosa cell model. Tartu, 2020, 139 p.
377. **Maarja Vaikre**. The impact of forest drainage on macroinvertebrates and amphibians in small waterbodies and opportunities for cost-effective mitigation. Tartu, 2020, 132 p.
378. **Siim-Kaarel Sepp**. Soil eukaryotic community responses to land use and host identity. Tartu, 2020, 222 p.
379. **Eveli Otsing**. Tree species effects on fungal richness and community structure. Tartu, 2020, 152 p.
380. **Mari Pent**. Bacterial communities associated with fungal fruitbodies. Tartu, 2020, 144 p.
381. **Einar Kärgenberg**. Movement patterns of lithophilous migratory fish in free-flowing and fragmented rivers. Tartu, 2020, 167 p.