Function of the Metazoan Mediator Kinase Module in Transcription

Emilia Kuuluvainen

Division of Genetics
Department of Biosciences
Faculty of Biological and Environmental Sciences

Research Programs Unit
Faculty of Medicine

University of Helsinki

ACADEMIC DISSERTATION

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in Haartman Institute, Lecture hall 2 (Haartmaninkatu 3, Helsinki) on September 1, 2017, at 12 noon.

Helsinki 2017
Supervisor
Professor Tomi P. Mäkelä
Founding Director
Helsinki Institute of Life Science HiLIFE
University of Helsinki, Finland

Thesis advisory committee
Professor Frank C.P. Holstege
Princess Máxima Cancer Research Institute
Utrecht, The Netherlands

Professor René H. Medema
Director of Research
Netherlands Cancer Institute
Amsterdam, The Netherlands

Reviewers
Docent Mikko Frilander
Institute of Biotechnology
University of Helsinki, Finland

Docent Pia Vahteristo
Research Programs Unit
Faculty of Medicine
University of Helsinki, Finland

Opponent
Professor Thomas G. Boyer
Department of Molecular Medicine
Institute of Biotechnology
The University of Texas Health Science Center at San Antonio
Texas, Unites States of America

Custos
Professor Juha Partanen
Division of Genetics
Department of Biosciences
Faculty of Biological and Environmental Sciences
University of Helsinki, Finland

http://ethesis.helsinki.fi

Unigrafia
Helsinki, 2017
And now for something completely different
- Monty Python
TABLE OF CONTENTS

TABLE OF CONTENTS

ABBREVIATIONS

LIST OF ORIGINAL PUBLICATIONS

SUMMARY

REVIEW OF THE LITERATURE

1. RNA polymerase II dependent transcription

2. The Mediator complex
   - The Mediator kinase module
   - Regulation of the Mediator kinase module

3. Regulation of transcription by CDK8-Mediator
   - Mediator and Pre-initiation complex assembly
   - Kinase module interactions with chromatin modifiers
   - An enhancer-associated kinase module
   - The kinase module and transcription elongation

4. Transcription factors regulated by the kinase module – implications for metazoan development and disease
   - A positive regulator of Wnt signaling

5. The Mediator kinase module in human disease
   - Developmental disorders caused by kinase module mutations
   - The kinase module in cancer
   - The kinase module in colorectal cancer

6. Shared or distinct functions of kinase module subunits

AIMS OF THE STUDY

RESULTS AND DISCUSSION

1. Metazoan CDK8 is dispensable for cell-autonomous survival but essential for pre-implantation mouse development
2. Cdk8 and CycC have distinct roles from Med12 and Med13 in transcription although Cdk8-CycC function is dependent on Med12 and Med13
3. Redundancy of human MED13 and MED13L and conserved co-regulation by MED12 and MED13/MED13L
4. Med12 and Med13 are required for activation of Serpent/GATA-dependent innate immunity in Drosophila
5. MED12, MED13 and MED13L are required for cancer-acquired super-enhancer associated gene expression and proliferation of colon cancer cells

CONCLUDING REMARKS

MATERIALS AND METHODS
1. Materials
2. Methods

ACKNOWLEDGEMENTS

REFERENCES
ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous polyposis coli</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>CCNC/CCND1</td>
<td>Cyclin C/cyclin D1</td>
</tr>
<tr>
<td>CDK7/8/9/19</td>
<td>Cyclin-dependent kinase7/8/9/19</td>
</tr>
<tr>
<td>CLL</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>CRPC</td>
<td>Castration-resistant prostate cancer</td>
</tr>
<tr>
<td>CTD</td>
<td>C-/Carboxy-terminal domain (of RNAPII)</td>
</tr>
<tr>
<td>CycC/CycH/ CycT1</td>
<td>Cyclin C/Cyclin H/CyclinT1</td>
</tr>
<tr>
<td>Dm</td>
<td>Drosophila melanogaster/fruit fly</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DptB</td>
<td>Diptericin B</td>
</tr>
<tr>
<td>dsRNA</td>
<td>Double-stranded RNA</td>
</tr>
<tr>
<td>E2.5-E12.5</td>
<td>Embryonic day 2.5-12.5</td>
</tr>
<tr>
<td>eRNA</td>
<td>Enhancer RNA</td>
</tr>
<tr>
<td>ES cell</td>
<td>Embryonic stem cell</td>
</tr>
<tr>
<td>H3K27ac</td>
<td>Histone 3 lysine 27 acetylation</td>
</tr>
<tr>
<td>H3K9me2</td>
<td>Histone r lysine 9 di-methylation</td>
</tr>
<tr>
<td>H3K27me3</td>
<td>Histone 3 lysine 27 tri-methylation</td>
</tr>
<tr>
<td>Hs</td>
<td>Homo sapiens/Human</td>
</tr>
<tr>
<td>ICD</td>
<td>Intracellular domain</td>
</tr>
<tr>
<td>IMD</td>
<td>Immune deficiency</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>LIF</td>
<td>Leukemia inhibitory factor</td>
</tr>
<tr>
<td>IncRNA</td>
<td>Long non-coding RNA</td>
</tr>
<tr>
<td>Luc</td>
<td>Luciferase</td>
</tr>
<tr>
<td>Iz</td>
<td>Lozenge</td>
</tr>
<tr>
<td>MDa</td>
<td>Megadalton</td>
</tr>
<tr>
<td>MED1-31</td>
<td>Mediator subunit 1-31</td>
</tr>
<tr>
<td>mESC</td>
<td>Mouse embryonic stem cell</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>mHSC</td>
<td>Mouse hematopoietic stem cell</td>
</tr>
<tr>
<td>miRNA</td>
<td>MicroRNA</td>
</tr>
<tr>
<td><em>Mm</em></td>
<td><em>Mus musculus</em> / Mouse</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td><em>Mtk</em></td>
<td><em>Metchnikowin</em></td>
</tr>
<tr>
<td>N-terminal</td>
<td>Aminoterminal</td>
</tr>
<tr>
<td>ncRNA-a</td>
<td>Non-coding RNA activating</td>
</tr>
<tr>
<td>NELF</td>
<td>Negative elongation factor</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PIC</td>
<td>Pre-initiation complex</td>
</tr>
<tr>
<td>PRC1/2</td>
<td>Polycomb repressive complex 1/2</td>
</tr>
<tr>
<td>pTEFb</td>
<td>Positive transcription elongation factor b</td>
</tr>
<tr>
<td>REST</td>
<td>RE1-silencing transcription factor</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNAi</td>
<td>RNA interference</td>
</tr>
<tr>
<td>RNAPII</td>
<td>RNA polymerase II</td>
</tr>
<tr>
<td>RNA-seq</td>
<td>RNA-sequencing</td>
</tr>
<tr>
<td>S2</td>
<td>Schneider 2</td>
</tr>
<tr>
<td>SEC</td>
<td>Super elongation complex</td>
</tr>
<tr>
<td>Shh</td>
<td>Sonic hedgehog</td>
</tr>
<tr>
<td>shRNA</td>
<td>Short hairpin RNA</td>
</tr>
<tr>
<td>siRNA</td>
<td>Short interfering RNA</td>
</tr>
<tr>
<td>Srb8-11</td>
<td>Suppressors of RNA polymerase B 8-11</td>
</tr>
<tr>
<td>srp</td>
<td>serpent</td>
</tr>
<tr>
<td>T-ALL</td>
<td>T-cell acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>TAF</td>
<td>TBP associated factor</td>
</tr>
<tr>
<td>TBP</td>
<td>TATA-box binding protein</td>
</tr>
<tr>
<td>TF</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>TFIIA-H</td>
<td>Transcription factor II A-H</td>
</tr>
<tr>
<td>TSS</td>
<td>Transcription start site</td>
</tr>
<tr>
<td>wt</td>
<td>wild type</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS


Submitted manuscript

The author's contribution to each publication

I. EK planned, performed and analyzed experiments, assisted in writing materials and methods. This publication is also included in the thesis of Dr. Thomas Westerling.

II. EK planned, performed and analyzed the majority of the experiments and was the main author of the publication.

III. EK planned and performed all experiments, analyzed the majority of the experiments and was the main author of the manuscript.
SUMMARY

Precise control of transcription, the copying of the genetic DNA code to an expressed RNA molecule, is fundamental for all processes of an organism. Transcriptional regulation is dependent on the activity of proteins and protein complexes including general and cell specific transcription factors and co-activators. Studying how these transcriptional regulators function is crucial for understanding processes such as development and disease.

This PhD thesis focuses on the function of one evolutionarily conserved transcriptional co-activator, called the Mediator complex, and in particular on the role of its reversibly associating kinase module. This kinase module consists of four proteins: Cdk8, cyclin C, Med12 and Med13 in lower metazoans and CDK8 or CDK19, cyclin C, MED12 or MED12L and MED13 or MED13L in vertebrates. The Mediator kinase module regulates transcription through various mechanisms, including association with transcription factors and regulation of enhancer-dependent transcription. Kinase module deregulation is implicated in developmental disorders and cancer, but the molecular mechanisms underlying these diseases remain poorly understood. Comparative studies of all kinase module subunits in metazoan species have essentially been missing. Thus, the role of the other kinase module subunits in functions linked to a particular subunit have often remained unknown.

This thesis studies the role of the metazoan Mediator kinase module subunits in transcription and their requirement for cell and organism survival. Cdk8 was found to be dispensable for cell-autonomous survival but required for mouse embryonic development at the pre-implantation stage. Cdk8-CycC-mediated regulation of transcription was dependent on Med12-Med13 while Cdk8 and CycC depletion caused distinct and even opposite effects on gene expression as compared to Med12 and Med13 depletion in fruit fly cells. This work identified highly similar effects on transcription after depletion of fruit fly Med12 or Med13, suggesting limited Med12-independent functions for Med13. Kinase module subunit hierarchy was conserved in human colon cancer cells where depletion of MED12 or double depletion of the redundant MED13 and MED13L also resulted in highly similar transcriptional responses. Med12 and
Med13 were found to be activators of innate immunity genes that are
dependent on the serpent/GATA transcription factor in fruit fly cells and larvae.
In human colon cancer cells, MED12, MED13 and MED13L were
disproportionally required for the expression of genes associated with cancer-
acquired super-enhancers. MED12 or MED13 and MED13L depletion caused a
dramatic decrease in the expression of the super-enhancer associated MYC
oncogene and impaired proliferation of colon cancer cells, suggesting that
targeting of these kinase module subunits is a possible future therapeutic
opportunity.

This thesis extends the present understanding of the relationships
between Mediator kinase module subunits in metazoan species and identifies
kinase module-dependent functions in development, immunity and cancer-
acquired transcription. The results support the notion of context-dependent
roles for the Mediator kinase module that are reliant on the presence of specific
signaling transcription factors (e.g. GATA factor serpent) or active regulatory
elements such as super-enhancers. These context-dependent functions can in
part explain the varying phenotypes linked to kinase module mutations in
cancer. Further research should be directed at studying the molecular
mechanisms of kinase module functions in vivo and at the potential to target
this complex with the goal of treating human diseases such as cancer.
REVIEW OF THE LITERATURE

1. RNA polymerase II dependent transcription

Although the cells of a multicellular, metazoan animal contain the same genetic material, only a subset of its genes are expressed in any given context. The gene expression signature of a particular cell is fundamental for its function and consequently gene expression is extensively regulated. Interpretation of the information residing in the genes begins with the process of transcription. Transcription is the copying of the genetic DNA code into a complementary RNA molecule by a class of enzymes called polymerases. RNA can function as part of organelles (e.g. ribosomal RNA or rRNA) or as signaling molecules regulating the expression of other RNAs (e.g. long non-coding RNA, IncRNA or micro RNA, miRNA). One class of RNA, called messenger RNA (mRNA), contains the information of the amino acid composition of proteins, which are crucial building blocks but also signaling molecules of cells and organisms. mRNAs are synthetized by the multi-subunit enzyme RNA polymerase II (RNAPII) in a complex process involving several steps.

RNAPII-mediated transcription can be divided into the following steps: formation of the pre-initiation complex (PIC), transcription initiation, promoter escape, elongation and termination (Liu et al., 2013). All of these steps are highly regulated and ultimately determine the rate and amount at which mRNAs are produced. For transcription to take place, RNAPII needs to be recruited to the promoter, a stretch of DNA upstream of the transcribed sequence, that contains binding sites for transcription factors and the transcription start site (TSS). When transcribing, RNAPII moves forward along the DNA synthesizing a pre-mRNA. The pre-mRNA undergoes co-transcriptional processing including 5’-capping, splicing of introns and poly-adenylation of the 3’ end to produce a mature mRNA, which is translated into a protein in the process of translation. The multiple steps of RNAPII-mediated transcription are precisely coordinated by the actions of a wide spectrum of proteins and multi-
protein complexes such as specific and general transcription factors, co-activators, co-repressors and chromatin modifiers.

2. The Mediator complex

The Mediator complex is an important multi-subunit co-activator that regulates RNAPII-mediated transcription at several steps. In mammals this complex is composed of up to 30 protein subunits and is more than twice the size of the RNAPII enzyme. Most Mediator subunits (Figure 1) are conserved in eukaryotes and assemble into four modules based on their location: three core modules, the head, the middle and the tail, and the kinase or CDK8 module (Figure 1). This overall structural of Mediator is also well conserved from yeast to humans (Allen and Taatjes, 2015; Bourbon et al., 2004; Poss et al., 2013).

Mediator can exist in several forms with varying subunit composition and not all subunits are expressed in all cells or required for general transcription. The minimal composition of Mediator needed to support transcription is not well defined in vivo. However, many core subunits that are though to associate with RNAPII or that are important for Mediator assembly are essential for viability in yeast Saccharomyces cerevisiae (Nozawa et al., 2017; Plaschka et al., 2016). For example, Med17, which is located in the RNAPII-interacting head module, is required for expression of 93% of yeast mRNAs (Holstege et al., 1998). Med14 was recently shown to have an essential function as a backbone for interactions between the head, middle and tail modules (Cevher et al., 2014; Plaschka et al., 2015; Tsai et al., 2017). The middle subunit Med21 is also required for viability in yeast (Chao et al., 1996) and for early embryonic development in mice, suggesting its essential function is conserved in mammals (Tudor et al., 1999).
Figure 1. The Mediator complex

Model of the Mediator complex indicating the subunit composition of the Core, comprised of the head (light blue), middle (blue) and tail (blue gray) modules and the reversibly associating kinase module (turquoise). White subunit names denote essentiality in yeast (S. cerevisiae) whereas underlined subunit names denote a vertebrate-specific subunit that can associate with the kinase module in a mutually exclusive manner with its paralog. When subunit’s metazoan and yeast name differ, they are separated by a forward slash (metazoan/yeast). The size and relative position of subunits is approximate and based on the following publications: (Bourbon et al., 2004; Cevher et al., 2014; Lariviere et al., 2012; Plaschka et al., 2015; Plaschka et al., 2016; Poss et al., 2013; Tsai et al., 2013; Tsai et al., 2017). Sometimes core Mediator is described as only the head and middle modules to distinguish essential modules from the regulatory tail and kinase modules (Nozawa et al., 2017).

In contrast to the essential subunits, other Mediator subunits are required for transcription of specific sets of genes in a context-dependent manner and are not essential for survival of cells or unicellular organisms such as yeast (van de Peppel et al., 2005). Although knockouts of Mediator subunits leads to lethality in mice due to developmental defects, this is suggested to be a consequence of the requirement of a given subunit for a specific transcriptional response during development (Yin and Wang, 2014). For example, knockout of the middle module subunit Med1 is embryonic lethal with defects in specific tissues such as the placenta, heart and erythroid cells and display impaired activation
of target genes of the transcription factor GATA1 and the nuclear hormone receptors PPARγ (Peroxisome proliferator-activated receptor 𝛽) and TR (Thyroid hormone receptor) (Ito et al., 2000; Stumpf et al., 2006; Zhu et al., 2000). Similarly, tail and kinase module subunits are not required for global transcription as deletion mutants for these subunits are viable and show defects in regulation in only a small percentage of yeast genes (van de Peppel et al., 2005).

- The Mediator kinase module

Although the composition of the Mediator complex can vary with respect to many of its subunits, the complex is present in cells mainly in two distinct forms. These are the large (Fondell et al., 1996; Gu et al., 1999) roughly 1,5 MDa complex called CDK8-Mediator, which contains all modules (head, middle, tail and kinase) and a smaller (Malik et al., 2000), 500-700 kDa complex containing only the core Mediator (head, middle and tail) but lacking the kinase module. The Mediator kinase module is a four-protein complex (Borggreve et al., 2002) that in yeast and lower metazoans such as flies contains the cyclin-dependent kinase 8, Cdk8, its cognate cyclin, cyclin C (CycC) as well as the two large proteins Med12 and Med13. In vertebrates, the alternative subunits CDK19, MED12L and MED13L can associate with the kinase module in a mutually exclusive fashion with their paralogs CDK8, MED12 and MED13, respectively (Allen and Taatjes, 2015; Bjorklund and Gustafsson, 2005; Poss et al., 2013; Sato et al., 2004). A stoichiometry of 1:1:1:1 (Knuesel et al., 2009b) of one of each type of protein in addition to cyclin C is though to comprise the Mediator kinase module (Figure 1). Since the kinase module can be purified from cells without Mediator it is possible that it could function independently (Knuesel et al., 2009b). However, genome-wide co-localization of core and kinase module subunits both in yeast and mammalian cells suggests that this happens rarely, if ever (Jeronimo et al., 2016; Whyte et al., 2013).

The kinase module associates with core Mediator via binding to the middle module. The binding has been suggested to involve the subunits MED1, MED7
and MED19 and other subunits positioned at the end of the middle module (Tsai et al., 2013; Tsai et al., 2017). Both human and yeast core Mediator bind to the kinase module through the MED13/MED13L subunits (Med13 in yeast) although binding between Cdk8 and core Mediator has also been observed in yeast (Knuesel et al., 2009a; Tsai et al., 2013). Within the kinase module MED13/MED13L interacts with MED12, which in turn binds the kinase-cyclin pair, CDK8/CDK19-cyclin C, through cyclin C (Figure 1) (Tsai et al., 2013). CDK8 and CDK19 are the only catalytically active subunits of the Mediator kinase module and especially CDK8 has been shown to phosphorylate a variety of substrates, including the RNAPII C-terminal domain (CTD) (Hengartner et al., 1998), other Mediator subunits (Poss et al., 2016; van de Peppel et al., 2005) and transcription factors (Bancerek et al., 2013; Fryer et al., 2004; Morris et al., 2008).

- Regulation of the Mediator kinase module

Depletion of both MED13 and MED13L leads to dissociation of the kinase module from core Mediator in human HeLa cells (Tsai et al., 2013). Accordingly, regulation of MED13/MED13L protein abundance through post-translational modifications provides means for cells to regulate kinase module dependent functions. MED13 and MED13L are regulated by the ubiquitin ligase FBW7 that ubiquitinates MED13 and MED13L thereby targeting them for degradation by the proteasome complex (Davis et al., 2013). MED13 and MED13L levels are possibly also regulated via phosphorylation by CDK8/CDK19. Human MED13 is a substrate for CDK8 in vitro (Knuesel et al., 2009b) and inhibition of CDK8/CDK19 kinase activity in human cells results in decreased MED13/MED13L phosphorylation accompanied by a modest increase in MED13/MED13L protein levels (Poss et al., 2016). The same study also identified MED12 as a potential substrate of CDK8/CDK19.

Due to its central position within the kinase module, MED12 is needed for association of CDK8/CDK19 and cyclin C with the core Mediator (Tsai et al., 2013) and for in vitro kinase activity of human CDK8 towards the RNAPII CTD.
(Knuesel et al., 2009b). In contrast, yeast Cdk8 is able to phosphorylate RNAPII CTD in vitro in the presence of cyclin C only, and does thus not need Med12 for kinase activity (Hengartner et al., 1998). In addition to cyclin-binding, cyclin-dependent kinases are typically activated via phosphorylation of the so-called T-loop threonine (e.g. T160 in human CDK2). However, CDK8 carries an aspartate at this position that can mimic the phospho-threonine (Hoeppner et al., 2005) and thus its activation is independent of a CDK-activating kinase.

CDK8 protein levels are regulated upon changes in nutrient availability in various organisms. In yeast, Cdk8 levels are downregulated upon nitrogen deprivation (Nelson et al., 2003) whereas starvation of fruit fly larvae results in the upregulation of Cdk8 protein levels (Xie et al., 2015). Consistent with this, re-feeding of starved mice leads to downregulation of CDK8 levels in the liver and in starved human cells CDK8 is similarly downregulated upon exposure to nutrient-rich conditions. Although the effect of starvation and re-feeding on CDK19 has not been addressed, CDK19 kinase activity is likely to be affected, as cyclin C levels are co-regulated with CDK8 in these conditions (Feng et al., 2015; Zhao et al., 2012).

Protein levels or activity of kinase module subunits can also be regulated by non-coding RNAs in specific contexts. In the mouse heart, MED13 is negatively regulated by expression of a miRNA, miR208a. Expression of miR208a promotes insulin resistance and obesity (Grueter et al., 2012). Transcription-activating IncRNAs, called ncRNA-as or eRNAs for enhancer RNAs, that interact specifically with MED12 have been shown to stimulate the kinase activity of CDK8 towards histone H3 (Lai et al., 2013), a suggested substrate (Knuesel et al., 2009b; Meyer et al., 2008) for CDK8 in human cells.

The existence of the alternative kinase module subunits CDK19, MED12L and MED13L in vertebrates provides another potential mechanism of regulation of kinase module function. It is possible that kinase modules composed of different subunits may have distinct functions. Thus, regulation of abundance of one kinase module subunit might provide a way to affect the association of its
paralog with the kinase module. In addition, cyclin C is not only required for the kinase activity of CDK8 and CDK19 but also activates another cyclin-dependent kinase, CDK3, which has Mediator-unrelated functions in cell cycle regulation (Li et al., 2014; Ren and Rollins, 2004). However, it is not known if there is competition between Mediator CDK’s and CDK3 for their shared cyclin partner in cells.

3. Regulation of transcription by CDK8-Mediator

Core Mediator was originally identified as a set of RNAPII-associating proteins that together with RNAPII and its general transcription factors were able to mediate activated transcription in vitro. This protein assembly was named the RNAPII holoenzyme (Kim et al., 1994; Koleske and Young, 1994). The requirement of Mediator for activated transcription is thought to be due to the association of Mediator with specific activators. Importantly, whereas Mediator interacts directly with gene-specific transcription factors, RNAPII is unable to do so. Therefore, Mediator is considered to form a bridge between distally bound transcription factors and RNAPII (Figure 2). As different transcription factors interact with specific Mediator subunits, this provides an additional level of regulation through Mediator subunit composition diversity (Yin and Wang, 2014).

Soon after the discovery of the Mediator complex, Cdk8 and CycC were shown to associate with the RNAPII holoenzyme and stimulate phosphorylation of the RNAPII CTD in vitro (Leclerc et al., 1996; Liao et al., 1995). Cdk8-Mediator was suggested to function as a repressor of transcription based on the ability of Cdk8 loss-of-function mutations to rescue lethality of CTD-truncating mutations in yeast. Accordingly, Cdk8-dependent phosphorylation of the CTD on the serine-5 residues of the CTD heptapeptide repeat (52 repeats of the sequence YSPTSPS in humans) was shown in vitro to suppress transcription prior to, but not after, assembly of the PIC (Hengartner et al., 1998). Furthermore, cdk8 deletion in yeast causes mainly upregulation of transcripts, indicating a
repressive role for Cdk8 in regulating transcription of a subset of genes (Holstege et al., 1998).

Mammalian CDK8-Mediator blocks transcription in vitro (Knuesel et al., 2009a; Pavri et al., 2005), thus supporting a repressive role for the Mediator kinase module in transcription. However, subsequent studies in fruit flies and human cells have shown that depletion of CDK8, MED12 and MED13 can cause downregulation of gene expression and that kinase module subunits are recruited to chromatin upon gene activation (Carrera et al., 2008; Donner et al., 2007; Kim et al., 2006), thus suggesting a positive role in transcription. The kinase module has since been implicated in both repression and activation of transcription at several steps of the transcription cycle through different mechanisms such as regulation of RNAPII binding (Elmlund et al., 2006), association with transcription factors (Alarcon et al., 2009; Zhou et al., 2006) and chromatin modifiers (Ding et al., 2008) and regulation of enhancer-promoter interactions (Kagey et al., 2010).

- Mediator and Pre-initiation complex assembly

Before transcription of a gene can initiate, the PIC, consisting of RNAPII and its general transcription factors called TFIIs, is assembled on the promoter. TFIID, which consists of the TATA binding protein, TBP, and TBP associating factors, TAFs, is required for promoter recognition, whereas the binding of TBP to DNA is stabilized by TFIIA and TFIIF. TFIIB is important for polymerase recruitment whereas TFIIE and TFIIH are required for opening of the DNA double helix, resulting in formation of the open promoter complex or transcription bubble. TFIIH also contains a cyclin-dependent kinase, CDK7, which phosphorylates the RNAPII CTD on its serine-5 residues upon transcription initiation (Liu et al., 2013; Sainsbury et al., 2015) (Figure 2).

The Mediator co-ordinates assembly of the PIC via direct association with RNAPII and its general transcription factors (Figure 2). Mediator binds RNAPII both through the CTD-containing Rpb1 subunit and through other polymerase
subunits leading to major conformational changes in Mediator structure (Plaschka et al., 2015; Poss et al., 2013; Tsai et al., 2017). Furthermore, Mediator has been shown to facilitate binding of general transcription factors and thus PIC stabilization has been suggested as the explanation for the requirement of Mediator for basal transcription (Allen and Taatjes, 2015; Nozawa et al., 2017). In addition, Mediator binding stimulates the kinase activity of TFIH towards RNAPII by stabilizing the CDK7 kinase complex close to the RNAPII CTD (Plaschka et al., 2015). CTD phosphorylation contributes to the disruption of the interaction between Mediator and RNAPII. As the polymerase escapes the promoter, Mediator is left behind in the scaffold complex, which is thought to facilitate re-initiation of transcription (Allen and Taatjes, 2015).

Mediator has been shown to interact with RNAPII in the PIC in its small form, lacking the kinase module (Samuelsen et al., 2003) (Figure 2). Consistent with this, presence of the kinase module inhibits transcription in vitro and the kinase module is released from Mediator upon PIC assembly in yeast (Hengartner et al., 1998; Jeronimo et al., 2016; Pavri et al., 2005). An explanation for this repressive function was given when the kinase module was shown to block the binding of RNAPII with core Mediator both in yeast and human cells (Elmlund et al., 2006; Knuesel et al., 2009a; Tsai et al., 2013). The kinase module was further shown to interfere with the binding of Mediator to the RNAPII CTD, providing an explanation as to why kinase module mutants can rescue the lethality of yeast with a truncated CTD (Hengartner et al., 1998; Tsai et al., 2013). However, more precise structural studies have shown that Mediator-RNAPII binding occurs mostly through subunits in the Mediator head domain (Lariviere et al., 2012; Plaschka et al., 2015; Tsai et al., 2017), suggesting that concurrent binding of the kinase module, via the middle module, and RNAPII to core Mediator could be possible (Figure 2).

Although core Mediator has been shown to be required for basal transcription (Holstege et al., 1998), Mediator is perhaps best known for its importance in activated transcription. Upon binding of gene-specific transcription factors to
their DNA elements, the association of specific Mediator subunits with these transcription factors is thought to affect both RNAPII recruitment to the correct genes and subsequent transcription elongation (Conaway and Conaway, 2011). The binding of transcriptional activators induces conformational changes in Mediator, creating new binding surfaces for other Mediator-interacting factors (Ebmeier and Taatjes, 2010; Taatjes et al., 2002). Kinase module subunits interact with transcription factors as well, resulting in either transcriptional repression or activation of specific sets of genes (Fryer et al., 2004; Kim et al., 2006).

![Image](image.png)

**Figure 2. Pre-initiation complex formation and transcription initiation**

RNAPII-dependent transcription begins with the recruitment of the PIC components to chromatin. The PIC is formed at promoters located near the transcription start site. The Mediator complex (blue) facilitates the formation of the PIC by stabilizing the association of RNAPII (purple) with general transcription factors (green). Mediator stimulates the phosphorylation of the RNAPII CTD by the TFIH kinase CDK7 occurring at transcription initiation. Following CTD phosphorylation, the association between Mediator and RNAPII is disrupted and promoter escape occurs. Mediator forms a bridge between RNAPII and transcription factors (pink) and subunit-TF interactions are needed for transcription of specific sets of genes. The relative size of different components is not drawn to scale.
- Kinase module interactions with chromatin modifiers

DNA exists in the cell within chromatin in which DNA is tightly wrapped around nucleosome complexes (Figure 2). Two copies of each of the histone proteins H2A, H2B, H3 and H4 form the nucleosome core octamer whereas linker histones, such as histone H1, are involved in regulating higher order chromatin structures. Histones are post-translationally modified through various mechanisms including methylation, acetylation, phosphorylation and ubiquitination. Specific epigenetic modifications of amino acids within the histone tails, which extend from the nucleosome, define the state of the chromatin and function to activate or repress transcription e.g. via recruitment of other transcriptional regulators or by regulating chromatin compaction. Thus, the enzyme complexes that are responsible for histone modification, the chromatin modifiers, play an important role in transcription (Tessarz and Kouzarides, 2014; Teves et al., 2014).

The Mediator kinase module has been shown to regulate transcription via recruitment of chromatin modifiers (Figure 4). MED12 interaction with the histone methyltransferase G9a/EHMT2 and the RE1 silencing transcription factor, REST, is required for repression of neuronal target genes via dimethylation of histone H3 lysine 9 (H3K9me2) by G9a/EHMT2 (Ding et al., 2008). Human CDK8 and CDK19 can associate with the histone arginine methyltransferase PRMT5 and its interacting protein WDR77 in HeLa cells and are needed for promoter binding of PRMT5 at certain genes. PRMT5 represses transcription by symmetrical dimethylation of histone H4 arginine 3 (H4R3me2s) and in HeLa cells activation of C/EBPβ leads to the loss of PRMT5, CDK8/CDK19 and H4R3me2s on target gene promoters (Tsutsui et al., 2013).

CDK8 has also been implicated in transcriptional repression via the Polycomb repressive complex 2 (PRC2). Knockout of Cdk8 leads to the reduction of repressive histone H3 lysine 27 trimethylation (H3K27me3) in intestinal epithelial cells and to the decreased binding of EZH2 on de-repressed genes (McCleland et al., 2015). EZH2 is the enzymatic component of PRC2 responsible
for catalyzing H3K27 trimethylation. CDK8 and CDK19 have also been found to interact with SUZ12, another component of PRC2, in a yeast-two-hybrid screen, and with SUZ12 and EZH2 in vitro (Fukasawa et al., 2015; Fukasawa et al., 2012). In addition, fly Med12 and Med13 have been implicated in Polycomb (PcG)-mediated repression of the Hox gene Ubx (Gaytan de Ayala Alonso et al., 2007).

The Mediator kinase module can also interact with epigenetic activators. In HeLa cells, CDK8 containing Mediator associates with a complex containing the histone acetyltransferase GCN5L. In vitro this GCN5L and CDK8 containing complex can stimulate acetylation of lysine 14 (H3K14ac) and phosphorylation of serine 10 of histone H3 (H3S10p), which are associated with transcriptional activation (Meyer et al., 2008). A function for CDK8-mediated H3S10p for transcription regulation has, however, not yet been demonstrated in cultured cells or in vivo. Both CDK8 and MED12 do nonetheless bind to sites of active transcription and co-localize with active histone marks such as acetylation of lysine residues on histones H3 and H4 and methylation of H3 lysine 4 both on genes and on enhancers (Aranda-Orgilles et al., 2016; Donner et al., 2010; Galbraith et al., 2013; Hnisz et al., 2013).

- An enhancer associated kinase module

Transcription factors binding to their respective DNA elements control where and when RNAPII initiates transcription. Transcription factors can bind both close to the promoter, at proximal elements, or at distal elements called enhancers. The distance between the TSS and enhancers can be tens or even hundreds of thousands of base pairs and consequently the chromatin needs to form 3D structures or loops for enhancer-bound factors to be able to interact with the RNAPII machinery on promoters. The Mediator localizes to enhancers both in mammalian cells and in yeast and especially the kinase module has been implicated in activating transcription through facilitating chromatin looping (Andrau et al., 2006; Jeronimo et al., 2016; Kagey et al., 2010; Kuras et al., 2003). (Figure 3.)
In mESCs, depletion of MED12 disrupts promoter-enhancer looping at loci involved in maintaining stem cell pluripotency (Kagey et al., 2010; Phillips-Cremins et al., 2013). The mechanism behind this is suggested to involve MED12 and cohesin, a ring shaped protein complex known to be important for maintaining chromatin 3D structures. In mESCs MED12 interacts with the cohesin-loading factor NIPBL and depletion of either MED12 or the cohesin subunit SMC1A results in similar defects in expression of various genes including those required for pluripotency (Kagey et al., 2010). In zebrafish, mutations in nipbl or med12 cause similar defects in fin development and gene expression and both Med12 and Nipbl are required for chromatin looping within the hoxD locus (Muto et al., 2014). The association between MED12 and cohesin also appears to be conserved in human cells. In human colorectal cancer cells, MED1 and MED12 co-localizes with cohesin on enhancers, although the possible requirement for these Mediator subunits for looping events in this context has not been established (Yan et al., 2013). However, in other human cancer cells, depletion of MED12 does disrupt chromatin looping of MED12-activated eRNA/ncRNA-a enhancer loci and their target genes (Lai et al., 2013).

In recent years, the Mediator and its kinase module has been implicated in regulation of transcription via exceptionally large and active enhancer clusters called super-enhancers. Super-enhancers are characterized as enhancer regions containing exceptionally high amounts of Mediator, transcription factors and active histone marks such as acetylation of histone H3 lysine 27 (H3K27ac) and mono-methylation of histone H3 lysine 4 (H3K4me1). Although the distinction between typical enhancers and super-enhancers is somewhat arbitrary, this group of high-activity enhancer clusters does on average contain larger amounts of these factors and is more often associated with genes typical for the identity of the cell in question as compared to regular enhancers (Hnisz et al., 2013; Loven et al., 2013; Whyte et al., 2013). Inhibition of CDK8 and CDK19 causes de-repression of super-enhancer associated genes in mouse myeloid cells derived from an animal model of acute myeloid leukemia (AML),
suggesting that they are negative regulators of super-enhancer activity (Pelish et al., 2015). In contrast, depletion of MED12 in mESCs or deletion of Med12 in mHSCs (mouse hematopoietic stem cells) disproportionally decreases expression of super-enhancer associated genes as compared to other genes (Aranda-Orgilles et al., 2016; Whyte et al., 2013), suggesting that MED12 is needed for super-enhancer activity.

It is not well understood why MED12 is disproportionally required for super-enhancer associated gene expression in stem cells. Interestingly, cohesin and NIPBL also associate more frequently with super-enhancers than with other enhancers (Hnisz et al., 2013), implying MED12-cohesin cooperation as a possible mechanism. Other super-enhancer associating factors, such as the histone acetyltransferase activity-containing co-activator p300, the BET-family co-activator/epigenetic reader BRD4 and the BRD4 interacting positive transcription elongation factor pTEFb have also been implicated in kinase module dependent activation of enhancers. MED12 and p300 have both been linked to the activation of enhancers in prostate cancer cells and in mHSCs deletion of Med12 was recently shown to cause decreased levels of p300 and active histone marks on a subset of enhancers (Aranda-Orgilles et al., 2016; Wang et al., 2011). In mESCs, inhibition of BRD4 leads to the loss of Mediator, as defined by MED1 binding, at super-enhancers (Loven et al., 2013). Also, in mouse AML cells, BRD4 inhibition causes MED1 loss at enhancers and depletion of MED12 or BRD4 results in similar gene expression defects. In these cells, MED12 depletion was also shown to cause decreased binding of RNAPII and the pTEFb kinase CDK9 on the super-enhancer known to activate the MYC gene (Bhagwat et al., 2016).
Figure 3. The kinase module associates with active enhancers and promotes chromatin looping and transcription elongation.

Mediator (blue) and its kinase module (turquoise) binds to active enhancer and super-enhancer regions where MED12 is needed for enhancer activity and transcription of non-coding eRNAs/ncRNA-αs. Active enhancers are characterized by specific histone marks exemplified here by acetylation (Ac) that can be recognized by p300 and BRD4. BRD4 stimulates the recruitment of Mediator and pTEFb to enhancers. MED12 associates with the cohesin (red ring) loading factor NIPBL and promotes looping of enhancers to promoters. Transcription regulation by many transcription factors (pink) depends on association with kinase module subunits or phosphorylation by CDK8/CDK19. Transcription elongation is triggered by phosphorylation of RNAPII (purple) CTD, NELF and DSIF by CDK9, the kinase of pTEFb. Mediator can stimulate elongation via association of pTEFb, other super elongation complex components (light green) and BRD4 with CDK8 and MED26. Vertebrate specific subunits CDK19, MED12L and MED13L (see Figure 1) are left out of the model for simplicity. The relative size of different components is not drawn to scale.
- The kinase module and transcription elongation

The Mediator kinase module has also been suggested to interact with CDK9 and BRD4 during transcription elongation (Donner et al., 2010; Galbraith et al., 2013). As transcription is initiated, RNAPII begins to synthesize the pre-mRNA transcript and does so for about 30-60 bp until stalling in a process called promoter proximal pausing. For the polymerase to continue with transcription elongation, Negative elongation factor (NELF), DRB sensitivity inducing factor, (DSIF) and the RNAPII CTD (specifically at its serine 2 residues) needs to be phosphorylated by CDK9, the kinase of pTEFb. These signals allow the polymerase to proceed into productive elongation and further synthesis of the transcript (Jonkers and Lis, 2015).

Promoter-proximal pausing and signaling factor induced pause release was first described for the heat shock genes in Drosophila melanogaster and long thought to be a rare event. However, in the last decade it has become evident that pausing is a feature of most RNAPII-transcribed genes both in fruit flies and mammals, making entry into transcription elongation an important regulatory step (Jonkers and Lis, 2015). Many transcription factors stimulate the release of RNAPII into elongation and Mediator has been implicated in regulation of elongation through the core subunit MED26 and through its kinase module (Galbraith et al., 2013; Takahashi et al., 2011).

CDK8 associates with immediate early genes upon serum stimulation in colon cancer cells and is required for the recruitment of CDK9, BRD4 and CDK7 to these genes. As CDK8 depletion does not affect recruitment of RNAPII but does affect elongation rates in vitro, this has been suggested to be a result of impaired transcription elongation (Donner et al., 2010). Similarly, upon hypoxia, CDK8-Mediator is recruited to Hypoxia-inducible factor-1-alpha (HIF1A) target genes in colon cancer cells. In CDK8-depleted cells transcription elongation and recruitment of CDK9, BRD4 and AFF4, an interactor of pTEFb within the super elongation complex (SEC), is reduced at HIF1A target genes upon response to hypoxia (Galbraith et al., 2013). The role of CDK8 in
elongation has been proposed to depend on a direct interaction between CDK8-Mediator and CDK9 (Donner et al., 2010; Galbraith et al., 2013). Indeed, both CDK9 and other pTEFb/SEC components cyclin T1 and AFF4 have been found to interact preferentially with the kinase module containing form of Mediator (Ebmeier and Taatjes, 2010), supporting these findings.

The Mediator middle domain subunit MED26 interacts directly with SEC proteins and is needed for SEC recruitment and elongation at specific genes including MYC and HSP70 (Takahashi et al., 2011). Recruitment of MED26 to HIF1A target genes is impaired in CDK8-depleted colorectal cancer cells, suggesting that CDK8-Mediator dependent association with the SEC might also be mediated through MED26 (Galbraith et al., 2013). Interestingly, pTEFb and BRD4 recruitment has been shown to depend on the Mediator kinase module both on enhancers and during elongation (Bhagwat et al., 2016; Donner et al., 2010; Galbraith et al., 2013). It is thus tempting to speculate that the kinase module could coordinate enhancer-activated transcription elongation via chromatin looping. In agreement with this notion, knockdown of cohesin, a MED12-interacting protein complex that is crucial for enhancer-promoter looping, has been shown to result in decreased elongation and increased pausing of RNAPII (Schaaf et al., 2013).

4. Transcription factors regulated by the kinase module – implications for metazoan development and disease

Although the Mediator kinase module is involved in regulation of many steps of the transcription cycle, it is not considered to be essential for transcription of all genes. In yeast, deletion of the non-essential kinase module subunits lead to defects, mainly upregulation, of a few percent of mRNA coding genes (Holstege et al., 1998; van de Peppel et al., 2005). Yeast Cdk8 has been shown to negatively regulate specific transcriptional programs, such as response to low nutrients or high iron, through repressive phosphorylation of transcription factors (or via Mediator subunits interacting with transcription factors) regulating these responses. Degradation or nuclear exclusion of Cdk8-
phosphorylated transcription factors has been demonstrated as a plausible mechanism of Cdk8-mediated repression in yeast (Chi et al., 2001; Nelson et al., 2003; van de Peppel et al., 2005).

The metazoan kinase module has also been shown to directly regulate and interact with specific transcription factors (Figure 4). As in yeast, mammalian CDK8 can inhibit transcription factor function through phosphorylation. Phosphorylation of the NOTCH intracellular domain (ICD) by CDK8 and CDK19 leads to its degradation and subsequent repression of NOTCH target genes. In mice, knockout of CCNC in hematopoietic cells leads to de-repression of NOTCH-signaling, mediated through CDK8 as well as CDK19 (and CDK3) and to the development of T-cell acute lymphoblastic leukemia (T-ALL) (Fryer et al., 2004; Li et al., 2014). Human CDK8 can also repress the transcription factors E2F1 (Morris et al., 2008) and SREBP-1c (Zhao et al., 2012) through phosphorylation with implications for cancer cell proliferation (E2F1) (Firestein et al., 2008) and fatty liver disease (SREBP-1c) (Feng et al., 2015). The role of Cdk8 in Srebp and E2f signaling is conserved in the fruit fly where Cdk8-mediated repression of these transcription factors is needed for regulation of eye development (E2f) and lipogenesis (Srebp) (Morris et al., 2008; Zhao et al., 2012).

Conversely, CDK8 dependent phosphorylation of SMAD transcription factors of the BMP and TGFβ pathways results in their activation as well as degradation (Alarcon et al., 2009). Also, phosphorylation of STAT-1 by CDK8 activates STAT-1-dependent transcription and thus CDK8 is required for the interferon response in human cells (Bancerek et al., 2013). CDK8 and CDK19 inhibition furthermore leads to loss of phosphorylation of many other transcription factors (Poss et al., 2016) that have not yet been shown to be direct targets of the Mediator kinases. In fruit flies, Cdk8 and CycC are required for activation of ecdysone hormone-dependent signaling. Although Cdk8-Mediator interacts with the ecdysone nuclear hormone receptor EcR (Xie et al., 2015), the receptor has not been shown to be a target for phosphorylation by Cdk8. The positive role for human CDK8 in HIF1A-induced transcription is also suggested to depend on interaction between HIF1A and CDK8-Mediator although it is
unclear which kinase module subunit is responsible for the interaction (Galbraith et al., 2013).

MED12 also regulates specific transcriptional responses through physical association with transcription factors (Figure 4). Interaction of MED12 and GLI3 has a repressive role in Sonic hedgehog (Shh) signaling in human cells (Zhou et al., 2006). Fruit fly Med12 and Med13 can also interact with the GLI3 homolog cubitus interruptus (Ci) in overexpression assays (Mao et al., 2014) and are known to regulate Ci target genes during development of the wings and eyes (Janody et al., 2003; Mao et al., 2014; Treisman, 2001). Also Notch signaling is disturbed during wing development in kinase module mutant fruit flies although a mechanism for this has not been established. In contrast to the repressive regulation of NOTCH ICD by CDK8/CDK19-cyclin C in mammals, the role of fruit fly kinase module subunits in Notch signaling is less clear and only studied on the level of a few genes (Janody and Treisman, 2011).

MED12-dependent activation of transcription factors has been shown to be crucial for stem cell identity and development of specific cell linages. MED12 associates with NANOG and activates its target genes in mESCs (Tutter et al., 2009), providing a second mechanism for the requirement of MED12 for pluripotency, in addition to its interaction with NIPBL (Kagey et al., 2010). In flies, Med12 and Med13 are required for the development of GATA/RUNX-dependent (Serpent/Lozenge) hematopoietic crystal cells and transcription of a reporter gene activated by these factors (Gobert et al., 2010). A requirement for Med12 for hematopoietic cells has also been described in vertebrates. In zebrafish med12 mutants have impaired neutrophil development (Keightley et al., 2011) whereas in mouse, MED12 is required already for hematopoietic stem cell survival (Aranda-Orgilles et al., 2016). Moreover, med12 mutant zebrafish have defects in development of the nervous system and cartilage due to the failure to activate genes that are dependent on the Sox9 protein (Rau et al., 2006; Wang et al., 2006), another transcription factor that physically interacts with Med12 (Zhou et al., 2002). MED12 is also implicated in neuronal development in mammals as it is required for the maintenance of stem cell.
identity of neuronal stem cells (NSCs) (Kim et al., 2016) and for the
development of myelinating glia cells in mouse due to an interaction with a
second Sox transcription factor, SOX10 (Vogl et al., 2013). Interestingly,
mutations in MED12 are known to cause neurodevelopmental disorders
(Spaeth et al., 2011), demonstrating that transcriptional control by the
Mediator kinase module is important in the development of human disease.

Figure 4. Transcription factors and chromatin modifiers interacting with the Mediator
kinase module. The subunits of the Mediator kinase module interact with many transcription
factors (pink and light green) and chromatin modifiers (dark green) with implications for
development, cancer, metabolism and immunity. Only factors that have been shown to directly
bind the kinase module or to be phosphorylated by it are shown. Pink transcription factors are
repressed by kinase module activity whereas green transcription factors and chromatin
modifiers are activated and drawn on top of the kinase module subunit with which a direct
binding event is implicated. The letter P denotes phosphorylation and repressive histone
modifications catalyzed by chromatin modifiers are indicated. A dashed line indicates that the
mechanism of activation or repression is unknown. Black or grey font denotes association in a
vertebrate species or fruit fly, respectively, while bold black font denotes association in both.
Association with both alternative subunits (e.g. CDK8 and CDK19) is indicated by a thicker black
lining of the factor.
- A positive regulator of Wnt signaling

Mediator kinase module subunits have been implicated in the activation of canonical Wnt signaling in different contexts. This pathway is activated by the binding of extracellular Wnt proteins to transmembrane receptors of the Frizzled family. Receptor binding results in the inactivation of GSK3β kinase by Dishevelled and the subsequent translocation of β-catenin to the nucleus. In the absence of Wnt binding, β-catenin is phosphorylated by GSK3β, which is part of the APC-containing destruction complex. GSK3β-mediated phosphorylation targets β-catenin for degradation by the proteasome and thus keeps Wnt-target genes silent. Upon nuclear translocation of β-catenin, it associates with DNA binding transcription factors of the TCF/LEF family together with co-factors Pygopus and Legless (or their mammalian homologues) and transcription of target genes is activated (Clevers and Nusse, 2012).

An activating role for the kinase module in Wnt signaling has been discovered independently in various studies (Figure 4). In Drosophila, the observation that mutant Med12 or Med13 showed mis-expression of Wnt pathway (wingless, wg) target genes in the fruit fly wing disc (Janody et al., 2003) led to the discovery of an interaction between the kinase module proteins and the Wnt signaling co-activator Pygopus (Carrera et al., 2008). Consistent with this, in cultured fruit fly cells, depletion of Med12 or Med13 results in decreased activity of a Wnt luciferase reporter gene (Carrera et al., 2008). Similarly, MED12 is also required for Wnt luciferase reporter activity in human cervical cancer HeLa cells and is recruited to Wnt target genes upon activation of the pathway. This study also showed that MED12 associates directly with β-catenin (Kim et al., 2006). In agreement with a β-catenin-activating role for mammalian MED12, mouse Med12 hypomorphic embryos show lost expression of canonical Wnt signaling target genes Axin2, Myc, Dkk1 and Ccnd1 (Rocha et al., 2010). Wnt signaling dysregulation has also been found in human tumors caused by mutations in MED12 (Lae et al., 2016; Makinen et al., 2011; Mehine et al., 2016).
In addition to MED12, mammalian CDK8 has also been implicated as an activator of Wnt signaling. In human colon cancer cells, CDK8 depletion results in decreased Wnt-reporter activity and expression of Wnt target genes (Firestein et al., 2008). The mechanism behind this is thought to be different from that of MED12-mediated Wnt-activation and to be dependent on antagonistic effects on β-catenin by E2F1, which in turn is repressed by CDK8-mediated phosphorylation (Morris et al., 2008) (Figure 4). CDK8-activated Wnt signaling has been suggested to promote epithelial to mesenchymal transition (EMT) of pancreatic cancer cells (Xu et al., 2015b) and tumorigenesis in colon cancer (Firestein et al., 2008).

5. The Mediator kinase module in human disease

- Developmental disorders caused by kinase module mutations

The importance of the Mediator kinase module in specific transcriptional programs is highlighted by the consequences of mutations found in proteins of this complex in human disease. Specific missense mutations in human MED12 cause several mental retardation syndromes that usually affect males because of the location of MED12 on the X-chromosome (Graham and Schwartz, 2013). FG or Opitz-Kaveggia syndrome is characterized by distinctive facial features, macrocephaly, problems in the gastro-intestinal tract, behavioral and mental disability, hypotonia and abnormality of the corpus callosum. FG syndrome is caused by a single amino acid change, R961W, in MED12 (Risheg et al., 2007). Another missense mutation in MED12, resulting in the amino acid change N1007S, causes the related Lujan syndrome, which shares many features with FG syndrome (Schwartz et al., 2007) whereas yet other MED12 mutations cause Ohdo syndrome (Vulto-van Silfhout et al., 2013). In addition to MED12, MED13L and CDK19 have also been linked to mental retardation. Different mutations in MED13L are found in patients with intellectual disability as well as physical malformations (Adegbola et al., 2015). Mutations in MED13L also cause congenital heart disease due to transposition of the great arteries (Asadollahi et al., 2013; Muncke et al., 2003). Disruption of CDK19 has been found in one
patient with mental retardation as well as microcephaly and malformation of the retinas (Mukhopadhyay et al., 2010).

Although the molecular mechanisms underlying these complex syndromes are not yet well understood, some important findings have been made. Firstly, the specific MED12 sites mutated in FG and Lujan syndromes are required for the interaction of MED12 with G9a and repression of REST target genes by MED12, suggesting epigenetic de-repression of neuronal genes as the cause for these syndromes (Ding et al., 2008). Similarly, MED12 mutations underlying Ohdo syndrome were shown to cause de-repression of REST target genes (Vulto-van Silfhout et al., 2013). Secondly, studies on lymphoblast cells from human FG and Lujan syndrome patients revealed the loss of CDK8 on Shh/GLI3 target genes that are normally repressed by MED12 in a CDK8 kinase-activity-dependent manner (Zhou et al., 2012). Both Shh- and REST-dependent transcription are important in neuronal development and disease, including mental retardation (Kramer and van Bokhoven, 2009; Vaillant and Monard, 2009), providing a rational to how MED12-mutations may cause neurodevelopmental disorders. Thirdly, MED12 FG mutations were found to disrupt the association of MED12 with eRNAs needed for chromatin looping, implicating that lost enhancer-promoter association could contribute to MED12-linked mental retardation syndromes (Lai et al., 2013). It is interesting to note that mutations in the MED12-interacting protein NIPBL and cohesin, required for enhancer-promoter looping, also cause developmental syndromes (Cohesinopathies; Cornelia de Lang syndrome, Roberts syndrome etc.) characterized among other features by mental retardation (Remeseiro et al., 2013).
The kinase module in cancer

In addition to developmental syndromes, alterations in kinase module subunits have also been found in cancer and tumorigenesis (Clark et al., 2015) (Table 1). Specific missense mutations in the N-terminus of MED12, mostly affecting amino acid G44, cause about 70% of all cases of uterine leiomyoma, a benign but clinically relevant tumor of the uterus (Makinen et al., 2011), and are found at lower frequency in malignant leiomyosarcoma (Kampjarvi et al., 2012). Whereas knockout of Med12 in the mouse endometrium does not cause tumors, knock-in of mutant Med12 in a Med12-deficient background does, thus confirming MED12 mutation as the cause of these tumors (Mittal et al., 2015). In human cells, these mutations lead to loss of cyclin C, CDK8 and CDK19 from the kinase module, suggesting that loss of Mediator kinase activity drives tumor formation (Turunen et al., 2014). It is not yet clear, however, if other MED12-regulated functions play a role in these tumors as well. MED12-mutated uterine leiomyomas are distinct from other leiomyomas and show defects in signaling pathways such as Wnt and prolactin signaling (Mehine et al., 2016). The same mutations in MED12, that mostly affect G44, have been found to cause 60% of benign fibroadenomas and phylloides tumors (benign or malignant) of the breast (Cani et al., 2015; Lim et al., 2014). MED12-mutant fibroadenomas show dysregulated estrogen signaling, providing a possible mechanism. MED12 has been shown to regulate estrogen receptor (ER)-dependent transcription together with cohesin in breast cancer cells (Prenzel et al., 2012). Interestingly, in addition to fibroadenomas, leiomyomas are also estrogen-dependent, suggesting a possible common mechanism for development of these tumors caused by mutation in MED12 (Mehine et al., 2014).

In addition to these hormone-dependent stromal tumors, MED12 missense mutations have been found at lower percentage in other cancers. Mutations, most commonly L1224F, have been reported to occur in up to 5% of prostate cancers (Barbieri et al., 2012). In contrast to the N-terminal leiomyoma/fibroadenoma MED12 mutations, the mutations found in prostate cancer do not affect MED12 binding to cyclin C or CDK8/CDK19 or their kinase
activity. The mutations do, however, cause a decrease abundance of core subunits MED1, MED14 (middle), MED17 (head), MED15 and MED24 (tail) in MED12 mutant Mediator preparations (Kampjarvi et al., 2016). In addition to these missense mutations, MED12 overexpression has been reported to occur in 40% of metastatic and 21% of local-recurrent castration-resistant prostate cancer (CRPC). Interestingly, both MED12 and MED12L are amplified in prostate cancer, suggesting they could both promote tumorigenesis (Taylor et al., 2017). Overexpression of MED12 correlates with activation of the TGFβ-signaling pathway and depletion of MED12 in prostate cancer cell lines reduces proliferation and expression of the TGFβ target vimentin, suggesting a mechanism for the role of MED12 in CRPC (Shaikhibrahim et al., 2014). In contrast to this, MED12 has been suggested to repress TGFβ signaling independently of MED13 and CDK8 in other cancer cell lines, resulting in drug resistance (Huang et al., 2012).

Somatic missense mutations in MED12 have further been found in hematopoietic cancers. In around 5% of chronic lymphocytic leukemia (CLL) cases studied, mutations are found in or near the same sites as in leiomyoma/fibroadenoma, suggesting that these mutations should lead to the loss of CDK8/CDK19-cyclin C from Mediator (Kampjarvi et al., 2015). In addition, loss-of-function mutations have been reported, although rarely, in T-cell acute lymphoblastic leukemia (T-ALL). T-ALL MED12 mutations have been found to cause loss of the cyclin C binding domain (splice site mutation) and nuclear exclusion of MED12 (nonsense mutation)(Heikkinen et al., 2017; Spinella et al., 2016). Consistent with this, loss of the CCNC and CDK19-containing locus is found in T-ALL where cyclin C has been identified as a haploinsufficient tumor suppressor (Li et al., 1996; Li et al., 2014). Cyclin C has further been suggested to function as a tumor suppressor in osteosarcoma, as one study reported loss of the CCNC-containing locus in 74% of studied cases (Ohata et al., 2006).
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uterine leiomyoma and leiomyosarcoma</strong></td>
<td><strong>MED12 missense mutation</strong></td>
</tr>
<tr>
<td></td>
<td>CDK8/CDK19/cyclin C loss, ER-, Wnt and prolactin signaling deregulation</td>
</tr>
<tr>
<td><strong>Breast fibroadenoma and phylloid tumors</strong></td>
<td><strong>MED12 missense mutation</strong></td>
</tr>
<tr>
<td></td>
<td>CDK8/CDK19/cyclin C loss ER signaling deregulation</td>
</tr>
<tr>
<td><strong>T-cell acute lymphoblastic leukemia</strong></td>
<td><strong>CCNC/CDK19 locus deletion</strong></td>
</tr>
<tr>
<td></td>
<td>CDK8/CDK19/CDK3 kinase activity loss, NOTCH activation (MED12 nuclear exclusion, loss of cyclin C binding)</td>
</tr>
<tr>
<td><strong>(MED12 missense &amp; splice site mutation)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Chronic lymphocytic leukemia</strong></td>
<td><strong>MED12 missense mutation</strong></td>
</tr>
<tr>
<td></td>
<td>CDK8/CDK19/cyclin C loss</td>
</tr>
<tr>
<td><strong>Prostate cancer</strong></td>
<td><strong>MED12 amplification, overexpression</strong></td>
</tr>
<tr>
<td><strong>(MED12 missense mutation)</strong></td>
<td>TGFβ activation (Reduced core Mediator binding)</td>
</tr>
<tr>
<td><strong>Osteosarcoma</strong></td>
<td><strong>(CCNC locus deletion)</strong></td>
</tr>
<tr>
<td><strong>Colorectal cancer</strong></td>
<td><strong>CDK8 amplification, overexpression</strong></td>
</tr>
<tr>
<td></td>
<td>Wnt signaling activation MYC target gene activation</td>
</tr>
<tr>
<td><strong>(CCNC amplification)</strong></td>
<td>(CDK8/CDK19/cyclin C loss)</td>
</tr>
<tr>
<td><strong>(MED12 missense mutation)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Breast cancer</strong></td>
<td><strong>CDK8 overexpression</strong></td>
</tr>
<tr>
<td></td>
<td>Cell cycle acceleration, p27 deregulation, ER signaling activation, STAT-1 activation</td>
</tr>
<tr>
<td><strong>(CDK19 overexpression)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td><strong>CDK8 overexpression</strong></td>
</tr>
<tr>
<td></td>
<td>Cell cycle progression, STAT-1 activation</td>
</tr>
</tbody>
</table>

**Table 1. Mutations and mis-expression of kinase module subunits in cancer.** The most studied mutations and mis-expressions of kinase module subunits found in human cancer (left column) are listed. When known, changes in signaling linked to the mutation or mis-expression is indicated (right column). Mutations in parenthesis occur at low frequency.
Although the role of cyclin C-CDK8/CDK19 complexes is tumor suppressive in leukemia and possibly osteosarcoma, CDK8 has also been implicated as an oncogene in different cancers. In melanoma, CDK8 is upregulated and drives cell cycle progression as a consequence of loss of the histone variant macroH2A1 (Kapoor et al., 2010). In breast cancer, CDK8 and CDK19 are overexpressed, correlating with poor survival (Broude et al., 2015). CDK8 overexpression in breast cancer is also promoted by mH2A1 suppression, resulting in decreased levels of the CDK inhibitor p27 and subsequent accelerated cell cycle progression (Xu et al., 2015a). A cancer promoting role of CDK8 in breast cancer could also be due to an activating role for CDK8 in estrogen receptor-dependent transcription (McDermott et al., 2017), or activating phosphorylation of STAT-1, which can drive proliferation of both breast cancer and melanoma cells (Putz et al., 2013). Furthermore, both under- and overexpression of kinase module subunits have been detected in various cancers (Clark et al., 2015), although in most cases the possible consequences of this, if any, for tumor progression have not been studied. The role of CDK8 gain, however, has been extensively studied in colorectal cancer, where CDK8 can function as an oncogene (Firestein et al., 2008).

- The kinase module in colorectal cancer

CDK8 is both amplified and overexpressed in colorectal cancer (CRC) (Firestein et al., 2008) and high levels of CDK8 in colon cancer correlate with poor survival (Firestein et al., 2010). In addition, CCNC amplification has been correlated with poor prognosis in CRC (Bondi et al., 2005). Depletion of CDK8 has been shown to reduce the proliferation of colon cancer cells and to prevent the growth of xenograft tumors derived from these cells in mice in vivo (Adler et al., 2012). The mechanism underlying CDK8 oncogenic function in colon cancer cells has been proposed to involve secondary activation of Wnt signaling due to repression of E2F1 by CDK8 (Firestein et al., 2008; Morris et al., 2008) (Figure 4, Table 1). In addition, CDK8 knockdown leads to the decreased expression of MYC target genes in embryonic stem cells and to impaired activation of immediate-early genes and HIF1A-induced genes upon serum stimulation and
hypoxia respectively in colon cancer cells (Adler et al., 2012; Donner et al., 2010; Galbraith et al., 2013). The possible contribution of these pathways to CDK8 oncogenic function however, is not yet known.

Whereas the impact of CDK8 overexpression in the intestinal epithelium in vivo is not known, deletion of Cdk8 is insufficient to reduce the tumor burden of the APC<sup>Min</sup> mouse model (McCleland et al., 2015) in which Wnt pathway activation causes adenomas of the intestine (Moser et al., 1990). In fact, Cdk8 knockout was found to increase tumorigenesis of the APC<sup>Min</sup> mouse and result in de-repression of PRC2 target genes (McCleland et al., 2015). The effect of inhibition of CDK8 kinase activity on cancer cell proliferation has been addressed in several studies. Because of the close homology of CDK8 and CDK19, many of these inhibitors target both kinases (Clarke et al., 2016; Porter et al., 2012; Poss et al., 2016). CDK8 (and CDK19) inhibition can reduce proliferation of colon cancer cells as well as other cancer cells including breast cancer and AML cells (He et al., 2013; Pelish et al., 2015; Porter et al., 2012). However, CDK8/CDK19 inhibition has also been shown to cause severe side effects in mice, calling into question the possibility of targeting these kinases in cancer treatment (Clarke et al., 2016).

The possible role of other kinase module subunits in CRC has not been as extensively studied. MED12 missense mutations have nonetheless been identified in a few percent of studied CRC tumors (Je et al., 2012; Kampjarvi et al., 2012; Seshagiri et al., 2012; Siraj et al., 2017). These mutations are spread out over the MED12 protein and it is not currently known which of them if any contribute to CRC tumor formation. Interestingly, identified CRC MED12 mutations include a few N-terminal leiomyoma/fibroadenoma hot spot mutations, suggesting that CDK8/CDK19 activity is lost in these tumors (Kampjarvi et al., 2012). Cases of misexpression and mutations of other kinase module subunits have been reported in CRC as well, but the possible consequences of this has not been studied (Clark et al., 2015).
6. Shared or distinct functions of kinase module subunits

The contradictory tumor suppressive and promoting outcome of kinase module subunit mutations, gain and loss in cancer highlight the notion of a context-dependent complex. It is unlikely that the Mediator kinase module exerts all of its suggested repressive and activating effects on transcription factors, chromatin modifiers and general factors simultaneously or in the same cell. Thus, the consequence of kinase module mutations or manipulations on transcription is clearly dependent on the presence and activity of the factors it regulates. In addition, the function of alternative kinase module subunits is likely to influence the outcome of kinase module depletion in vertebrate tissues in which both paralogs are expressed.

The extent to which the vertebrate alternative subunits have distinct or shared functions with CDK8, MED12 and MED13 is not well understood. Expression profiling of human cells has shown that the depletion of CDK8 or CDK19 leads to deregulation of both different and overlapping target genes (Tsutsui et al., 2011). Shared functions for CDK8 and CDK19 have further been reported in the repression of NOTCH- and C/EBPβ-dependent transcription (Li et al., 2014; Tsutsui et al., 2013). Interestingly, CDK8 and CDK19 have overlapping target genes in response to glucose deprivation, whereas only CDK8 regulates the response to hypoxia in the same cells (Galbraith et al., 2013). As CDK19 was recognized as a Mediator kinase much later than CDK8 (Sato et al., 2004), its possible role in CDK8-dependent functions has not been addressed in most cases. Redundancy of the kinases has, however, been demonstrated in mouse AML cells, in which knockdown of both CDK8 and CDK19 is needed to reduce proliferation (Bhagwat et al., 2016).

Even less is known about the function of MED12L and MED13L, as studies comparing the effects of their depletion on genome-wide transcription to that of MED12 and MED13 depletion are missing. Although depletion of both MED13 and MED13L is required to abolish kinase module binding with Mediator (Tsai et al., 2013) the severe outcome of dysfunction of either subunit in the
mammalian heart suggests that one subunit can not compensate for the loss of the other. Knockout of Med13 in the mouse heart disturbs metabolism, causing obesity and diabetes (Grueter et al., 2012), whereas a frameshift deletion in one allele of MED13L in human patients causes defects in heart and neurological development (Asadollahi et al., 2013). In mouse AML cells, depletion of MED13 alone is sufficient to decrease proliferation (Bhagwat et al., 2016), suggesting that MED13L can not compensate for the loss of MED13. Depletion of MED12 alone has repeatedly been shown to be sufficient to cause significant transcriptional defects (Aranda-Orgilles et al., 2016; Bhagwat et al., 2016; Kagey et al., 2010). However, both MED12 and MED12L can bind to SOX10 (Vogl et al., 2013) and amplification of both paralogs has been identified in prostate cancer (Taylor et al., 2017), implying shared functions for these kinase module subunits.

In yeast, deletion of any kinase module subunit, cdk8, cycC, med12 or med13, causes highly similar phenotypes, suggesting shared functions in transcription likely to be mediated through Cdk8 kinase activity (van de Peppel et al., 2005). Structural and biochemical studies have predicted that MED12 and MED13 are required for Mediator-dependent functions of CDK8 and cyclin C both in yeast and human cells (Knuesel et al., 2009a; Tsai et al., 2013). In agreement with this, human CDK8 and MED12 are both required for repression of Shh signaling and for activation of SOX10-dependent transcription (Vogl et al., 2013; Zhou et al., 2012). Furthermore, knockdown of mouse CDK8/CDK19, MED12 or MED13 all trigger differentiation of AML cells, implying shared functions (Bhagwat et al., 2016). In flies, mutating any of the four subunits causes similar defects in external sensory organ development (Loncle et al., 2007). However, there are also reports of MED12-independency of CDK8 both in human colon cancer cells (Adler et al., 2012) and in fruit fly cells (Chen et al., 2012), suggesting kinase module independent functions of CDK8.

In addition, studies of mutant fruit flies and tissues have shown that metazoan Med12 and Med13 have evolved Cdk8/CycC-independent functions. In development of Drosophila eyes, legs and wings Med12 and Med13 mutants
share phenotypes (Carrera et al., 2008; Janody et al., 2003; Treisman, 2001) that are more severe than phenotypes of Cdk8 or CycC mutants (Janody and Treisman, 2011; Loncle et al., 2007). There are also reports of MED13-independent functions for mammalian MED12 in both the repression of TGFβ signaling and the development of hematopoietic cells (Aranda-Orgiltes et al., 2016; Huang et al., 2012), although a possible compensatory role of MED13L has not been addressed in these studies. In fact, the role of the other kinase module subunits in a function described for a particular subunit remains unclear in most studies. Thus, the extent to which Mediator kinase module subunits share or have distinct functions has been largely unknown due to the lack of comparative studies of all kinase module subunits in metazoan species.
AIMS OF THE STUDY

At the time when this PhD project was initiated there were no studies on mammalian kinase module subunits *in vivo*. Thus, the **first** aim of this study was to investigate the *in vivo* function of mammalian CDK8 and its possible requirement for development and survival. As no genome-wide comparisons of all metazoan kinase module subunits existed prior to this study, the **second** aim of this thesis was to clarify the extent to which the subunits share or have distinct functions in transcription regulation. The **third** aim of this study was to identify signaling pathways activated or repressed by the metazoan kinase module subunits and the possible physiological relevance of identified functions. During the course of this thesis work, the kinase module has been implicated in cancer and a role in the activation of super-enhancer associated genes has been discovered for MED12 in stem cells. Thus, the **fourth** aim of this thesis was to investigate the possible requirement of the kinase module subunits for colon cancer acquired super-enhancer associated gene expression and proliferation of cancer cells.
RESULTS AND DISCUSSION

1. Metazoan CDK8 is dispensable for cell-autonomous survival but essential for pre-implantation mouse development

To study the in vivo function of mammalian CDK8, mice with a loss-of-function allele of Cdk8 were generated by aggregations of wt CD1 morulas and ES cells containing a β-Geo gene trap allele in the fourth exon of Cdk8, causing loss of the kinase domain (I, Fig. 1). The gene trap insertion results in expression of a mutant CDK8-β-Geo fusion protein that could be detected by anti-β-galactosidase western blotting of Cdk8+/− ES-cells. Heterozygote Cdk8 mice were phenotypically normal, demonstrating that the Cdk8 mutant allele does not act as a dominant gain-of-function allele. Cdk8 heterozygote mice were intercrossed to produce homozygous null Cdk8−/− mice. Although these intercrosses produced Cdk8 wt and heterozygous offspring at the Mendelian ratio, no Cdk8−/− genotype could be detected by PCR genotyping of weaned mice (I, Table 1). As this suggested embryonic lethality of Cdk8 homozygous deletion, embryos were sacrificed at the E12.5 midgestation stage to study the possible requirement of mouse CDK8 for organogenesis. LacZ staining of Cdk8−/− embryos at E11.5 demonstrated ubiquitous expression of the fusion protein at this stage. However, Cdk8−/− embryos were not detected and there were not any visible signs of spontaneous abortion, indicating requirement of CDK8 prior to embryo implantation. Embryos were thus analyzed at pre-implantation stages E2.5, E3.0 and E3.5 leading to the detection of Cdk8−/− embryos at E2.5 and E3.0. At E2.5 Cdk8−/− embryos displayed fragmented blastomeres and no compacted homozygous null embryos could be detected, indicating lethality prior to compaction (I, Fig. 2). This early lethality was surprising with regards to the limited knowledge of Cdk8 function (e.g. non-essential repressor in yeast) at the time of this study. To investigate whether metazoan CDK8 was required for cell-autonomous survival, CDK8 was depleted using RNA interference (RNAi) by dsRNA in cultured fruit fly and human cells. Transfection of fruit fly S2 cells with dsRNA targeting Cdk8 resulted in efficient knockdown of the Cdk8 protein.
but did not affect the growth of the cells, even when dsRNA transfection was repeated and cells followed for 11 days. Consistent with this, knockdown of human CDK8 using shRNA in 293FT cells did not affect colony formation capacity (I, Fig. 3). Taken together, these results suggest that similarly as in yeast (Holstege et al., 1998), metazoan CDK8 is not required for cell-autonomous survival although it is essential for pre-implantation embryonic development in mice.

Knockdown of other fruit fly kinase module components, Med12 and Med13, in S2 cells also did not affect cell-autonomous growth (Figure 5.), suggesting that the metazoan kinase module is not required for proliferation or general transcription. In agreement with this, later studies have demonstrated that knockout of mouse Cdk8 is dispensable for survival of mouse intestinal epithelial cells in vivo (McCleland et al., 2015) whereas knockout of Med12 does not affect viability of zebrafish embryonic cells (Shin et al., 2008) or mesenchymal cells in the mouse uterus (Mittal et al., 2015). Similarly as CDK8, mouse MED12 and cyclin C have also later been shown to be essential for early embryonic development. MED12 is required for gastrulation at E7.5, whereas Ccn3 null embryos die between E9.5 and E10.5 (Li et al., 2014; Rocha et al., 2010). In addition, knockout of core Mediator subunits causes embryonic lethality due to specific developmental defects, consistent with the notion that different subunits regulate specific transcriptional responses (Yin and Wang, 2014). The early lethality of Cdk8\(^{-/-}\) embryos suggests that the loss of CDK8 leads to the aberrant expression of genes crucial for development just prior to compaction. Recent work describing Cdk8 conditional knockout in the mouse intestine has implicated CDK8 in activation of the PRC2 methyltransferase EZH2 in vivo (McCleland et al., 2015). Interestingly, EZH2 is also required at early development and high expression already in the oocyte suggests a role for EZH2 during pre-implantation (O’Carroll et al., 2001). Thus, it is possible that de-repression of EZH2 target genes could contribute to Cdk8\(^{-/-}\) embryonic lethality. In addition, depletion of either Cdk8 or Med12 in cultured mESCs leads to decreased expression of the pluripotency transcription factors Oct4 and Nanog (Adler et al., 2012; Kagey et al., 2010). Whereas this does not
recapitulate in ES cells harboring the truncated Med12-mutant allele used to generate Med12 null and hypomorphic mutants (Rocha et al., 2010), it remains to be elucidated if Cdk8−/− embryos have lost expression of Oct4 and Nanog, which could explain the early lethality.

Figure 5. *Drosophila* Med12 and Med13 are dispensable for proliferation
S2 cells were transfected with 20μg of indicated dsRNA on day 0. Cell amounts were measured by counting a sample of cells by a Coulter Counter on days 0, 1, 3, 5 and 7. Columns and error bars show mean and s.d. of two experiments except for day seven, which represents one experiment. In the longer (7 day) experiment cells were split and dsRNA transfection repeated on days 3 and 5 and cell amounts were estimated in relation to the starting amount. The ratio of cells in Med12 and Med13 dsRNA samples compared to the GFP dsRNA sample was not affected at day 5 by repeated transfection on day 3.
2. Cdk8 and CycC have distinct roles from Med12 and Med13 in transcription although Cdk8-CycC function is dependent on Med12 and Med13

Concurrently with our mouse CDK8 study, *Drosophila* Cdk8, CycC, Med12 and Med13 were shown to be required for fruit fly development (Loncle et al., 2007), although dispensable for cell survival (Figure 5. and Treisman, 2001). As flies lack the alternative vertebrate-specific kinase module subunits CDK19, MED12L and MED13L (Bourbon et al., 2004), *Drosophila* cells were used to study the relationships between metazoan Cdk8, CycC, Med12 and Med13. To identify kinase module-regulated genes, expression profiling was performed by microarrays following depletion of Cdk8, CycC, Med12 or Med13 by dsRNA in S2 cells (II). Depletion of any kinase module subunit resulted in both up- and downregulation of transcripts, suggesting repressive and activating roles in transcription, respectively. Expression profiles of Cdk8 and CycC depleted cells were highly similar but surprisingly different from expression profiles of Med12 or Med13 depleted cells. Only a small subset of kinase module-regulated transcripts were similarly changed by depletion of each subunit, whereas the majority of Cdk8-CycC-regulated genes were unaffected or even oppositely regulated by Med12 or Med13 depletion (II, Fig. 1). This suggests that unlike in yeast, where all kinase module subunits are needed for the same set of genes (van de Peppel et al., 2005), metazoan Cdk8-CycC and Med12-Med13 pairs have mostly distinct functions.

The observed co-regulation between Med12 and Med13 was striking and suggested that Med13 rarely functions independently of Med12, although the kinase module hierarchy suggests this could be possible as Med13 can bind Mediator without Med12 (Figure 1. and Tsai et al., 2013). Consistent with this, Med12 and Med13 mutant fruit flies also display highly similar defects in development of specific organs (Janody et al., 2003; Treisman, 2001). The majority of Med12/Med13-regulated genes were unaffected by Cdk8 or CycC depletion, supporting the notion that differently from yeast, metazoan Med12 and Med13 have evolved kinase independent functions (Loncle et al., 2007). Structural and biochemical studies have shown that Cdk8 and CycC bind
Mediator through Med12 and Med13 (Knuesel et al., 2009a; Tsai et al., 2013; Turunen et al., 2014). Taking this into account, the significant set of Cdk8-CycC-regulated genes that were not affected by Med12 or Med13 depletion (II, Fig.1) was surprising. To address if Cdk8-CycC can function independently of Med12-Med13 in transcriptional regulation, the expression of Cdk8-CycC-regulated genes was measured following a simultaneous depletion of all four kinase module subunits. Importantly, this experiment revealed a dependency of Cdk8-CycC on Med12-Med13 for all assayed genes, as Cdk8-CycC-mediated effects were lost when Med12-Med13 were simultaneously depleted (Figure 6. and II, Fig. 2). This experiment further demonstrated that Med12-Med13-dependent gene regulation can be masked by opposite effects of Cdk8-CycC and suggested that opposite regulation by the two pairs is common.

![Figure 6. Med12-Med13 is epistatic to Cdk8-CycC](image)

Med12-Med13-dependent transcriptional regulation can be masked by opposite effects of Cdk8-CycC. The model shows an example gene, which is activated by Med12-Med13 and repressed by Cdk8-CycC. Cdk8-CycC depletion causes de-repression, resulting in an increase of expression level. Cdk8-CycC binding to core Mediator is dependent on Med12-Med13. Thus, Med12-Med13 depletion results in loss of Med12-Med13-mediated activation but also loss of Cdk8-CycC-mediated repression. Concomitant loss of both opposing signals results in unchanged expression. Dependency of Cdk8-CycC on Med12-Med13 is revealed by depletion of all four subunits: Cdk8-CycC depletion has no effect on gene expression in the absence of Med12-Med13.
3. Redundancy of human MED13 and MED13L and conserved co-regulation by MED12 and MED13/MED13L

Studies comparing genome-wide transcriptional effects of kinase module subunits in mammalian systems are limited to comparisons of CDK8 and CDK19 (Galbraith et al., 2013; Tsutsui et al., 2011) or CDK8 and MED12 (Adler et al., 2012). To identify mammalian kinase module-regulated genes and address subunit relationships, RNA-sequencing was performed following siRNA-mediated depletion of CDK8, MED12, MED13 or MED13L individually or following double depletion CDK8 together with CDK19 and MED13 together with MED13L in human colon cancer HCT116 cells (III). Whereas CDK8 depletion caused minor changes in gene expression (134 genes), CDK8 and CDK19 double depletion had stronger effects (452 genes) that significantly overlapped with gene expression changes observed after MED12 or MED13 and MED13L double depletion (III, Fig.1). Opposite direction gene expression changes by depletion of CDK8 and CDK19 compared to that of depletion of MED12 and MED13 together with MED13L was observed here only for 0.15 % (2 out of 1315 genes) of significantly regulated genes (compared to 4.74 % in S2 cells, 11 out of 232 genes, II, Fig.1). However, the epistatic relationship of Med12-Med13 to Cdk8-CycC observed in S2 cells (Figure 6 and II, Fig.2) suggests that MED12/MED13/MED13L-mediated regulation is likely to be masked by opposite effects of CDK8/CDK19 in colon cancer cells as well (up to 19% of significantly regulated genes, 250 out of 1315 genes changed by CDK8/CDK19 siRNA but not MED12 and MED13/MED13L siRNA).

Individual depletion of MED13 or MED13L resulted in surprisingly limited gene expression changes (siMED13 156 genes, siMED13L 177 genes), while their simultaneous depletion caused strong effects (1998 genes) (III, Fig.1). This demonstrates redundancy of MED13 and MED13L in colon cancer cells and is in line with the observation that both subunits need to be depleted to abolish kinase module association with core Mediator in HeLa cells (Tsai et al., 2013). However, these results do not exclude the possibility of MED13 or MED13L-specific functions in other cell types. Importantly, the MED13/MED13L redundancy observed in this study suggests that other previously identified
functions for MED12 that are not affected by MED13 depletion (Aranda-Orgilles et al., 2016) are still likely to be Mediator kinase module-dependent. MED12 depletion caused highly similar gene expression changes (1971 deregulated genes) compared to depletion of MED13 and MED13L (III, Fig.1). This striking correlation between MED12 and MED13/MED13L depletion RNA-seq profiles further suggests very limited MED12-independent functions for MED13 and MED13L, similarly to what was observed for Med12 and Med13 in Drosophila S2 cells (II, Fig.1). Taken together, this study demonstrates genetically a conserved hierarchy of Mediator kinase module subunits that supports the structural model of the metazoan Mediator, in which the MED13/MED13L subunit links the CDK-cyclin pair to core Mediator via a centrally located MED12 (Knuesel et al., 2009a; Tsai et al., 2013; Turunen et al., 2014).
4. Med12 and Med13 are required for activation of Serpent/GATA-dependent innate immunity in *Drosophila*

To understand which signaling pathways the *Drosophila* Mediator kinase module regulates, the expression profiles of Cdk8, CycC, Med12 and Med13 knockdown cells (II) were compared to available S2 cell expression profiles following depletion of relevant transcriptional regulators. Cdk8- and CycC-activated genes correlated significantly with genes shown to be dependent on NELF (Muse et al., 2007), suggesting a role for Cdk8-CycC in NELF-regulated transcription. Interestingly, inhibition of human CDK8/CDK19 was subsequently shown to cause the decrease of a phosphorylated form of the NELF-A (S-363 and S-360) subunit (Poss et al., 2016), suggesting that NELF could be directly regulated by CDK8 phosphorylation.

About one third of Med12-Med13-activated genes were co-regulated by the Drosophila GATA transcription factor Serpent (Srp) (II, Fig.3), a master regulator of hematopoiesis and immunity (Fossett, 2013; Petersen et al., 1999). Med12-Med13/Srp-co-activated genes were more strongly decreased by Srp depletion than other Srp-dependent genes. Furthermore, promoter analysis revealed enrichment of GATA binding sites (HGATAABV sequence) in promoters of genes co-activated by Srp and Med12-Med13, suggesting that these genes are direct targets of Srp (Figure 7 and II, Fig.3). During the preparation of this study, Med12 and Med13 were shown to activate a Srp/Lz reporter gene and to be specifically required for the differentiation of hematopoietic Lz-dependent crystal cells (Gobert et al., 2010). Our study, however, demonstrated that Med12/Med13-mediated activation of Srp target genes does not require the presence of Lz (II, Fig.4). Med12-Med13 and Srp co-regulation thus comprises a significant set of Srp-activated genes, including novel Srp target genes such as *CG14629*, a predicted *Drosophila* homolog of Leukemia inhibitory factor, LIF (Cheng et al., 2009). To further study the function of Med12 and Med13 in Srp-activated transcription, the predicted *CG14629* promoter was fused to a luciferase reporter gene. Another luciferase reporter containing the promoter of the *Mtk*, a previously identified Srp target gene.
gene (Senger et al., 2004), was also used. Both the CG14629 (II, Fig.4) and the Mtk (II, Fig.5) luciferase reporter gene constructs were decreased upon Med12, Med13 or Srp depletion. However, CG14629 and Mtk reporters with mutated GATA sites showed reduced response to Med12, Med13 or Srp depletion compared to wt reporters. Thus, Med12/Med13-dependent activation of CG14629 and Mtk was partially mediated through functional GATA binding sites (II, Fig. 4 and 5). It remains to be established if Med12/Med13-dependent Srp target gene activation further depends on a direct interaction of these Mediator subunits and Srp. However, coinciding with our study, the kinase module was shown to co-immunoprecipitate with over expressed Srp and Lz in fruit fly Kc167 cells (Gobert et al., 2010), suggestive of a physical interaction.

Several Med12/Med13-activated genes are implicated in the regulation of innate immunity, including the Serpent-dependent IMD pathway-responsive antimicrobial peptide genes Mtk and DptB. To investigate the possible role of Med12-Med13 in innate immunity, S2 cells were treated with heat-inactivated E. coli bacteria to activate the IMD pathway. Interestingly, Med12 and Med13 knockdown cells were unable to fully activate IMD target gene expression in response to E. coli treatment. This was reproduced in vivo as tissue-specific knockdown of Med12 or Srp in larval fatbodies using the Fb-Gal4 driver decreased the expression of Mtk and DptB upon septic injury (II, Fig.5). Taken together, this study identified a novel role for Med12 and Med13 in GATA-dependent transcription and innate immunity (Figure 7.).

Other studies have also linked the kinase module to immune response regulation via different mechanisms. In mouse embryonic fibroblasts (MEFs), CDK8 can activate the interferon response via phosphorylation of STAT-1 (Bancerek et al., 2013) whereas CDK8 and CDK19 repress C/EBPβ-target immune genes in HeLa cells (Tsutsui et al., 2013). In our study, fruit fly Cdk8 and CycC were required for expression of a subset of Med12-Med13 and Srp-dependent genes including IMD-pathway genes Mtk and DptB. In addition, limited co-regulation was observed for Cdk8-CycC and the Drosophila STAT transcription factor STAT92E, suggesting that Cdk8-dependent activation of
STAT might be conserved. A role for the mammalian Mediator kinase module in GATA-activated transcription has not been directly demonstrated. However, in mHSCs, Med12 knockout was recently shown to cause decreased expression of genes dependent on the GATA2 transcription factor (Aranda-Orgilles et al., 2016). Furthermore, MED12 co-localizes with GATA2 on super-enhancers in mHSCs and Med12 knockout leads to loss of H3K27ac and p300 binding. MED12 is thus essential for the survival of these cells and was also recently shown to be required for the maintenance of the undifferentiated state and for proliferation of mouse AML blasts (Bhagwat et al., 2016). Investigating GATA2-dependent enhancer activity in AML and MED12-mutated hematopoietic cancers could thus be an interesting area for further research.

![Figure 7. Med12 and Med13 are required for activation of Serpent-dependent genes and innate immunity in Drosophila.](image)

One third of Med12-Med13 dependent genes in S2 cells require the GATA transcription factor Serpent (Srp). Enrichment of GATA binding sites implicates Med12-Med13 and Srp co-regulated genes as direct Srp targets. Med12 and Med13 activated both novel (eg. CG14629) and known (eg. IMD pathway) Serpent target genes with implications for innate immunity activation. A physical interaction between the kinase module and Serpent has been suggested in another study (Gobert et al., 2010).
5. MED12, MED13 and MED13L are required for cancer-acquired super-enhancer associated gene expression and proliferation of colon cancer cells

Recent research has established a role for MED12 in activation of super-enhancer-dependent transcription in non-malignant stem cells (Aranda-Orgilites et al., 2016; Whyte et al., 2013). Cancer cells acquire new super-enhancers that drive expression of oncogenes. Thus, targeting super-enhancer-dependent transcription has showed potential in cancer drug research (Chipumuro et al., 2014; Hnisz et al., 2013; Loven et al., 2013). In colon cancer, CDK8 is considered to be an oncogene (Firestein et al., 2008), whereas the role of other subunits is largely unknown. To investigate the possible role of the Mediator kinase module in colon cancer super-enhancer associated gene (SE gene) transcription, the expression of previously identified SE genes (Hnisz et al., 2013), was examined using RNA-seq in HCT116 cells following depletion of multiple kinase module subunits (III). CDK8/CDK19 did not repress SE-genes in colon cancer cells, as reported previously in AML (Pelish et al., 2015), but instead they were partially required for SE-gene activation (III, Fig.1). However, SE genes were not more strongly down regulated by CDK8/CDK19 depletion compared to down regulated non-SE genes. Interestingly, the expression of SE genes was more sensitive to depletion of MED12 or double depletion of MED13 and MED13L compared to other genes as both MED12 or MED13/MED13L depletion resulted in stronger decrease of SE genes compared to non-SE genes (III, Fig.2). Thus, MED12, MED13 and MED13L are disproportionally required for expression of SE genes in colon cancer cells (Figure 8).

BRD4 is required for SE gene activation in cancer cells (Chapuy et al., 2013; Loven et al., 2013) and thus, BRD4 targeting has been suggested as a therapeutic opportunity in hematopoietic cancer and in triple negative breast cancer, TNBC (Roderick et al., 2014; Roe et al., 2015; Shu et al., 2016). In colon cancer cells, BRD4 can interact with CDK8-Mediator (Donner et al., 2010) and BRD4 inhibition impairs proliferation and expression of the SE gene MYC (McCleland et al., 2016). Thus, RNA-seq was performed after BRD4 depletion in HCT116 cells to compare the effect of BRD4 depletion with that of kinase
module subunit depletions on SE gene transcription. BRD4 depletion resulted mostly in decrease of SE gene expression, indicative of a positive role for SE gene transcription. However, the effect of BRD4 depletion on SE gene expression was not stronger as compared to the effect on expression of other BRD4-activated genes (III, Fig.2). Importantly, only MED12 and MED13/MED13L double depletion had a specific effect on cancer-acquired SE genes, i.e. genes associated with a super-enhancer only in colon cancer cells but not in normal colon, whereas BRD4 depletion also resulted in decreased expression of control genes, associated with a super-enhancer only in normal colon (Figure 8 and III, Fig.2). Furthermore, whereas MED12, MED13 and MED13L were required for expression of highly expressed SE genes, this was not seen for BRD4. Taken together, these results suggest that depletion of MED12 or MED13 and MED13L provides a strategy to target highly expressed SE genes in colon cancer cells and imply that this should have more cancer specific effects than inhibition of BRD4.

Colon cancer super-enhancers are sensitive to Wnt pathway manipulation and are bound by high amount of the β-catenin-interacting Wnt pathway transcription factor TCF4 (Hnisz et al., 2015). To investigate if MED12-dependent Wnt pathway activation, reported previously in HeLa cells (Kim et al., 2006), could explain the requirement of MED12, MED13 and MED13L for SE gene expression, the siMED12 and siMED13/siMED13L RNA-seq profiles were compared to RNA-seq results following β-catenin depletion in HCT116 cells (Moffa et al., 2016). Interestingly, MED12/MED13/MED13L-activated SE genes showed enrichment for dependency on β-catenin compared to MED12/MED13/MED13L-dependent non-SE genes (III, Fig.2). Thus, kinase module recruitment to super-enhancers via β-catenin might explain the disproportional requirement of MED12, MED13 and MED13L for SE gene expression. In addition, the possible contribution of kinase module interactions with cohesin and pTEFb, both implicated in SE gene activation in other cells (Bhagwat et al., 2016; Hnisz et al., 2013), would be interesting to study.
Figure 8. MED12, MED13 and MED13L are required for expression of colon cancer acquired super-enhancer associated genes.
Cancer cells acquire super-enhancers that drive high expression of oncogenes such as MYC. Super-enhancers are characterized by high levels of transcription factors (dark gray), co-factors (light gray) and Mediator (turquoise). In colon cancer cells, highly expressed cancer acquired super-enhancer associated genes are especially sensitive to depletion of MED12 or MED13 and MED13L compared to genes that are not associated with a super-enhancer.

MED12 or MED13 and MED13L depletion in colon cancer cells caused a dramatic loss of expression of the cancer acquired SE gene MYC and its target genes (III, Fig.3). Strong decrease of MYC could be detected already one day after siRNA transfection whereas knockdown of MED12 or MED13 and MED13L did not affect MYC expression in a non-malignant colon-derived control cell line CCD841 CoN. Accordingly, MED12 or MED13 and MED13L depletion resulted in reduced proliferation of HCT116 and DLD1 cells (another colon cancer cell line), while CCD841 CoN cell numbers were unaffected (III, Fig.3). The effect on MYC expression following BRD4 depletion was not as apparent. In addition to MYC, also other SE genes, such as EREG and LIF, were more dependent on MED12, MED13 and MED13L as compared to BRD4. On the other hand, some SE genes, such as SUSD2, were more sensitive to BRD4 depletion. This implied partly independent mechanisms in SE gene activation for BRD4 and MED12/MED13/MED13L. In agreement with this, combining BRD4 depletion with depletion of MED12 or MED13 and MED13L resulted in a robust decrease
of all examined cancer SE genes and additive effects on colon cancer cell proliferation (III, Fig.4). Similarly, combined depletion of MED12 or MED13/MED13L with inhibition of BRD4 by the BET inhibitor JQ1 resulted in an increased growth-inhibitory effect, suggesting dual targeting as a possible future therapeutic opportunity. Interestingly, in TNBC, resistance to BRD4 inhibition has been demonstrated to involve BET-domain independent recruitment of BRD4 by Mediator to gained super-enhancers (Shu et al., 2016). Similarly, not all MED1 bound super-enhancers are sensitive to BRD4-inhibition in mouse AML cells (Bhagwat et al., 2016). Thus, dual targeting of the Mediator kinase module and BRD4 might cause additive effects on proliferation not only in colon cancer cells, but also in other cancers, including TNBC and AML.
CONCLUDING REMARKS

Two decades of research has modified the understanding of Mediator kinase module function substantially. A complex that was initially regarded as a repressor of PIC formation is now known as a context-dependent regulator of specific signaling pathways and enhancers important in development and malignancy. The first reports of an activating function for the kinase module were controversial and seemed contradictory with structural studies that had shown mutually exclusive binding of the kinase module and RNAPII to core Mediator. However, high-resolution crystal structures have led to a more precise map of subunit location within Mediator, establishing the middle module as the kinase module-binding site at one end of the core, whereas RNAPII binds mostly through the head module. Thus, although the kinase module associates with core Mediator without RNAPII in purified complexes in vitro, the new model of Mediator subunit arrangement does not exclude simultaneous association of the kinase module and RNAPII with core Mediator in vivo.

The CDK8 null mouse (I) was the first study of mammalian kinase module function in vivo and established CDK8 as an essential gene for metazoan development. At the time when the CDK8 null mouse was generated, further studies that could have explained the mechanism causing early lethality seemed laborious. Today however, modern single-cell sequencing techniques could be undertaken to study the mechanism underlying CDK8 requirement in pre-implantation development. Subsequently generated conditional knockout alleles of Cdk8, CCNC and Med12 have provided important insights into kinase module function in development and disease. These studies have further shown that kinase module function in regulation of chromatin modifiers, transcription factors and enhancer-promoter looping first identified in cultured cells recapitulates in vivo. Further studies of mammalian kinase module subunit knockout in different tissues as well as studies of the specific mutations that cause developmental disorders and tumorigenesis in their physiological context will be needed to understand the in vivo function of the kinase module.
The Mediator kinase module has been shown to interact with a wide spectrum of transcription factors and to either activate or repress their function. In this thesis, an activating role was identified for fruit fly Med12 and Med13 in Serpent/GATA-dependent transcription with implications for innate immunity regulation (II). Whether or not Mediator is recruited to GATA-activated genes via a direct interaction between Serpent and the kinase module remains unclear. However, the requirement of MED12 for GATA2-occupied super-enhancers in mouse HSCs suggests that if such an interaction takes place, as implied by experiments using overexpressed Serpent, it might be conserved in mammals. The identification of a role for MED12 in super-enhancer activation is important and may explain the context dependency of kinase module function. The presence of context dependent kinase module-interacting transcription factors; GATA and NOTCH in hematopoietic cells, SOX factors and GLI3 in neuronal tissues, β-catenin and HIF1α in colorectal cancer cells, could thus specifically recruit CDK8-Mediator to cell identity gene enhancers and super-enhancers containing high amounts of binding sites for these factors. On enhancers, CDK8-Mediator can subsequently regulate transcription of target genes via interaction with cohesin, RNAPII, elongation factors and chromatin modifiers (Figure 3).

In this thesis, an activating function for super-enhancer associated genes was identified not only for MED12 but also for MED13 and MED13L and to a lesser extent CDK8 and CDK19 (III). Importantly, the disproportional requirement of MED12, MED13 and MED13L for cancer-acquired super-enhancer associated gene expression implies that targeting these kinase module subunits might provide a future therapeutic opportunity. Ever since CDK8 was identified as a colorectal cancer oncogene, the possibility of targeting CDK8/CDK19 in cancer treatment has been investigated. However, results have been contradictory and both other studies and this study have identified distinct effects of CDK8 and CDK19 depletion compared to their inhibition. Furthermore, the kinase module CDKs and cyclin can have opposing effects from MED12/MED13/MED13L on the same genes both in mammalian cells and in flies (II). The epistatic
relationship of Med12-Med13 (and MED12 and MED13/MED13L based on kinase module structure) to Cdk8-CycC suggests that opposite regulation is common. The results of this thesis from comparing transcriptional effects following depletion of multiple metazoan kinase module subunits do not support the existence of MED12/MED13/MED13L-independent functions for CDK8/CDK19/cyclin C or MED13/MED13L independent functions for MED12 in the fruit fly and human cells used here (II, III). This study does however not excluded that such independent functions could exist in other contexts or that the kinase module could regulate transcription independently of core Mediator. Contribution of other Mediator proteins, within the kinase module and core, in functions or disease linked to a specific kinase module subunit thus remain an interesting area for further research.
MATERIALS AND METHODS

Materials and methods used in this study are listed in the tables below. More detailed protocols can be found in the original publications referred to with Roman numerals.

1. Materials

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Description</th>
<th>Source/Reference</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-β-Gal</td>
<td>mouse monoclonal, U Z3781</td>
<td>Promega</td>
<td>I</td>
</tr>
<tr>
<td>anti-BRD4</td>
<td>(Hs) rabbit polyclonal, H250 sc-48772</td>
<td>Santa Cruz</td>
<td>III</td>
</tr>
<tr>
<td>anti-CDK8</td>
<td>(Dm/Hs/Mm) rabbit polyclonal, K-35</td>
<td>Dr. E. Nigg (Tassan et al., 1995)</td>
<td>I, II, III</td>
</tr>
<tr>
<td>anti-Cyclin C</td>
<td>(Dm) rabbit polyclonal</td>
<td>Dr. P. Leopold (Leclerc et al., 1996)</td>
<td>II</td>
</tr>
<tr>
<td>anti-Cyclin C</td>
<td>(Hs) rabbit polyclonal, A301-989A</td>
<td>Bethyl Laboratories</td>
<td>III</td>
</tr>
<tr>
<td>anti-CDK19</td>
<td>(Hs) rabbit polyclonal, HPA007053</td>
<td>Sigma-Aldrich</td>
<td>III</td>
</tr>
<tr>
<td>anti-Gapdh</td>
<td>(Hs) rabbit monoclonal, 14C10 2118S</td>
<td>Cell signaling</td>
<td>III</td>
</tr>
<tr>
<td>anti-Med12</td>
<td>(Dm) guinea pig polyclonal, anti-Kto</td>
<td>Dr. J. Treisman (Janody et al., 2003)</td>
<td>II</td>
</tr>
<tr>
<td>anti-MED12</td>
<td>(Hs) rabbit polyclonal, A300-774A</td>
<td>Bethyl Laboratories</td>
<td>III</td>
</tr>
<tr>
<td>anti-Med13</td>
<td>(Dm) rabbit polyclonal, anti-Skd</td>
<td>Dr. J. Treisman (Janody et al., 2003)</td>
<td>II</td>
</tr>
<tr>
<td>anti-MED13L</td>
<td>(Hs) rabbit polyclonal, A301-278A</td>
<td>Bethyl Laboratories</td>
<td>III</td>
</tr>
<tr>
<td>anti-MYC</td>
<td>(Hs) mouse monoclonal, 9E10 MMS-150R</td>
<td>Babco</td>
<td>III</td>
</tr>
</tbody>
</table>
### Cell and fruit fly lines

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Source/Reference</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td>293FT</td>
<td><em>Hs</em> embryonic kidney</td>
<td>Invitrogen</td>
<td>I</td>
</tr>
<tr>
<td>CCD841 CoN</td>
<td><em>Hs</em> colon CRL-1790</td>
<td>ATCC</td>
<td>III</td>
</tr>
<tr>
<td><em>Cdk8</em>- ES</td>
<td>RRS314 Cdk8 gene trap</td>
<td>BayGenomics</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Mm embryonic stem cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLD-1</td>
<td><em>Hs</em> colorectal adenocarcinoma</td>
<td>Mäkelä lab</td>
<td>III</td>
</tr>
<tr>
<td>HCT-116</td>
<td><em>Hs</em> colorectal carcinoma</td>
<td>Mäkelä lab</td>
<td>III</td>
</tr>
<tr>
<td>S2</td>
<td><em>Dm</em> Schneider 2 R690-07</td>
<td>Invitrogen</td>
<td>I, II</td>
</tr>
<tr>
<td>FB-Gal4</td>
<td>Fatbody specific fly line</td>
<td>Dr. V. Hietakangas</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>FBti0013267</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Med12 RNAi</td>
<td>v23142: FBst0454848,</td>
<td>VDRC and Bloomington stock center</td>
<td>II</td>
</tr>
<tr>
<td>fly</td>
<td>34588: FBst0034588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Srp RNAi fly</td>
<td>v109521: FBst0481207</td>
<td>VDRC</td>
<td>II</td>
</tr>
<tr>
<td>w118</td>
<td>Control fly line</td>
<td>Dr. V. Hietakangas</td>
<td>II</td>
</tr>
</tbody>
</table>

### Datasets

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Source/Reference</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-catenin RNA-seq</td>
<td>siCTNNB1, control, <em>Hs</em> HCT116, SOLiD sequencing</td>
<td>Array Express E-MTAB-651, (Moffa et al., 2016)</td>
<td>III</td>
</tr>
<tr>
<td>BRD4 RNA-seq</td>
<td>siBRD4, siNT, <em>Hs</em> HCT116, Illumina NextSeq 500</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>BRD4 RNA-seq</td>
<td>shBRD4, shNTC, <em>Hs</em> HCT116, Illumina HiSeq 2000</td>
<td>GEO GSE73317, (McCleland et al., 2016)</td>
<td>III</td>
</tr>
<tr>
<td>Catalogue of super-enhancers</td>
<td>Super-enhancers identified by H3K27ac ChIP-seq in <em>Hs</em> HCT116 cells and sigmoid colon</td>
<td>(Hnisz et al., 2013)</td>
<td>III</td>
</tr>
<tr>
<td>CDK8-module RNA-seq</td>
<td>siCDK8, siCDK8+siCDK19, siMED12, siMED13, siMED13L, siMED13+, siMED13L, siNT, <em>Hs</em> HCT116, Illumina NextSeq 500</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Description</td>
<td>Source/Reference</td>
<td>Used in</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Cdk8 module RNAi microarray</td>
<td>Cdk8, Cyc, Med12, Med13, GFP, Luc dsRNA, Dm S2 cells, Affymetrix Drosophila Genome 2.0 Microarray</td>
<td>GEO GSE52343, (II)</td>
<td>II</td>
</tr>
<tr>
<td>Cdk9 RNAi expression microarray</td>
<td>Cdk9 dsRNA, control, Dm S2 cells, Affymetrix Drosophila Genome 2.0 Microarray</td>
<td>(Bonke et al., 2013)</td>
<td>II</td>
</tr>
<tr>
<td>E2f1 RNAi microarray</td>
<td>E2f1 dsRNA, control, Dm S2 cells, Affymetrix Drosophila Genome 2.0 Microarray</td>
<td>(Bonke et al., 2013)</td>
<td>II</td>
</tr>
<tr>
<td>H3K27ac ChIP-seq</td>
<td>Anti-H3K27ac, Abcam ab4729 ChIP seq, Hs HCT116 analyzed in (Hnisz et al., 2013)</td>
<td>GSM945853, (Consortium, 2012)</td>
<td>III</td>
</tr>
<tr>
<td>GSEA Hallmark gene sets</td>
<td>Broad Institute, 50 Hallmark gene sets</td>
<td>(Mootha et al., 2003; Subramanian et al., 2005)</td>
<td>III</td>
</tr>
<tr>
<td>JQ1 RNA-seq</td>
<td>JQ1, DMSO, Hs HCT116, Illumina HiSeq 2000</td>
<td>GEO GSE73318, (McCleland et al., 2016)</td>
<td>III</td>
</tr>
<tr>
<td>Nelf RNAi microarray</td>
<td>Nelf-B, Nelf-E, LacZ dsRNA Dm S2 cells, Affymetrix Drosophila Genome 2.0 Microarray</td>
<td>GEO GSE6714, (Muse et al., 2007)</td>
<td>II</td>
</tr>
<tr>
<td>Nipped-B RNAi microarray</td>
<td>Nipped-B dsRNA, Mock, Dm BG3 cells, Affymetrix Drosophila Genome 2.0 Microarrays</td>
<td>GEO, GSE16152, (Schaaf et al., 2009)</td>
<td>II</td>
</tr>
<tr>
<td>Srp RNAi microarray</td>
<td>Srp dsRNA, control, Dm S2 cells, Affymetrix Drosophila Genome Microarray</td>
<td>(Kuuluvainen et al., 2014)</td>
<td>II</td>
</tr>
<tr>
<td>Stat92E RNAi microarray</td>
<td>Stat92E dsRNA, control, Dm S2 cells, Affymetrix Drosophila Genome 2.0 Microarray</td>
<td>(Bonke et al., 2013)</td>
<td>II</td>
</tr>
<tr>
<td>Name</td>
<td>Description</td>
<td>Source/Reference</td>
<td>Used in</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Cdk8 dsRNA</td>
<td>dsRNA targeting exon 2 (dsRNA_/1 and dsRNA_/2) of Dm Cdk8</td>
<td>I, II, generated by author</td>
<td>I, II</td>
</tr>
<tr>
<td>CycC dsRNA</td>
<td>dsRNA targeting exon 1 (dsRNA_/1) and 2 (dsRNA_/2) of Dm CycC</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td>GFP dsRNA</td>
<td>dsRNA targeting EGFP</td>
<td>I, II, generated by author</td>
<td>I, II</td>
</tr>
<tr>
<td>Luc dsRNA</td>
<td>dsRNA targeting Renilla luciferase</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td>Lz dsRNA</td>
<td>dsRNA targeting exon 2 of Dm Lozenge</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td>Med12 dsRNA</td>
<td>dsRNA targeting exon 2 (dsRNA_/1) and 3 (dsRNA_/2) of Dm Med12</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td>Med13 dsRNA</td>
<td>dsRNA targeting exon 7 (dsRNA_/1) and 10 (dsRNA_/2) of Dm Med13</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td>Srp dsRNA</td>
<td>dsRNA targeting exon 7 (dsRNA_/1) and 1 (dsRNA_/2) of Dm Serpent Hs</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td>siBRD4</td>
<td>siBRD4: L-004937-00, siBRD4_/1: J-004937-06, siBRD4_/2: J-004937-07</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>siCDK8</td>
<td>siCDK8: L-003242-00, siCDK8_/1: J-003242-10, siCDK8_/2: J-003242-09</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>siCDK19</td>
<td>siCDK19: L-004689-00, siCDK19_/1: J-004689-05, siCDK19_/2: J-004689-08</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>siMED12</td>
<td>siMED12: L-009092-00, siMED12_/1: J-009092-05</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>siMED13</td>
<td>siMED13: L-019908-00, siMED13_/1: J-019908-05, siMED13_/2: J-019908-06</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>siMED13L</td>
<td>siMED13L: L-027126-00, siMED13L_/1: J-027126-05, siMED13L_/2: J-027126-06</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>siNT</td>
<td>siNT pool of 4 D-001810-10, siNT_/1: single D-001810-1, siNT_/2: single D-001810-3</td>
<td>Dharmacon</td>
<td>III</td>
</tr>
<tr>
<td>shCdk8</td>
<td>shRNA targeting Mm Cdk8 in pDSL-hpUGlH</td>
<td>BCH knockdown library, I</td>
<td>I</td>
</tr>
<tr>
<td>Name</td>
<td>Description</td>
<td>Source/Reference</td>
<td>Used in</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>control shRNA</td>
<td>shRNA targeting <em>Mm</em> Cdk8 with 4bp deletion in pDSL-hpUGIH</td>
<td>BCH knockdown library, I</td>
<td>I</td>
</tr>
<tr>
<td><em>Dm</em> qPCR oligos</td>
<td>Designed to amplify cDNA of target genes</td>
<td>II, designed by author</td>
<td>II</td>
</tr>
<tr>
<td><em>Mm</em> genotyping oligos</td>
<td>For detecting Cdk8 wt and Cdk8- alleles</td>
<td>I, designed by T.W.</td>
<td>I</td>
</tr>
<tr>
<td><em>Hs</em> qPCR oligos</td>
<td>Designed to amplify cDNA of target genes</td>
<td>III, designed by author</td>
<td>III</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Source/Reference</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CG14629</em>-luc</td>
<td>978bp promoter of <em>Dm</em> <em>CG14629</em> driving firefly luciferase in in pGL3-basic</td>
<td>II, generated by author</td>
<td>II</td>
</tr>
<tr>
<td><em>CG14629-ΔGATA</em>-luc</td>
<td><em>CG14629</em>-luc with GATA&gt;GCCA</td>
<td>II, generated by M.S.E.</td>
<td>II</td>
</tr>
<tr>
<td>GST-CTD</td>
<td>Triple repeat of RNAPII CTD fused to GST</td>
<td>Dr. R. Young</td>
<td>I</td>
</tr>
<tr>
<td><em>Mtk</em>-luc</td>
<td>343 bp promoter of <em>Dm Metchnikowin</em> driving firefly luciferase in pGL3- basic</td>
<td>Dr. M. Boutros (Gesellchen et al., 2005)</td>
<td>II</td>
</tr>
<tr>
<td><em>MtkΔGATA</em>-luc</td>
<td><em>Mtk</em>-luc with GATA&gt;AATA mutations</td>
<td>II, generated by H.H.</td>
<td>II</td>
</tr>
<tr>
<td>P4xPO45-Fluc</td>
<td>4x <em>Dm PO45</em> enhancer in pGLA.23[luc2/minP]</td>
<td>Dr. L. Waltzer (Gobert et al., 2010)</td>
<td>II</td>
</tr>
<tr>
<td>pAc-Lz-V5</td>
<td>Full length <em>Dm Lozenge</em> in pAc5.1/V5-HisA</td>
<td>Dr. L. Waltzer (Gobert et al., 2010)</td>
<td>II</td>
</tr>
<tr>
<td>pAc5.1/V5-HisA</td>
<td>Empty vector control</td>
<td>Life Technologies</td>
<td>II</td>
</tr>
<tr>
<td>pCMV-β-gal</td>
<td>Cytomegalovirus promoter driving β-Galactosidase</td>
<td>(MacGregor and Caskey, 1989)</td>
<td>I</td>
</tr>
<tr>
<td>pRLnull-copiaLTR</td>
<td><em>Dm copia</em> LTR promoter driving renilla luciferase</td>
<td>Dr. S. Crews (Emmons et al., 1999)</td>
<td>II</td>
</tr>
</tbody>
</table>

Plasmids and peptides
2. Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Used and described in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdk8 immunoprecipitation and kinase assay</td>
<td>I</td>
</tr>
<tr>
<td>cDNA synthesis</td>
<td>II, III</td>
</tr>
<tr>
<td>Cell culture</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Colony formation assay</td>
<td>I</td>
</tr>
<tr>
<td>Dual luciferase assay</td>
<td>II</td>
</tr>
<tr>
<td>EdU incorporation assay</td>
<td>III</td>
</tr>
<tr>
<td>Expression profiling by Affymetrix Microarrays</td>
<td>II</td>
</tr>
<tr>
<td>Generation of <em>Cdk8</em> mutant mice, husbandry and embryo manipulation</td>
<td>I</td>
</tr>
<tr>
<td>Generation of <em>CG14629</em>-luc construct</td>
<td>II</td>
</tr>
<tr>
<td>Generation of <em>Dm</em> dsRNA knockdown constructs</td>
<td>I, II</td>
</tr>
<tr>
<td>Generation of shCDK8 knockdown constructs</td>
<td>I</td>
</tr>
<tr>
<td>Gene Set Enrichment Analysis</td>
<td>III</td>
</tr>
<tr>
<td>Larval and S2 cell infection assay</td>
<td>II</td>
</tr>
<tr>
<td>mRNA <em>in vitro</em> synthesis</td>
<td>I</td>
</tr>
<tr>
<td>Mutagenesis of <em>CG14629</em>-luc and <em>Mtk</em>-luc reporters</td>
<td>II</td>
</tr>
<tr>
<td>PCR genotyping</td>
<td>I</td>
</tr>
<tr>
<td>Promoter analysis</td>
<td>II</td>
</tr>
<tr>
<td>Quantitative PCR (qPCR)</td>
<td>II, III</td>
</tr>
<tr>
<td>RNAi by dsRNA</td>
<td>I, II</td>
</tr>
<tr>
<td>RNAi by shRNA</td>
<td>I</td>
</tr>
<tr>
<td>RNAi by siRNA</td>
<td>III</td>
</tr>
<tr>
<td>RNA sequencing by NextSeq500</td>
<td>III</td>
</tr>
<tr>
<td>S2 cell counting</td>
<td>I, Results of this thesis</td>
</tr>
<tr>
<td>Southern blotting</td>
<td>I</td>
</tr>
<tr>
<td>Total RNA extraction</td>
<td>II, III</td>
</tr>
<tr>
<td>Western blotting</td>
<td>I, I, III</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

The Research Program Unit and the Genome Scale Biology Research Program at the Faculty of Medicine and the Institute of Biotechnology at the University of Helsinki are acknowledged for providing excellent research facilities and infrastructure. This thesis has also been supported by the Faculty of Biological and Environmental Sciences, the Doctoral Program in Biomedicine at the Doctoral School in Health Sciences, the Academy of Finland, Sigrid Juselius Foundation, Biocentrum Helsinki, Cancer Society of Finland, Svenska Kulturfonden, Biomedicum Helsinki foundation, K. Albin Johansson foundation, Oskar Öflund foundation, Ida Montin foundation and Paulo foundation.

I would like to thank Professor Thomas G. Boyer for traveling the long way from Texas to Helsinki to be my opponent. I have followed your important work on the Mediator kinase module for over a decade and I’m looking very much forward to meeting you in person. I’m sure the thesis defense will be an exiting experience. Professor Juha Partanen is acknowledged for serving as my custos, for efficient replies to my e-mails and for kindly making easy questions for my final exam. Mikko Frlander and Pia Vahteristo, thank you for pre-examining my thesis and agreeing to my tight schedule. Your comments considerably improved the readability of my thesis. To Frank Holstege and René Medema, thank you for taking the time to be my thesis committee members. Your feedback was always useful and constructive. I hope we will meet again in the future, regarding science or fishing.

To my supervisor Tomi Mäkelä, it has been a privilege to work in your group and I am proud to be a Makelab PhD graduate. I know I’m not alone to think that the training we have received in Tomi’s school is extraordinary. I have especially appreciated the long writing sessions during which you have really thought me critical thinking and scientific writing. You have made me a scientist and for this I’m very grateful. You expect high standard from your lab members but have also always been very supportive of all unscientific events, something I think is crucial for creating the special Makelab spirit.

Thomas Westerling guided me through my first years in the lab and your mentoring and support is what made me stay in the lab those first years. Your endless enthusiasm made you a great teacher and many times I have found myself repeating your wisdom as well as stupid jokes to students of my own. Thank you also for bringing me onboard the Cdk8 mouse project, without that paper this PhD project would have felt endless.

Perhaps the most enjoyable period of my PhD studies was when I worked together with Heini Hakala. Heini was a super student form day one and your efficient hands where crucial for finalizing the fly project. I know you will have a great career no matter where you go. Your laughter and energy really boosted the atmosphere during your years in the lab.
Working with a fly project in a mouse lab was not always easy and would have been even more difficult without the contribution of our expert collaborators. Ville Hietakangas, thank you for your input on the manuscript and for letting me use your facilities and group members to learn in vivo fly experiments. Essi Havula was the one who thought me everything about flies and without your help I would never have dared to take that step. Thank you for all the time you spent away from your own studies while dissecting those fat bodies for me, I really enjoyed our collaboration. Thank you also to Mika Rämet for your crucial input on the immunity experiments and for sharing with us your microarray data that really set the direction for the fly paper.

Michelle Sahal Estimé, having you in the lab performing luciferase assays and mutating constructs not only moved the project forward but also allowed me the break I needed during my first maternity leave. I would have loved to work longer with you. Eva Domenéch-Moreno is a bright young scientist without whom RNA-seq analysis would have been difficult. Thank you, you R great.

The incredible people of Makelab, no doubt, are what I will miss the most. Luckily I know that many of you will remain as dear friends. Pekka Katajisto only supervised me during the first summer but have remained an important mentor ever since. Thank you for everything ranging from Gene Spring to lemon curry. Katja Helenius, sharing a bench, excitement, frustration, hotel rooms and conference after parties with you was a thrill. Susanna Suski Räsänen, Kari Vaahntomeri, Anou Londesborough, Thomas and Pekka, thanks for bringing me along on all your brilliant spare time activities. Thank you as well as Saana, Bianca, Kaisa, Saara, Elina, Tea, Sushil, Yan, Jing, Iris, Niina and Eeva O for the interruptions to pipetting, and for letting me disturb you, which certainly mostly extended my thesis but made it so much more fun. Ying, Tima, Katja, Thomas, Heinu and Elina, thank you for sharing the ups and downs of transcription, excitement towards large excel sheets and transcriptional Cdk’s. To all the fantastic technicians, Saana, Birgitta, Kirsi, Suski, Outi, Markus, Jenny and Sari, thank you for keeping things rolling and for your help whenever pipetting schedules got too unrealistic. All your great personalities and enthusiasm both in science and social events has been the backbone of the Makelab spirit. A special thank you also to Satu (Dr.) Sankkila and Anu Taulio for all your help, to Alisa for efficient pipetting and to Ryan for all your efforts with data analysis. Thank you also Ryan, Elina and Iris for proof reading and improving my thesis. To all Makelab members, past and present, thank you for sharing the journey, hope to see you still many times at Makelab reunions.

It has been a privilege to work in the kind of community created by the people at Biomedicum Helsinki and at the Institute of Biotechnology. The helpful staff at Biomedicum Functional Genomics Unit and Genome Biology Unit is acknowledged for excellent services. To the labs of Päivi Ojala, Juha Klefström and Mikko Frilander, to Tapsa and Rähkonen: thank you for always helping with reagents and equipment and for sharing all the fun. Thank you Päivi for always having time to talk. And thank you Johanna, Anni and Annika for being fabulous team mates in birding and fishing competitions.
A broad social safety net of friends, in-laws and family has been essential for my well being and has allowed me to make this career choice. My friends from the biology studies: Miimu, Jenny, Julia, Minja, Milja, Anna and Marie have remained an important group of peer support. I greatly value your friendship. Sharing laughs while catching up on development of kids and careers is something we need to do more often. Laura, you are the kind of friend I know I can always count on. Thank you for being there for me all these years.

I owe my interest in biology to summers spent in the archipelago and Friday nights spent with il était une fois la vie. All of these moments I shared with my sister, in whose footsteps it was always easy to follow. Because of you, Hannele, I started studying biology and did not miss out on field courses despite my interest in molecules. Thank you for all your support and encouragement and for being one of my best friends. Mom and Dad, Marika and Stefan, you did your best to answer all our questions on good and bad guys of the human body. I promise that one day, I will answer your questions on what it is that I do. Your upbringing inspired curiosity and debate, which are really the fundamentals of science. Thank you for your endless love and support and for looking after our kids so many times when I was working or networking.

To my wonderful children, Nelly and Kevin, keep asking questions, I admire your curious minds and stubborn personalities. You are the most important persons in my life and after having you bad days at work don't seem to matter that much anymore. Finally, Kim, my husband, partner and love of my life. You have always been supportive of my choice of career and at times of doubt you were always the one who told me that what I do makes a difference. Thank you for the balance you bring and for all the wonderful moments we have shared. To have a family with you is the smartest thing I have ever done.

Kasnäs, 27.7.2017

[Signature]

Emilia
REFERENCES


Clarke, P.A., Ortiz-Ruiz, M.J., TePoele, R., Adeniji-Popoola, O., Box, G., Court, W., Czasch, S., El Bawab, S., Esdar, C., Ewan, K., et al. (2016). Assessing the


The histone variant macroH2A suppresses melanoma progression through regulation of CDK8. Nature 468, 1105-1109.


Malik, S., Gu, W., Wu, W., Qin, J., and Roeder, R.G. (2000). The USA-derived transcriptional coactivator PC2 is a submodule of TRAP/SMCC and acts synergistically with other PCs. Mol Cell 5, 753-760.


Xu, W., Wang, Z., Zhang, W., Qian, K., Li, H., Kong, D., Li, Y., and Tang, Y. (2015b). Mutated K-ras activates CDK8 to stimulate the epithelial-to-mesenchymal