

# Mediator and cell cycle regulation

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This thesis is dedicated to my family



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## ABSTRACT

The multiprotein Mediator complex is an evolutionarily conserved coregulator of eukaryotic transcription. Mediator functions as a bridge between gene-specific transcription factors and the RNA polymerase II transcription machinery. Mutations affecting Mediator function have been associated with a large number of diseases from cancer to neurodegenerative disorders. In the present thesis we address how Mediator affects cell cycle progression using the fission yeast *Schizosaccharomyces pombe* as a model.

The Cyclin dependent kinase 8 (Cdk8) and its partner Cyclin C (CycC) are both components of Mediator. The Cdk8-CycC pair is recruited together with Mediator to genes periodically transcribed during cell cycle progression. Deletion of Cdk8 or inactivation of the Cdk8 associated kinase activity results in delayed mitotic entry and delayed activation of mitotic genes. An important target for the Cdk8 kinase activity is Fkh2, a gene specific transcription activator required for periodic transcription of mitotic genes in fission yeast. Fkh2 mutations that abolish Cdk8-phosphorylation delay mitotic progression, whereas mutations that mimic protein phosphorylation cause early entry into mitosis.

Cdk8 activity is regulated by two other Mediator components, Med12 and Med13, which connect the Cdk8-CycC pair to the core Mediator. Loss of Med12 and Med13 leads to the formation of a free pool of Cdk8 that can

stimulate early entry into mitosis, i.e. an effect directly opposite to that observed upon loss of kinase activity.

In our work, we have also investigated the link between cell cycle progression and transcription. We report that transcription of periodically expressed genes relies solely on the activity of the master cell cycle regulator, Cdk1, independent of the current stage of cell cycle progression.

In conclusion, our observations firmly establish Mediator as a regulator of mitotic progression in fission yeast. Our work also provides a framework of ideas that may be explored to better understand how mutations affecting Mediator function in human cells may lead to disturbed cell cycle control and the development of cancer.

**Keywords:** mediator, cell cycle, cdk8, fission yeast, cancer

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# SAMMANFATTNING PÅ SVENSKA

Mediatorn är ett multiproteinkomplex, som spelar en viktig roll vid reglering av transkription i eukaryota celler. Komplexet fungerar som en brygga mellan genspecifika transkriptionsaktivatorer och det basala transkriptionsmaskineriet vid promotorn. Mutationer i gener vilka kodar för subenheter hos Mediatorn har associerats med en rad olika sjukdomar, särskilt cancer och neurodegenerativa tillstånd. I den föreliggande avhandlingen undersöker vi hur Mediatorn påverkar cellcykeln i jästen *Schizosaccharomyces pombe*.

Cdk8 är ett cyklinberoende kinas, som tillsammans med Cyklin C (CycC) utgör en del av Mediatorn. Dessa två proteiner rekryteras tillsammans med övriga Mediatorkomponenter till gener som uttrycks på ett periodiskt vis under cellcykeln. Förlust av Cdk8, eller mutationer som inaktiverar dess kinasaktivitet, leder till försenat inträde i den fas då cellkärnan delas (mitos) och försenad aktivering av de gener, som uttrycks under denna del av cellcykeln. Cdk8 har förmågan att direkt fosforylera Fkh2, en genspecifik transkriptionsaktivator, som tidigare visats reglera mitotiska gener hos *S. pombe*. Om de aminosyror som fosforyleras av Cdk8 muteras, så kan det leda till för tidig eller försenad celledelning.

Aktiviteten hos Cdk8 regleras av två andra subenheter, Med12 och Med13, vilka förankrar Cdk8-CycC paret till Mediatorkomplexet. Förlust av antingen Med12 eller Med13 leder till att en fri pool av Cdk8 bildas, vilken i sin tur kan orsaka för tidigt inträde i mitos, d.v.s. en effekt som är rakt motsatt den, vilken observeras när kinaset inaktiveras.

I våra studier har vi också undersökt den generella kopplingen mellan cellcykelns progression och reglerad gentranskription. Vi har funnit att periodiskt transkription är direkt kopplad till aktiviteten hos det cyklinberoende kinaset Cdk1, men i övrigt oberoende av cellcykeln.

Sammanfattningsvis så etablerar våra undersökningar Mediatorn som en viktig regulator av cellcykelprogression hos *S. pombe*. Vårt arbete bidrar också med grundläggande förståelse, som kan användas för senare

undersökningar av cancerassocierade mutationer och deras effekter på cellcykeln i humana celler.

# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Cyclin-dependent kinase 8 regulates mitotic commitment in fission yeast.**  
Szilagyi Z, Banyai G, Lopez MD, McNerny CJ, Gustafsson CM.  
*Mol Cell Biol.* 2012 Jun;32(11):2099-109.
  
- II. Mediator can regulate mitotic entry and direct periodic transcription in fission yeast.**  
Banyai G, Lopez MD, Szilagyi Z, Gustafsson CM.  
*Mol Cell Biol.* 2014 Nov;34(21):4008-18.
  
- III. Cyclin C influences the timing of mitosis in fission yeast.**  
Banyai G, Szilagyi Z, Baraznenok V, Khorosjutina O, Holmberg S, Gustafsson CM.  
*MANUSCRIPT*
  
- IV. Cdk1 activity acts as a quantitative platform for coordinating cell cycle progression with periodic transcription.**  
Banyai G, Baidi F, Coudreuse D, Szilagyi Z.  
*MANUSCRIPT (submitted)*



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# ABBREVIATIONS

APC	Anaphase promoting complex
CAK	Cdk activating kinase
Cdc	Cell division cycle
CDK	Cyclin-dependent kinase
ChIP	Chromatin Immunoprecipitation
CKI	Cyclin-dependent kinase inhibitor
CPC	Chromosomal passenger complex
CTD	C-terminal domain
CycC	Cyclin C
DNA	Deoxyribonucleic acid
EM	Electron microscopy
GTF	General transcription factor
L Mediator	Large Mediator complex
MAPK	Mitogen activated protein kinase
MBF	MCB-binding factor
MCM	Mini chromosome maintenance
mRNA	Messenger RNA
ORC	Origin recognition complex
PCB	Pombe cell cycle box
PIC	Pre-initiation complex

Pol II	RNA Polymerase II
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
S Mediator	Small Mediator complex
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i> , budding yeast
<i>S. pombe</i>	<i>Schizosaccharomyces pombe</i> , fission yeast
SBF	SCB-binding factor
TAF	TBP associated factor
TBP	TATA binding protein
TF	Transcription factor
tRNA	Transfer RNA
TSS	Transcription start site

## DEFINITIONS IN SHORT

Cell cycle	A series of events that coordinates growth and proliferation of cells.
Checkpoint	A control mechanism capable of halting cell cycle progression at a certain stage.
Cytokinesis	The process of cell separation at the end of the cell cycle.
G0 phase	Also called quiescent phase, in which cells are outside of the cell cycle and not preparing for the next division.
G1 phase	Cell cycle phase after mitosis, in which cells prepare for the next reproduction cycle and synthesize the material needed for DNA replication.
G2 phase	Cell cycle stage following DNA replication in S phase, where cells grow and prepare for mitosis.
Kinase	A protein that can phosphorylate other proteins and thereby regulate their functions.
M phase	The cell cycle phase in which duplicated chromosomes are relocated into the newly formed nuclei.
Mediator	A multiprotein complex involved in the control of gene transcription.
Mitosis	Process that ensures the controlled segregation of replicated chromosomes into newly formed nuclei during the cell cycle.

Phosphatase	A protein that can remove a phosphate group from another protein, a process called dephosphorylation.
Phosphorylation	Attachment of a phosphate group to an amino acid of a given protein.
S phase	The cell cycle phase in which DNA replication takes place, resulting in two identical copies of the genome.
Transcription	Process of copying (transcribing) the genetic information stored in DNA molecule into a complementary strand of RNA (messenger RNA, mRNA).
Transcriptome	The collection of all RNA molecules isolated from a population of cells.
Translation	The process whereby proteins are synthesized based upon an mRNA sequence.

# 1 INTRODUCTION

Present estimates suggest that life has existed for almost 4 billion years on our planet. Early organisms began to produce copies of themselves, always adjusting to the environment, which eventually resulted in the extremely wide range of species we observe today. In a sense, this biological diversity is the consequence of cell cycle progression through billions of years.

The cell cycle is a series of molecular events that coordinates the growth and proliferation of cells. The cell cycle is responsible for the self-renewal of individual organisms, and the principles of the cell cycle are conserved between different organisms, even if its mechanisms can vary. The cell cycle can operate with relatively low coordination, as seen in prokaryotic cells that duplicate by binary fission, or can be extremely complex as seen in human cells, which are capable of differentiation as well as proliferation and thus can adapt to a wide range of stimuli (Morgan, 2007).

## 1.1 The eukaryotic cell cycle

Cellular reproduction and proliferation is pivotal to life. During cell division, the chromosomes are copied to form identical sets that are equally distributed between the daughter cells. Genome replication is also a source of variability that is used by populations of cells to adapt to environmental changes, which is the basis of evolution. Eukaryotic cells store most of their genetic information in the nucleus. This information is defined by four different nucleotides containing the bases adenine, thymine, guanine or cytosine, which are joined together forming deoxyribonucleic acid (DNA). The bases form specific base pairs (adenine with thymine, and guanine with cytosine), and two complementary DNA strands are coiled around each other to form a stable double helix. Since the DNA is extremely long, it must be compacted in order to fit into the nucleus. To this end, DNA is packaged together with ribonucleic acid (RNA) and protein to form chromatin. The basic unit of chromatin is the nucleosome, which is composed of DNA wrapped around a protein core consisting of 8 histone proteins. Nucleosomal DNA is in turn folded into a series of higher order structures, eventually forming a chromosome.

The information in DNA is copied dynamically into RNA during the process of transcription. DNA packaging into chromatin not only compacts the genetic material, but also prevents unwanted gene transcription, thereby adding a layer of genetic control. During nuclear division, or mitosis, the genetic material is packaged into visible chromosomes. This is accompanied by the silencing of most genes and the ejection of transcription factors (TFs) from chromatin. During other phases of the cell cycle, chromatin packaging is looser, which allows DNA replication and transcription factors to access the genetic material (Morgan, 2007).

### **1.1.1 The start of the cell cycle and replication of DNA**

In general, cells can be present in two different stages: quiescent (resting, non-dividing) or proliferating (dividing). Directly after cell division, the newly-created cell enters a so-called gap phase. At this stage, depending on the environmental conditions, cells may either enter the next cycle and prepare for the upcoming mitosis, or remain in their current stage and wait for a signal to re-enter the cell cycle. The active gap phase is called G1 whereas the quiescent phase is G0. G1 is therefore important, since in this phase the cells commit to either starting the next cycle or stay in a resting state. As the cells sense optimal conditions for reproduction, they begin to grow and synthesize the material needed for the next stage. Once committed to the cell cycle, the next major step is the Synthesis (S) phase, where the duplication of the chromosomes takes place (Morgan, 2007).

Since the DNA molecules present in the nucleus are very long, it is not possible to replicate a chromosome from a single start site. Instead, replication is initiated from multiple origins of replication. These origins are recognized and bound by the multiprotein Origin Recognition Complex (ORC), thus forming the pre-replication complex (pre-RC). In late mitosis, the cell division cycle 6 (Cdc6) protein associates with ORC in an ATP-dependent process. In turn, the Cdc6 protein loads the mini chromosome maintenance (MCM) helicase complex onto the DNA. The role of MCM is to separate the two DNA strands, which will allow for DNA synthesis using the original strands as templates. Single-particle reconstruction of electron

microscopy (EM) images have revealed that ORC-Cdc6, just as the MCM complex, forms a ring structure around the DNA (Speck et al., 2005).

In the next step, the MCM complex is phosphorylated, leading to the removal of Cdc6 and the incorporation of Mcm10, which in turn recruits Cdc45. Cdc45 is responsible for the recruitment of key components of the replisome, including DNA Pol  $\alpha$  with its polymerase and primase subunits. The replisome will then initiate the synthesis of complementary DNA strands, resulting in two nearly identical copies of the genome (Morgan, 2007). The old and new copies are called sister-chromatids and are physically connected by cohesin complexes. The cohesin complex consists of 4 proteins: Smc1, Smc3, Scc1 and Scc3 that are bound together, forming a ring structure around the two chromatids (Haering et al., 2002). It is extremely important to keep the chromatids together until the end of nuclear division, otherwise the newly formed cells could receive an unequal amount of genetic material, which in humans may result in diseases such as Down syndrome (Yoshida et al., 2013).

### **1.1.2 G2 and Mitosis**

The S phase is followed by another gap phase in most organisms, called G2. More growth takes place during this phase as the cell prepares for mitosis. The G1 and G2 phases are not only important for growth, but also include regulatory processes that ensure a controlled and safe cell cycle progression. The G2 phase is followed by nuclear division or mitosis. Nuclear division is a complex process and requires a delicate machinery in order to correctly separate the newly synthesized chromosomes. First, cells enter prophase and chromosomes undergo condensation, which requires the condensin complex. The centrosomes are responsible for the coordination of mitosis via microtubules. By the beginning of prophase the centrosomes are duplicated and move towards the opposite poles of the cell. Molecular lassos composed of microtubules emanate from the centrosomes and form the mitotic spindle, which attaches to the central part of each sister chromatid (the centromere) through the kinetochore complex, so that sister chromatids become bi-oriented towards the two poles. In vertebrates there is a prometaphase stage in which the nuclear envelope is broken down, whereas this structure remains intact in other eukaryotes. In the next phase, termed metaphase, sister

chromatids are held in the middle of the cell, connected to centrosomes through the mitotic spindle and waiting for the signal to start the segregation. At this point, the chromatin is at its most compact and the condensed chromosomes are visible under a microscope. It is important to note that the kinetochore fibers are relatively loose by the time they are attached to the centrosomes and the centromeres. As the centrosomes move further towards the opposite poles of the cell, they begin to raise the tension of the spindle. The cell is capable of sensing this tension through the Aurora B kinase, located at the inner centromere as part of the chromosomal passenger complex (CPC). When the tension in the filaments increases, the cell proceeds with mitosis, bypassing the metaphase checkpoint and entering anaphase. At this stage, the anaphase promoting-complex (APC) directs nuclear separation. APC degrades an inhibitory chaperone called Securin, which results in the activation of the separase enzyme. Separase cleaves the cohesin molecules holding the sister chromatids together. This enables the spindles to pull the sister chromatids to opposite poles of the cell as they shorten their length. After the end of anaphase, the next and final “cleanup” stage, termed telophase, begins. The chromosomes begin decondensation, and in higher eukaryotes, new nuclear envelopes are formed. Mitotic spindles detach from the chromatids and dissociate while the interphase microtubule network starts to form (Morgan, 2007).

### **1.1.3 Cytokinesis**

Cytokinesis, the division of mother and daughter cells, immediately follows mitosis in most organisms. It begins with the choice of the division site at the central plane of the cell. The components of the cleavage furrow, including actin, myosin and other proteins form the so-called actomyosin ring at the division plane. Upon the segregation of the chromatids to the opposite poles (anaphase), this contractile ring will then ingress, creating a membrane barrier between the two daughter cells. The final step of cytokinesis is abscission, where the cleavage furrow completely separates the two daughter cells from each other, thus finishing the cell cycle (Morgan, 2007).

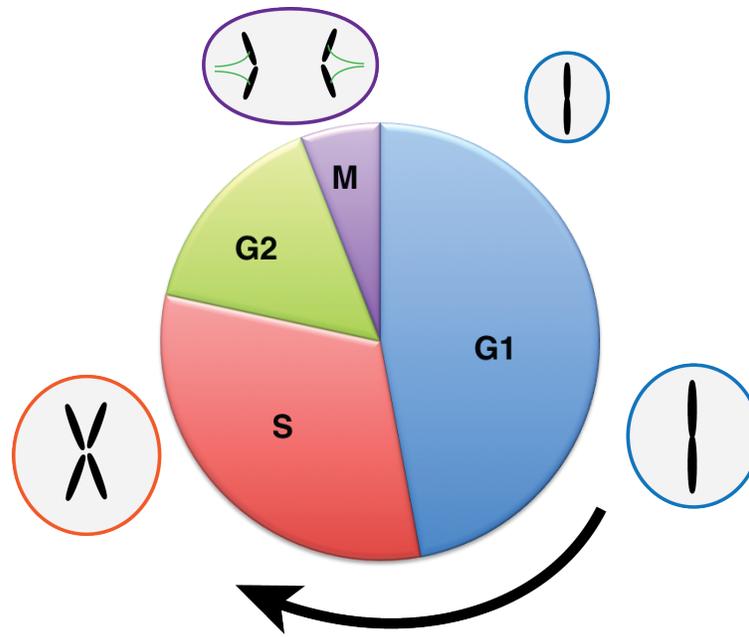


Figure 1. Human cell cycle stages

#### 1.1.4 Regulation of the eukaryotic cell cycle

The cell cycle is a highly regulated process, which relies on intricate mechanisms to ensure the faithful duplication and segregation of the genetic material. Studies conducted in the 1970s identified a number of factors required for proper cell cycle progression (Hartwell et al., 1973). These factors turned out to be evolutionarily conserved, with most being essential for cell viability (Lee and Nurse, 1987). Many of the most important proteins in cell cycle regulation belong to the Cyclin-dependent kinase (Cdk) family that can phosphorylate specific target proteins, thereby regulating their activity, which in turn leads to a precisely controlled progression through the cell cycle. The Cdks are unable to function on their own; they require delicate activation signals in order to fulfill their kinase activity at the right time. First, they must form a complex with so-called cyclins. Many of the Cyclin proteins, unlike Cdks, have varying concentrations throughout the cell cycle, peaking at specific cell cycle stages. Cdks usually also contain a special element termed the T-loop or activation loop. A Cdk associates with its

Cyclin partner, but in order to be active it must be phosphorylated at the T-loop by a Cdk activating kinase (CAK). These mechanisms are usually paired with positive feedback regulation, leading to rapid phosphorylation of target proteins.

Precise control of Cdk activities during the cell cycle ensures the execution of the different cell cycle events in the correct order. In addition, there are also control points or checkpoints, which are responsible for monitoring the cellular environment and may halt cell cycle progression if the conditions are not optimal. There are three main checkpoints in cell cycle regulation. The first is the restriction point or G1/S transition, where the cell decides whether or not to enter the cell cycle. The second checkpoint is the G2/M transition, where the cell makes sure that all components necessary for nuclear division are available, since the chromosomes will be compacted and transcription activity will be significantly lower from this point on. The third main checkpoint is called the spindle checkpoint, which monitors the metaphase to anaphase transition during mitosis to ensure that all the chromosomes are correctly attached to the mitotic spindle and separated correctly during anaphase.

### **1.1.5 The G2/M transition**

The transition from G2 to mitosis, also called mitotic entry, requires a number of proteins for precise regulation. At the core of this regulatory machinery is the Cdk1 kinase that can phosphorylate target proteins, leading to a cascade of events, pushing the cell into mitosis. Three things must happen in order to activate the kinase: (i) Cdk1 must bind to its cyclin partner, Cyclin B; (ii) the Cdk1 T-loop must be phosphorylated, and (iii) Cdk1 must be dephosphorylated at position Tyr15. While the first two steps occur at an earlier time during the cell cycle, the phosphorylation status of the Tyr15 position is crucial for the timing of mitosis. Two key enzymes are responsible for the control of Tyr15 phosphorylation: the Cdc25 phosphatase, which can remove the phospho-group and push cells into mitosis; and the Wee1 kinase, which can phosphorylate Tyr15, keeping cells in G2 phase. The delicate balance between Cdc25 and Wee1 activities ensures that the cell enters mitosis very quickly once all of the necessary factors required for nuclear division are available (Morgan, 2007). The basic elements of this

process was first discovered in fission yeast, but later studies have found that Cdk1 is evolutionarily conserved, as the human ortholog can complement a *cdc2* (the gene encoding Cdk1) mutant phenotype (Lee and Nurse, 1987).

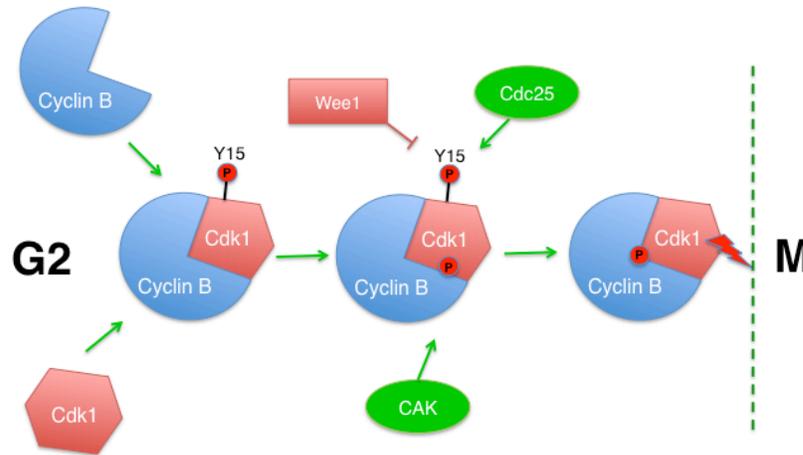


Figure 2. The control of mitotic entry by Cyclin-Cdk complex

### 1.1.6 Fission yeast as a model for cell cycle research

To better understand human diseases associated with perturbed cell cycle progression, such as cancer, it would seem most logical to use human cells. However, cell cycle studies in human cells are complicated. For instance, primary cell samples collected from human tissues have a limited regeneration capability and are somewhat difficult to culture. Alternatively, immortalized cell lines such as HeLa may be used, which are relatively easy to work with. However, these cells barely resemble normal human cells, due to their abnormal genome consisting of approximately 76-80 chromosomes and other extreme abnormalities (Landry et al., 2013).

Fortunately, the cell cycle process in eukaryotic cells is executed with stunning conservation. As a consequence the use of simple unicellular eukaryotic model organisms, such as yeasts, has been instrumental in understanding the cell cycle and its control. The two most common yeast model systems for cell cycle research are the baker's yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*. Although they

seem closely related, they are actually very distinct from each other and diverged on the evolutionary tree a couple of hundred million years ago, making them almost as different from each other as they are from human cells (Sipiczki, 2000). Baker's yeast is more widely used in the scientific community, mostly due to historical reasons, but in cell cycle research, fission yeast has been the preferred model system.

The fission yeast was discovered in the late 19<sup>th</sup> century as a microorganism fermenting beers in Africa. Since this fission yeast has been isolated from a limited number of sources, the scientific community uses only a few strains, which has resulted in better reproducibility in research. Fission yeast is a rod shaped unicellular eukaryote measuring 7-14  $\mu\text{m}$  in length and 3-4  $\mu\text{m}$  in diameter.



Figure 3. Microscope image of *Schizosaccharomyces pombe* cells

### 1.1.7 The fission yeast cell cycle

The *S. pombe* life cycle is relatively simple, since the cells spend most of their life in the haploid state. The cells can enter the sexual cycle once exposed to nitrogen starvation, forming diploid cells, which immediately undergo meiosis, resulting in 4 haploid spores. The cell itself will then transform into a rigid structure, called an ascus, which provides protection for the spores in harsh environments. If conditions become optimal for the cell, these spores will then be released and begin a normal cell cycle. *S. pombe* cells found in nature have two mating types (P and M) and they are capable

of switching between these. For practical reasons, scientists often use mutant strains that have defects in the genes responsible for mating type switching, thereby reducing the possibility of mating and genetic variability. As with most fungi, fission yeast is also encapsulated by a cell wall that provides an efficient protection against the environment. This wall is important in cell cycle progression, since *S. pombe* cells do not undergo cytokinesis directly after mitosis. Instead, the fission yeasts finish cell separation in S phase after the formation of a septum. This septum is formed behind the contractile ring during the contraction phase of cytokinesis (see 1.1.3). It consists of cell wall proteins and is therefore a more rigid structure than the septum observed during cell division in higher eukaryotes. The septum is also visible using light microscopy, which enables cell cycle progression to be monitored in real time, by looking at cells in S-phase. The fission yeast genome is relatively small (14 Mbp) and divided into only three chromosomes. Genetic manipulations, such as homologous recombination, are relatively simple in *S. pombe* and the entire genome was sequenced in 2002 (Wood et al., 2002).

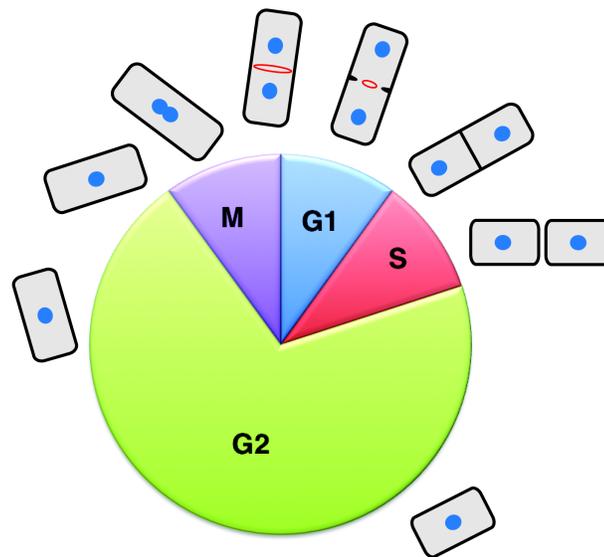


Figure 4. Fission yeast cell cycle and cell division

Fission yeast cells spend approximately 70 % of their lives in the G2 phase and pass very quickly through the M-G1-S phases (Figure 4). In fission yeast, cycle regulation is closely tied to cellular growth. The cells synthesize most

of the material needed for duplication during the G2 stage, making this an important phase for cell cycle control. Due to this trait, early mitotic entry will result in smaller cells, as seen in *wee1-50* mutants, whereas delayed mitosis will produce cells that are longer than their wild type counterparts (Morgan, 2007).

### **1.1.8 Cell cycle synchronization in fission yeast**

Cells in cultures are found in various stages of the cell cycle. In order to study processes affecting cell cycle progression it is necessary to synchronize cell populations. Doing so results in the majority of cells undergoing cell cycle stages at the same time, enabling subsequent experiments to be performed with sufficient amounts of material. Synchronization can be done using conditional mutants, which permit cell cycle progression at a permissive temperature, while blocking cells at a specific stage when inactivated at the restrictive temperature. A frequently used method to synchronize cells at the G2/M transition is the use of the *cdc25-22* mutant, which bears a conditional mutation in the key phosphatase which activates Cdk1 (Cdc2 in fission yeast) and thereby controls the timing of mitotic entry (see 1.1.5) (Fantes, 1981). The *cdc25-22* mutant cells are grown at 25 °C, until they reach an optimal density. The cells are then shifted to 36 °C for twice the generation time (4 hours) to ensure that virtually all of the cells are accumulating at the end of the G2 phase. Although the cells are unable to enter mitosis, they continue growing in G2, which results in longer cells. When the incubation time is over, the cells are cooled down immediately to 25 °C, which activates the Cdc25 phosphatase. This activation pushes the cells synchronously into mitosis and the subsequent events of the cell cycle. Samples are taken at certain time intervals to monitor a given process during cell cycle progression.

Another standard method to synchronize cells is to block the cell cycle in mitosis before the metaphase to anaphase transition or mitotic exit. To achieve this, we can use the conditional *nda3-KM-311* mutant. The *nda3* gene encodes a beta-tubulin protein that can be inactivated by cooling the cultured cells to 18 °C. The cells can next be grown as described for the *cdc25-22* mutant, but in this case they must be kept at 18 °C for 6 hours, due to their slower growth rate at the lower temperature. Spindle formation is

abolished, which activates the spindle checkpoint and halts the cell cycle before chromosome segregation. Shifting the temperature to the optimal 30 °C will enable cells to enter anaphase synchronously (Hiraoka et al., 1984; Umesono et al., 1983).

There are also chemical methods to synchronize cell cycle progression without the need to introduce new mutations into the cell. For instance, hydroxyurea is capable of halting DNA synthesis reversibly, allowing the block and release of cells in S phase. Cells can also be separated based on their size and shape using physical methods, such as elutriation centrifugation (Luche and Forsburg, 2009).

## 1.2 The gene transcription machinery

Our genetic information is stored in DNA. However, the information in itself is not what defines us as a living organism; it is the decoded message that will result in the color of our eyes or hair. DNA first needs to be transcribed into RNA. After RNA processing, including 5'-capping, splicing and polyadenylation, the mature messenger RNA (mRNA) is transported out from the nucleus. In the next step, mRNA is translated into proteins on free ribosomes or ribosomes associated with the rough endoplasmic reticulum (Katahira, 2015). The central dogma states that the flow of information is a one-way process, but RNA can also be transcribed back into DNA by reverse transcription, a mechanism utilized by retroviruses, such as HIV (Hurwitz and Leis, 1972). The amount of proteins in a cell depends on many factors, but mainly on the production of functional mRNA molecules and their regulated degradation. There are 3 major types of nuclear RNA polymerases (Pol) in eukaryotic cells: Pol I, II and III. Pol I is responsible for ribosomal RNA (rRNA) production, which accounts for 50% of the total RNA population. Pol III produces transfer RNAs (tRNAs) carrying amino acids to ribosomes during translation. Pol III is also involved in the synthesis of rRNA and other short RNAs. Pol II is responsible for the synthesis of mRNA, which requires both high throughput and precision (Vannini and Cramer, 2012). The level of a specific mRNA molecule in the cell usually correlates well with the resulting protein levels. There are however many exceptions to this rule, for instance caused by regulation at the level of

translation. Although higher eukaryotes have large and complex genomes, the transcription machinery is surprisingly similar to that found in lower eukaryotes, such as yeast. These similarities have made yeast a popular model for scientists interested in studying the molecular mechanisms of transcription.

Gene transcription can be regulated at many different levels. Quantitatively, the most important step in transcription regulation is at the initiation of transcription, which may be regarded as a committed step. Elongation and termination are also necessary, but are usually quantitatively less important for the control of gene expression. A DNA sequence that directs transcription initiation is termed a promoter. In most textbooks, the classical eukaryotic promoter contains an AT-rich sequence element (the TATA box) located 25 – 30 bp upstream of a transcription start site (TSS) surrounded by a relatively ill-defined initiation element. These two sequence elements together help the Pol II transcription machinery to associate with the correct DNA sequence. This promoter structure is however not so common in higher eukaryotes, where TATA-less promoters are more common (Yang et al., 2007). DNA also contains other regulatory elements termed enhancers and silencers. Gene-specific regulatory proteins, termed activators and repressors, can bind to these sequences promoting or inhibiting gene transcription, respectively. Transcription requires the ordered recruitment of Pol II together with a set of general transcription factors (GTFs), which form the pre-initiation complex (PIC). Today, Mediator is also seen as a constituent of the general transcription machinery. Activators are capable of binding to enhancer regions and recruit components of the PIC indirectly via the Mediator complex (Bjorklund and Gustafsson, 2005).

### **1.2.1 RNA polymerase II**

Pol II is a 500 kD complex composed of 12 subunits from Rpb1 to Rpb12. Its largest subunit, Rpb1, harbors an evolutionarily conserved C-terminal domain (CTD) consisting of a repeated sequence of 7 amino acids (YSPTSPS). The sequence itself is conserved between species, but the number of repeats varies from yeast (26 repeats) to human (52 repeats) (Meinhart et al., 2005). The CTD acts as a binding partner for a number of other factors, and changes in CTD phosphorylation directly affects

transcription regulation. The hypophosphorylated (IIo) form of Pol II is able to bind transcription factors and initiate transcription, whereas the hyperphosphorylated form (IIa) is involved in transcription elongation (Corden et al., 1985; Lu et al., 1991; Payne et al., 1989).

### 1.2.2 General transcription factors

Studies in the late seventies revealed that Pol II alone is unable to initiate transcription and produce mRNA molecules from a promoter-containing DNA template; it requires other components in order to function (Weil et al., 1979). Later studies identified six GTFs capable of *in vitro* transcription and a generally accepted model for transcription initiation emerged based on these findings. The 6 GTFs are transcription factor IIA (TFIIA), TFIIB, TFIID, TFIIE, TFIIIF and TFIIH. TFIID is a multiprotein factor composed of the TATA-binding protein (TBP) and a set of TBP associated factors (TAFs). TBP can initiate transcription without TAFs *in vitro* (Oelgeschlager et al., 1998). The function of TFIIA is controversial, since this factor is not necessary for *in vitro* transcription using recombinant TBP, but stimulates transcription in cell free extracts (Cortes et al., 1992; Ma et al., 1996; Ozer et al., 1994). The GTFs assemble at the promoter in a step-wise fashion. TFIIB binds to and stabilizes the DNA - TFIIA - TFIID complex. Next, Pol II (in complex with TFIIIF) is recruited to the promoter, followed by TFIIE. The last step of transcription initiation is the recruitment of the multimeric TFIIH. Together these proteins form the pre-initiation complex (PIC) (Roeder, 1996). TFIIH contains a kinase (Cdk7) that is capable of phosphorylating every fifth serine in the CTD heptads, thereby catalyzing the switch between PIC formation and active elongation (Buratowski, 2009).

### 1.3 Mediator complex

Promoter-dependent transcription was reconstituted in the 1980s using purified transcription factors from budding yeast. However, this system could not respond to gene-specific activators. In 1994, Roger D Kornberg and colleagues identified a factor that could support activated transcription *in vitro* (Kim et al., 1994). The multiprotein Mediator complex acts as a bridge between transcription activators and the transcription machinery. Today, Mediator is more than just a transcription regulator; it is involved in

numerous cellular processes ranging from cell cycle control to chromatin remodeling. Its composition differs between species, and the number of the subunits varies between 20 and 30, but its overall function appears to be evolutionarily conserved. The loss of Mediator subunits often leads to embryonic lethality in higher eukaryotes. Structural studies have revealed that Mediator has a “half-doughnut” shape and can be organized into 4 modules: head, middle, tail and a detachable CDK8 module (Cai et al., 2009).

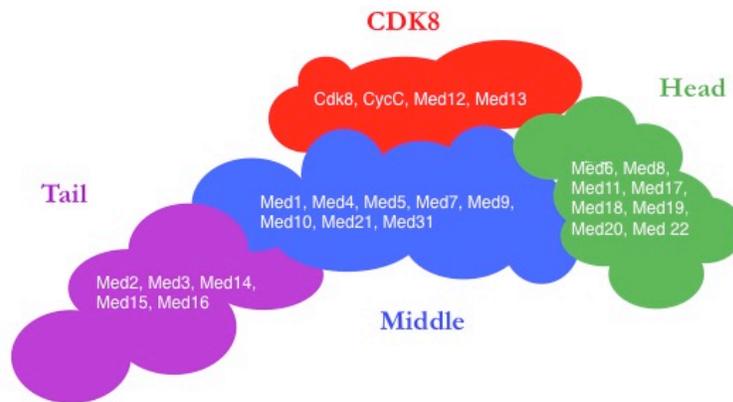


Figure 5. Modular composition of *S. cerevisiae* Mediator (based on (Guglielmi et al., 2004))

### 1.3.1 The modular organization of Mediator

Mediator can be isolated in two different forms: the core Mediator including the head, middle and tail modules, termed small Mediator (S-Mediator); and the large Mediator (L-Mediator) that also includes the CDK8 module. Electron microscopy and single particle reconstruction, in combination with *in vitro* biochemistry, suggest that the Mediator head module binds to Pol II (Elmlund et al., 2006; Takagi et al., 2006). Pol II only interacts with S-Mediator, since the CDK8 module competes with Pol II binding in the L-Mediator complex (Elmlund et al., 2006). The head module is not a major target for gene specific activators, but there are exceptions. The Gal4 activator interacts directly with Med17 (Koh et al., 1998). The middle module is the most evolutionarily conserved region of the Mediator complex. Similarly to the head module, the middle module can also interact with Pol II (Davis et al., 2002; Elmlund et al., 2006). The middle module also interacts

with the CDK8 module, via the Med1 subunit, and could be involved in regulating the presence/absence of CDK8 within Mediator. Interestingly, the middle domain is lost from Mediator isolated from a *med19Δ* strain. As a consequence, a stable head-tail complex without the middle domain is formed. However, this complex is unable to perform its function as a coregulator of Pol II transcription in yeast (Baidoobonso et al., 2007).

Most transcriptional activators interact directly with the Mediator tail module, and loss of this Mediator module leads to global changes in transcription patterns. Many Mediator components of the *S. cerevisiae* tail module, e.g. Med2 and Med3, lack clear homologues in higher cells. These tail module components have however been replaced by other proteins and a tail-like structure is still found also in mammalian Mediator (Baumli et al., 2005).

### 1.3.2 The CDK8 module

The CDK8 module consists of 4 proteins: CycC, Cdk8, Med12 and Med13; and L-Mediator containing these subunits has been identified by several groups (Gu et al., 1999; Hengartner et al., 1995). Early genetic and biochemical screens suggested that the CDK8 module has a negative regulatory role in transcription regulation, as it can suppress Pol II activity. The kinase component, Cdk8, can phosphorylate the CTD of Pol II prior to the formation of a pre-initiation complex and thereby inhibit transcription initiation (Hengartner et al., 1998). Others have demonstrated that Cdk8 can phosphorylate TFIIF and thereby inhibit Cdk7 activity, which is required for CTD phosphorylation and promoter escape (Akoulitchev et al., 2000). Later studies have found that in the absence of Cdk7, Cdk8 may also have a positive effect on transcription, suggesting that transcription regulation via the CDK8 module is a more complex process than first anticipated (Liu et al., 2004). Mutations affecting the CDK8 module are associated with various forms of cancers (see 1.3.2.4).

#### 1.3.2.1 Cdk8, the kinase subunit

Unlike other Cdks, Cdk8 does not require T-loop phosphorylation to be active (Hoepfner et al., 2005; Schneider et al., 2011). In mammals, CDK8 affects p21 gene expression. The p21 protein is a cyclin-dependent kinase

inhibitor (CKI) capable of negatively influencing the G1 transition by blocking the Cdk1 and Cdk2 complexes. The mRNA levels of the p21 gene display a linear correlation with *CDK8* gene expression. CDK8 and p21 can also interact directly, stimulating CDK8 activity and forming a positive feedback loop. This mechanism could provide a direct link to cancer development (Porter et al., 2012). In human cell lines, a drop in *CDK8* levels can lead to differentiation, whereas increased levels of the protein induces pluripotency (Adler et al., 2012).

CDK8 has also been linked to the Wnt/ $\beta$ -catenin pathway, which is a complex transcription regulator-signaling pathway that promotes the G1/S transition by altering gene expression. CDK8 can bind to the  $\beta$ -catenin dependent c-Myc promoter as part of the Mediator complex, and may thereby directly influence the cellular levels of  $\beta$ -catenin (Firestein et al., 2008).

CDK8 may also affect E2F1 activity. The E2F1 transcription factor activates a number of genes connected to S-phase progression. E2F1 also affects the Wnt/ $\beta$ -catenin pathway, since it stimulates the transcription of factors that promote  $\beta$ -catenin degradation and inhibits transcription of  $\beta$ -catenin-dependent genes. CDK8 can phosphorylate E2F1 at the S375 position, which is crucial for counteracting the repressive effect of E2F1 on  $\beta$ -catenin target genes (Zhao et al., 2013). E2F1 is a key element in cell-cycle regulation and apoptosis signaling. A similar pathway has been observed in *S. cerevisiae* cells, where two important factors are responsible for G1 control: SCB-binding factor (SBF) and MCB-binding factor (MBF). High-throughput studies show that Mediator is recruited to SBF-dependent cell cycle genes, such as the G1 cyclins *CLN1* and *CLN2* (Cosma et al., 2001). Besides its role in cell cycle control, Cdk8 might also have a role in regulating cellular differentiation. Nutrient deprivation causes yeast cells to start a differentiation program, resulting in special growth and morphological characteristics. For instance, nitrogen starvation leads to the formation of pseudohyphae, whereas the lack of glucose in the growth medium forces the cell to grow deeper into the agar plate. Nitrogen starvation in *S. cerevisiae* induces mating pheromone synthesis. These pheromones can activate a G-protein-coupled membrane complex, which triggers the mitogen activated protein kinase (MAPK) cascade. Two proteins downstream of this cascade,

Fus3 and Kss1, can activate the Ste12 transcription factor, which controls mating and filamentous growth. Cdk8 targets the previously described Ste12 and another transcription factor, Phd1, responsible for the expression of filamentous growth genes (Nelson et al., 2003; Raithatha et al., 2012). Cdk8-dependent phosphorylation of Ste12 and Phd1 decreases their stability, leading to normal growth. Mutations in any of these proteins will result in a differentiated filamentous growth phenotype.

### 1.3.2.2 Cyclin C, a special cyclin

In Mediator, Cdk8 interacts with CycC, an irregular cyclin, which does not fluctuate during the cell cycle (Leopold and O'Farrell, 1991; Schneider et al., 2011). *CYCC* was cloned more than 20 years ago as a regulator of the G1 transition, suggesting a role in cell cycle control (Leopold and O'Farrell, 1991). Studies in higher eukaryotes confirmed a link between CycC and cell cycle regulation, as CycC can cooperate with the c-Myc transcription factor and promote cell proliferation. (Liu et al., 1998). C-Myc can induce the expression of a number of target genes, facilitating cell proliferation (Grandori et al., 2000). It has also been shown that CycC can superinduce *cdc2* gene expression, thereby affecting both the G1/S and G2/M phases (Liu et al., 1998).

More recently, experiments in mice have revealed that CycC is also involved in the control of cell differentiation by affecting Notch 1 levels (Li et al., 2014). Notch 1 is a transmembrane receptor with a number of functions in human cells ranging from development to proliferation (Maillard and Pear, 2003). This study also demonstrated that null-mutant mice had difficulties re-entering the cell cycle, suggesting a role in G0 exit regulation (Li et al., 2014). In addition, CycC can act independently from Cdk8, translocating from the nucleus to the cytoplasm upon oxidative or ethanol stress, where it can induce mitochondrial hyperfission (Cooper et al., 2014; Cooper et al., 2012). Taken together, these results suggest that CycC is involved in diverse cellular processes, but the mechanistic explanations for many of these pleiotropic effects remain somewhat unclear.

### 1.3.2.3 Med12 and Med13, the largest subunits

The CDK8 module also contains the Med12 and Med13 proteins. Similarly to other components of the module, these subunits also play a role in repressing gene expression (Elmlund et al., 2006; Knuesel et al., 2009).

The functional role of Med12 and Med13 has been characterized in a number of different eukaryotic systems. Med12 coordinates differentiation processes both in humans and plants (Gillmor et al., 2014; Wu et al., 2014). *In vitro* studies demonstrated that the loss of Med12 results in the loss of CDK8 kinase activity. Interestingly, loss of Med13 fails to produce a similar effect and CDK8 can phosphorylate its targets in the absence of this protein. Med13 acts as a bridge between the CDK8 module and the core Mediator (Knuesel et al., 2009). In mammalian cells, MED13 stability is affected by Fbw7. This tumor suppressor ubiquitin ligase binds to L-Mediator and stimulates the destruction of MED13 in human cell lines (Davis et al., 2013). Furthermore, MED13 has been linked to the control of cell proliferation by acting as a co-factor for Retinoblastoma (Rb) (Angus and Nevins, 2012). Rb is a well known tumor suppressor that halts cell cycle progression in G1 phase by forming a complex with the transcription factor E2F (Morgan, 2007).

How Med12 and Med13 may function in transcription repression is not completely clear. As mentioned above, these proteins can, together with the other components of the CDK8 module, block Mediator interactions with Pol II (Elmlund et al., 2006). Alternatively, Med12 and Med13 may influence the activity of Cdk7, perhaps dependent upon input from gene-specific activators and repressors. More work is clearly required to understand the role of the Med12 and Med13 proteins in transcriptional regulation.

### 1.3.2.4 CDK8 module in health and disease

Mutations affecting the components of the CDK8 module can lead to the development of various forms of cancer. As previously described, the kinase subunit Cdk8 may act via the Wnt/ $\beta$ -catenin pathway, thereby influencing cell cycle progression. Mutations affecting the CDK8 kinase activity have been found in patients with colon cancer (Firestein et al., 2008). Abnormal CDK8 levels are also associated with malignant melanoma, a form of skin cancer.

Increased levels of *CDK8* expression may also contribute to malignant phenotypes. The histone isoform macroH2A is associated with the repression of transcription, and this protein acts to fine-tune gene expression programs required for developmental processes. Depletion of macroH2A causes increased malignancy and this effect seems to be mediated in part through direct transcriptional upregulation of *CDK8*. The expression levels of *CDK8* correlates inversely with macroH2A levels in melanoma patients, and knockdown of *CDK8* can suppress the growth stimulation effect caused by macroH2A depletion (Kapoor et al., 2010). CycC has also been associated with human diseases. One study demonstrated that the *CYCC* gene is overexpressed in 88% of all colon adenocarcinomas (Bondi et al., 2005). In another study, a haplo-insufficient *CYCC* deletion promoted tumorigenesis in patients with leukemia (Li et al., 1996).

Similarly to CDK8, Med12 is also involved in the Wnt/ $\beta$ -catenin pathway. In fact,  $\beta$ -catenin interacts directly with Med12 to activate transcription (Kim et al., 2006). Mutations in *MED12* are seen in a number of different cancers, including breast fibroadenomas (Lim et al., 2014). Interestingly, at least some of these mutations interrupt protein-protein interactions within the CDK8 module and cause decreased levels of Mediator associated kinase activity. *MED12* mutations may also impair interactions with S-Mediator components (Kampjarvi et al., 2012; Kim et al., 2006). Med12 is also involved in oestrogen-dependent breast cancer development, as it is required for expression of the *ESR1* gene (Prenzel et al., 2012). Although there are numerous papers showing *MED12* mutations in smooth muscle tumors, such as uterine sarcomas, the mechanism behind this is still unknown (Kampjarvi et al., 2014). The loss of Med12 will result in the activation of the TGF- $\beta$ R signaling, which is responsible for drug resistance. This can be a problem for patients diagnosed with colon or lung cancer undergoing chemotherapy (Huang et al., 2012). Med12 has also been associated with neurodegenerative diseases, such as Opitz-Kaveggia (FG) syndrome, Lujan syndrome and Ohdo syndrome (Maat-Kievit-Brunner type) (Risheg et al., 2007; Schwartz et al., 2007; Vulto-van Silfhout et al., 2013). Amplification of the *MED13* gene is associated with breast cancer development (Monni et al., 2001).

## 1.4 Gene expression during the cell cycle

Studies conducted in the 1980s demonstrated that some histone genes are transcribed periodically during the cell cycle (Hereford et al., 1981). In the upcoming years more and more genes were found to show a peak of gene transcription at a specific stage of the cell cycle. Modern technologies such as DNA microarrays and RNA-sequencing have allowed researchers to look at all mRNA molecules present in the cell at a given cell cycle stage. These studies have identified further genes that are expressed in waves during cell cycle progression in fission yeast as well as other in organisms including human cells. These investigations have revealed that approximately 20 % of the genes in a eukaryotic cell are transcribed periodically, and these fall into different clusters (Cho et al., 1998; Rustici et al., 2004; Spellman et al., 1998). Periodic activation of these clusters coincides with major cell cycle transitions and plays a crucial role in the control of cell proliferation. A number of regulatory factors, protein kinases and transcription factors have been implicated in the control of periodic gene expression, although its control mechanisms are far from completely understood. In fission yeast, approximately 500 genes were categorised into four clusters: cluster 1 genes are expressed near the G2/M transition, cluster 2 genes are transcribed around M/G1, cluster 3 genes are transcribed during S-phase, and cluster 4 genes are periodically expressed during G2 (Rustici et al., 2004).

A large part of this thesis is devoted to studies of the mechanisms regulating transcription of the G2/M gene cluster in fission yeast. Many of the genes required for this regulation have been implicated in mitotic progression and cytokinesis. These genes, such as *cdc15*, *ace2*, *plol*, *fin1*, *spo12* and *slp1* contain a promoter element termed Pombe cell cycle box (PCB) that interacts with regulatory transcription factors. In fission yeast, a TF complex called PBF binds to PCB and controls the transcription program. PBF contains two forkhead transcription factors, Sep1 and Fkh2, and the Mbx1 MADS box transcription factor. Microarray studies suggest that Sep1 stimulates transcription, whereas Fkh2 seems to have the opposite function, as deletion of the *fkh2* gene results in the upregulation of a subset of cluster 1 genes. Another transcription factor, Sak1, has recently been identified. Sak1 binds close to Fkh2 and acts as a positive regulator of mitotic gene transcription. Whereas Sep1 is required for the transcription of a subset of mitotic genes,

the essential Sak1 protein is required for activation of nearly all mitotic genes (Garg et al., 2015; Wu and McLeod, 1995).

The polo kinase Plo1 is part of the cluster 1 genes, however it can act as a positive feedback regulator of mitotic gene expression by regulating PBF activity (Anderson et al., 2002). Interestingly, overexpression of *fkh2* is lethal in wild type cells, but not in the *sep1Δ* background (Buck et al., 2004). This observation suggests that Sep1 and Fkh2 act in concert, and that Sep1 is required for Fkh2 function.

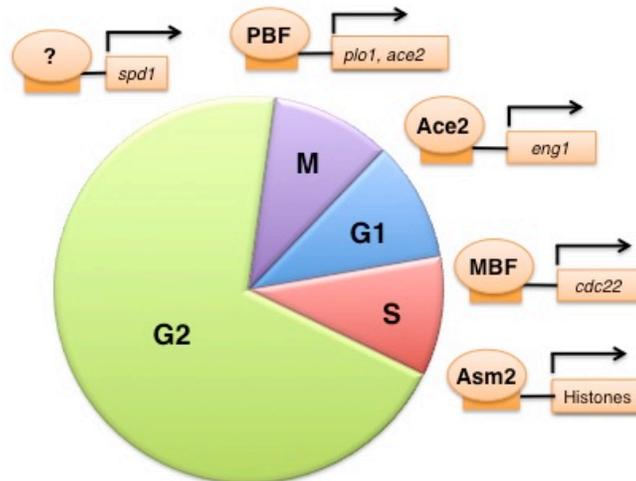


Figure 6. Regulators of periodic transcription in fission yeast (based on (McInerny, 2004))

The G2/M cluster is followed by the M/G1 cluster or cluster 2, which is partly controlled by the Ace2 transcription factor and includes genes (e.g. *eng1*) required for cell separation and the degradation of the division septum (Rustici et al., 2004). Another part of this cluster is controlled by the MBF complex and includes genes with functions in DNA replication and the G1/S transition, such as the ribonucleotide reductase gene *cdc22*. The next wave of periodic transcription coincides with S phase progression and includes histone genes regulated by the Ams2 transcription factor (Trickey et al., 2013). A schematic representation of the different clusters and their control is shown in Figure 6. Despite this, several factors have been found to be implicated in the control of periodic transcription, and the interplay between the networks that generate these transcriptional oscillations and the core cell

cycle machinery remains largely unexplored. In the budding yeast, it was found that a network of transcription factors could generate oscillation of the periodic clusters during the cell cycle, largely independently from the core cell cycle machinery (Orlando et al., 2008). Whether this type of control operates in fission yeast or in human cells is unknown.

## 2 AIMS OF THE THESIS

Mediator is required for the proper function of signalling pathways related to cellular differentiation and neuronal development (Lim et al., 2007; Loncle et al., 2007). Subunits of the CDK8 module have also been linked to cancer development (Firestein et al., 2008; Kampjarvi et al., 2015), but how Mediator affects cell cycle progression has not been studied in detail.

In fission yeast, the forkhead protein Sep1 is a component of the transcription regulatory network that governs mitotic transcription. Fission yeast propagates by medial fission, and mutations affecting Sep1 impair this process. Interestingly, a number of mutations in Mediator-encoding genes display cell separation phenotypes, implying that they may also affect mitotic transcription patterns (Grallert et al., 1999). In addition, mutations affecting the CDK8 module impair transcription of the mitotic *ace2* gene, which in turn is required for expression of factors required for cell separation (Linder et al., 2008). These results suggest a direct connection between periodic transcription and the Mediator complex. However, before we initiated the work described in this thesis, there was no direct evidence for the role of Mediator in periodic transcription and the mechanisms by which Mediator could influence cell cycle progression were unknown.

In the current work, we have aimed to address the role of Cdk8 and other Mediator components in the control of periodic transcription during mitotic progression. We have also tried to investigate how Mediator is recruited to cell cycle-dependent promoters and the role of specific Mediator subunits in this process. We have aimed to connect Mediator to the general cell cycle regulatory machinery and tried to find direct targets for the Cdk8 kinase activity. Finally, we have attempted to elucidate how other Mediator components regulate the specificity and activity of Cdk8.

In the last manuscript of the thesis, we have investigated how cell cycle progression is linked to periodic transcription. The work has been performed in collaboration with the Coudreuse lab. In our work, we have used a fission yeast strain lacking a number of cyclins and containing only a minimal cell cycle regulatory network (Coudreuse and Nurse, 2010). This simplified

model system provides an excellent opportunity to study the relationship between transcription and cell cycle progression.

## 3 RESULTS AND DISCUSSION

### 3.1 Paper I

In this paper we describe a possible mechanism for how cell cycle progression can be coordinated by the Cdk8 kinase via the Fkh2 transcription factor.

Previous studies had described a number of *S. pombe* genes that are periodically expressed during the cell cycle. In total, 87 cluster 1 genes are activated during mitosis and repressed during G1. These genes are under the control of Sep1, Fkh2, and the MADS-box protein Mbx1. The loss of *sep1* reduces their transcription, whereas the deletion of *fkh2* results in elevated gene transcription (Buck et al., 2004; Rustici et al., 2004). Fkh2 is phosphorylated during mitosis, however the responsible kinase had not been identified prior to our studies.

In our first experiments we investigated the effects of Cdk8 inactivation upon cell cycle progression. We used the previously described *cdk8-D158A* mutant *S. pombe* strain with a mutant version of Cdk8, which lacks kinase activity due to an aspartate to alanine point mutation (Elmlund et al., 2006). The loss of Cdk8 kinase function resulted in delayed mitotic entry. We also observed a delay in the dephosphorylation of the Tyr15 residue in Cdk1. These observations suggested that Mediator can influence cell cycle progression. We used Chromatin Immunoprecipitation (ChIP) to show that Mediator indeed binds to mitotic promoters, such as *ace2* and *slp1* in a periodic manner, thereby affecting periodic transcription. Interestingly, the loss of Cdk8 did not abolish binding to the promoter, but altered the timing. Our genetic studies showed an interaction between the genes encoding the Cdk8 kinase and the Fkh2 transcription factor, suggesting a kinase-substrate relationship between these factors. To test this hypothesis we analyzed Fkh2 phosphorylation in wild type and kinase-dead cells and found that the *cdk8-D158A* mutation altered the Fkh2 phosphorylation pattern during cell cycle progression. Mass Spectrometry analysis revealed that Cdk8 phosphorylated Fkh2 at two different positions *in vitro*, and that these sites correlated with phosphorylated positions *in vivo* (Beltrao et al., 2009). The introduction of

phospho-mimicking mutations at the two phosphorylated sites (S322E S375E) resulted in premature mitotic entry, whereas the non phosphorylatable S322A S375A mutations delayed mitosis. The Fkh2 protein contains a signal for quick proteolytic degradation termed the PEST domain. Our results demonstrated that the mitotic phosphorylation prevented the degradation of the protein. Altered levels of Fkh2 in turn affected promoter binding, as demonstrated by ChIP experiments. Finally, genetic analysis indicated that the Cdk8-Fkh2 pathway acts through the Wee1 kinase and thereby controls mitotic entry.

## 3.2 Paper II

In our next paper we addressed some questions that remained open regarding the role of Mediator during mitotic progression. We first wanted to identify genes bound by Mediator during mitosis. In order to do this, we used ChIP-sequencing technology in non-synchronous (G2) or mitotically-blocked cell cultures. We found a number of cell cycle-dependent genes, both in M-phase and in G1, which required Mediator for proper transcription. We also showed that Mediator recruitment coincides with *ace2* gene transcription and that Sep1 is required for this process. Loss of the *fkh2* gene did not abolish periodic transcription, but affected the levels and precise timing of gene transcription. For instance, *ace2* gene transcription was somewhat higher in the *fkh2Δ* mutant cells and the peak of transcription was earlier.

The tail and head modules are also necessary for periodic transcription, as *med15* and *med20* deletion affects mitotic gene expression. One of the most interesting findings of this paper is how the loss of Med12 and Med13 affects cell cycle control. Previous studies had demonstrated that abnormalities in the CDK8 module lead to a flocculating phenotype in fission yeast (Linder et al., 2008). Loss of either Med12 or Med13 produced inconclusive results. However, when we investigated the double mutant *med12Δmed13Δ* we observed a clear cell cycle effect with a premature mitotic entry, i.e. the opposite result to what had been seen in the Cdk8 kinase-dead mutant. In agreement with previous studies, demonstrating that Med12 and Med13 are required to anchor Cdk8 to the Mediator complex, we found that Cdk8 no longer associates with the *med12Δmed13Δ* Mediator and that Cdk8 is no

longer recruited to mitotic promoters in this mutant background (Knuesel et al., 2009). Interestingly, we also found an increase in Cdk8 protein levels in the *med12Δmed13Δ* mutant cells. Fkh2, a Cdk8 target, was prematurely phosphorylated in the mutant cells, which suggested that Cdk8 is active even outside of Mediator and is capable of phosphorylating target proteins. The finding that Cdk8 may function independently of Mediator may be relevant for our understanding of how disturbances in the CDK8 module architecture can promote cancer development.

### 3.3 Paper III

Our third paper focused on the structural composition of the CDK8 module, and the effects of CycC on cell cycle progression. Firstly, we showed that Med12 and especially Med13 are key components of CDK8 module integrity, and without their stabilizing effect Cdk8 and CycC are unable to form a stable complex *in vivo*. We found that CycC is recruited to mitotic promoters as part of the Mediator complex. Similarly to our observations with *cdk8Δ*, the loss of CycC leads to delayed mitosis and significantly decreased Cdk8 levels. Interestingly, the loss of the Med12 and Med13 subunits suppressed the effects of *cyccΔ* and caused an increase in Cdk8 levels. To further investigate the CycC-Cdk8 function, we fused the two proteins together, thereby abolishing the possibility of complex formation with other cyclins. The fusion protein was active, associated with the Mediator complex under normal conditions, and was lost from the complex upon deletion of *med12* and *med13*. We also tested whether an additional copy of the *cdk8* gene could push the cells into early mitosis, but this was not the case. The loss of Med12 and Med13, on the other hand, showed an unexpected effect on cell cycle progression. Although cells enter mitosis prematurely, they enter S phase at the same time as the wild type strain, suggesting that Cdk8 is not only an important regulator of mitosis in fission yeast, but also affects G1 control. This hypothesis needs to be addressed with more experiments.

### 3.4 Paper IV

In our last paper we investigated how periodic transcription is regulated throughout the cell cycle by the master regulator Cdk1. This is especially intriguing to study, since we have found an interesting interplay between the control of Cdk1 activity (namely, mitotic commitment) and the Cdk8/Mediator complex, which we have shown to be a global regulator of periodic genes in Paper II. This suggested that there may be an interesting, as yet-unknown interplay regarding how cells connect changes in Cdk1 activity to the control of periodic gene transcription on a more global scale. We performed *in vivo* studies on special *S. pombe* mutant cells, in which Cdk-activity is tightly controlled by chemical inhibitors. The aim was to study how gene expression of cell cycle genes changes after directed changes in Cdk1 activity.

We synchronized cells by blocking them in G2 phase, then released them into mitosis and significantly decreased the Cdk1 activity by the small molecule inhibitor 3-MBPP1 after mitotic exit, arresting them in G1 phase, whereas control cultures kept cycling. We found that none of the representative genes belonging to different clusters (*ace2*, *cdc22*, *hht1* or *spd1*) showed cell cycle independent periodic regulation. This is in sharp contrast to what was found in budding yeast. Nevertheless, the S-phase gene *hht1* showed increased expression similarly to the control culture, even though the blocked cells did not undergo replication, however its levels remained high throughout the whole experiment. These results therefore suggested that cell cycle independent oscillations of periodic gene transcription do not occur in fission yeast. Similar results were obtained when G2 cells were released into mitosis, or remained blocked by the addition of the Cdk1 inhibitor. Interestingly, the S phase cluster gene *hht1* did not show any changes in this case. We used microarray analysis to also address genome-wide changes in the transcription program, which confirmed previous findings on individual periodic genes. Taken together, these observations suggest that the fission yeast transcription program differs from the previously described budding yeast regulation. Periodic expression may therefore be linked to distinct cell cycle phases in fission yeast, rather than to distinct changes in Cdk1 activity.

Furthermore, we showed that the periodicity of transcription is not controlled by the cell cycle phases *per se*, but by changes in Cdk1 activity. For this, we blocked cells using the cold sensitive *nda3-KM311* mutant fission yeast strain. Interestingly, the mitotic block led to an accumulation of *ace2* and *cdc22* transcripts, rather than one or more periodic oscillations, whereas no induction of histone genes or *spd1* (G2 cluster) were seen, as expected. Next, we introduced this mutation to the minimal strain, allowing us to inhibit Cdk1 activity during the mitotic block. These cells were also blocked in mitosis, and thus accumulated transcripts as in the previous experiment. Intriguingly, inhibition of Cdk1 activity at this point did not result in exit from mitosis, but even a smaller decrease in Cdk1 activity resulted in quick downregulation of mitotic and MBF targets. Furthermore, stronger inhibition of Cdk1 derepressed histone gene expression. Therefore we were able to switch between periodic transcription programs in mitosis merely by changing Cdk1 activity. To confirm our finding, we blocked cells in G2 phase, lowered Cdk1 activity significantly, then mildly, allowing them to reset G1 and re-replicate their genome without an intervening mitosis. The transcription pattern of each gene correlated with the previous results, thereby confirming a simple model, where quantitative changes in Cdk1 activity can control periodic transcription.

Finally, we also showed that even if the cell cycle is so rewired that cells perform replication and mitosis simultaneously, periodic programs still followed the changes in Cdk1 activity. We therefore concluded that cell cycle phases do not interfere with nor control the Cdk1 activity-driven periodic transcription programs.

## 4 CONCLUSION

Our work demonstrates a connection between the Mediator complex and cell cycle progression. The observed effects are primarily an effect of the CDK8 module and the associated Cdk8 kinase. Based on our observations, we conclude that Cdk8 is capable of phosphorylating cell cycle regulatory targets, controlling mitotic entry and possibly the G1/S phase transition in fission yeast cells. One specific target identified was Fkh2. Future phosphoproteome analyses are planned to identify Cdk8 targets in mitosis and G2 cells. These results could provide further insight into the various signaling pathways involving Mediator. It would also be interesting to see if Cdk8 can interact with other cyclins *in vivo*. Furthermore, our data indicates a regulatory role for the Mediator components Med12 and Med13. These proteins may act to stabilize the Cdk8-CycC pair, and possibly direct Cdk8 activity to specific targets during cell cycle progression. Finally, we found evidence that the Cdk1 kinase activity can regulate periodic transcription in fission yeast independently of the actual cell cycle stage. It would be very interesting to see how the previously described deletions in the CDK8 module affect cell cycle progression in the minimal cell cycle model system.

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## REFERENCES

- Adler, A.S., McClelland, M.L., Truong, T., Lau, S., Modrusan, Z., Soukup, T.M., Roose-Girma, M., Blackwood, E.M., and Firestein, R. (2012). CDK8 maintains tumor dedifferentiation and embryonic stem cell pluripotency. *Cancer research* *72*, 2129-2139.
- Akoulitchev, S., Chuikov, S., and Reinberg, D. (2000). TFIID is negatively regulated by cdk8-containing mediator complexes. *Nature* *407*, 102-106.
- Anderson, M., Ng, S.S., Marchesi, V., MacIver, F.H., Stevens, F.E., Riddell, T., Glover, D.M., Hagan, I.M., and McNerny, C.J. (2002). Plo1(+) regulates gene transcription at the M-G(1) interval during the fission yeast mitotic cell cycle. *The EMBO journal* *21*, 5745-5755.
- Angus, S.P., and Nevins, J.R. (2012). A role for Mediator complex subunit MED13L in Rb/E2F-induced growth arrest. *Oncogene* *31*, 4709-4717.
- Baidooonso, S.M., Guidi, B.W., and Myers, L.C. (2007). Med19(Rox3) regulates Intermodule interactions in the *Saccharomyces cerevisiae* mediator complex. *The Journal of biological chemistry* *282*, 5551-5559.
- Baumli, S., Hoepfner, S., and Cramer, P. (2005). A conserved mediator hinge revealed in the structure of the MED7.MED21 (Med7.Srb7) heterodimer. *The Journal of biological chemistry* *280*, 18171-18178.
- Beltrao, P., Trinidad, J.C., Fiedler, D., Roguev, A., Lim, W.A., Shokat, K.M., Burlingame, A.L., and Krogan, N.J. (2009). Evolution of phosphoregulation: comparison of phosphorylation patterns across yeast species. *PLoS biology* *7*, e1000134.
- Bjorklund, S., and Gustafsson, C.M. (2005). The yeast Mediator complex and its regulation. *Trends in biochemical sciences* *30*, 240-244.
- Bondi, J., Husdal, A., Bukholm, G., Nesland, J.M., Bakka, A., and Bukholm, I.R. (2005). Expression and gene amplification of primary (A, B1, D1, D3, and E) and secondary (C and H) cyclins in colon adenocarcinomas and correlation with patient outcome. *Journal of clinical pathology* *58*, 509-514.
- Buck, V., Ng, S.S., Ruiz-Garcia, A.B., Papadopoulou, K., Bhatti, S., Samuel, J.M., Anderson, M., Millar, J.B., and McNerny, C.J. (2004). Fkh2p and Sep1p regulate mitotic gene transcription in fission yeast. *Journal of cell science* *117*, 5623-5632.
- Buratowski, S. (2009). Progression through the RNA polymerase II CTD cycle. *Molecular cell* *36*, 541-546.
- Cai, G., Imasaki, T., Takagi, Y., and Asturias, F.J. (2009). Mediator structural conservation and implications for the regulation mechanism. *Structure (London, England : 1993)* *17*, 559-567.
- Cho, R.J., Campbell, M.J., Winzler, E.A., Steinmetz, L., Conway, A., Wodicka, L., Wolfsberg, T.G., Gabrielian, A.E., Landsman, D.,

- Lockhart, D.J., *et al.* (1998). A genome-wide transcriptional analysis of the mitotic cell cycle. *Molecular cell* 2, 65-73.
- Cooper, K.F., Khakhina, S., Kim, S.K., and Strich, R. (2014). Stress-induced nuclear-to-cytoplasmic translocation of cyclin C promotes mitochondrial fission in yeast. *Developmental cell* 28, 161-173.
- Cooper, K.F., Scarnati, M.S., Krasley, E., Mallory, M.J., Jin, C., Law, M.J., and Strich, R. (2012). Oxidative-stress-induced nuclear to cytoplasmic relocalization is required for Not4-dependent cyclin C destruction. *Journal of cell science* 125, 1015-1026.
- Corden, J.L., Cadena, D.L., Ahearn, J.M., Jr., and Dahmus, M.E. (1985). A unique structure at the carboxyl terminus of the largest subunit of eukaryotic RNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America* 82, 7934-7938.
- Cortes, P., Flores, O., and Reinberg, D. (1992). Factors involved in specific transcription by mammalian RNA polymerase II: purification and analysis of transcription factor IIA and identification of transcription factor IIIJ. *Molecular and cellular biology* 12, 413-421.
- Cosma, M.P., Panizza, S., and Nasmyth, K. (2001). Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF. *Molecular cell* 7, 1213-1220.
- Coudreuse, D., and Nurse, P. (2010). Driving the cell cycle with a minimal CDK control network. *Nature* 468, 1074-1079.
- Davis, J.A., Takagi, Y., Kornberg, R.D., and Asturias, F.A. (2002). Structure of the yeast RNA polymerase II holoenzyme: Mediator conformation and polymerase interaction. *Molecular cell* 10, 409-415.
- Davis, M.A., Larimore, E.A., Fissel, B.M., Swanger, J., Taatjes, D.J., and Clurman, B.E. (2013). The SCF-Fbw7 ubiquitin ligase degrades MED13 and MED13L and regulates CDK8 module association with Mediator. *Genes & development* 27, 151-156.
- Elmlund, H., Baraznenok, V., Lindahl, M., Samuelsen, C.O., Koeck, P.J., Holmberg, S., Hebert, H., and Gustafsson, C.M. (2006). The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America* 103, 15788-15793.
- Fantes, P.A. (1981). Isolation of cell size mutants of a fission yeast by a new selective method: characterization of mutants and implications for division control mechanisms. *Journal of bacteriology* 146, 746-754.
- Firestein, R., Bass, A.J., Kim, S.Y., Dunn, I.F., Silver, S.J., Guney, I., Freed, E., Ligon, A.H., Vena, N., Ogino, S., *et al.* (2008). CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. *Nature* 455, 547-551.
- Garg, A., Futcher, B., and Leatherwood, J. (2015). A new transcription factor for mitosis: in *Schizosaccharomyces pombe*, the RFX transcription factor Sak1 works with forkhead factors to regulate mitotic expression. *Nucleic acids research* 43, 6874-6888.

- Gillmor, C.S., Silva-Ortega, C.O., Willmann, M.R., Buendia-Monreal, M., and Poethig, R.S. (2014). The Arabidopsis Mediator CDK8 module genes CCT (MED12) and GCT (MED13) are global regulators of developmental phase transitions. *Development (Cambridge, England)* *141*, 4580-4589.
- Grallert, A., Grallert, B., Zilahi, E., Szilagyi, Z., and Sipiczki, M. (1999). Eleven novel sep genes of *Schizosaccharomyces pombe* required for efficient cell separation and sexual differentiation. *Yeast (Chichester, England)* *15*, 669-686.
- Grandori, C., Cowley, S.M., James, L.P., and Eisenman, R.N. (2000). The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annual review of cell and developmental biology* *16*, 653-699.
- Gu, W., Malik, S., Ito, M., Yuan, C.X., Fondell, J.D., Zhang, X., Martinez, E., Qin, J., and Roeder, R.G. (1999). A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. *Molecular cell* *3*, 97-108.
- Guglielmi, B., van Berkum, N.L., Klapholz, B., Bijma, T., Boube, M., Boschiero, C., Bourbon, H.M., Holstege, F.C., and Werner, M. (2004). A high resolution protein interaction map of the yeast Mediator complex. *Nucleic acids research* *32*, 5379-5391.
- Haering, C.H., Lowe, J., Hochwagen, A., and Nasmyth, K. (2002). Molecular architecture of SMC proteins and the yeast cohesin complex. *Molecular cell* *9*, 773-788.
- Hartwell, L.H., Mortimer, R.K., Culotti, J., and Culotti, M. (1973). Genetic Control of the Cell Division Cycle in Yeast: V. Genetic Analysis of *cdc* Mutants. *Genetics* *74*, 267-286.
- Hengartner, C.J., Myer, V.E., Liao, S.M., Wilson, C.J., Koh, S.S., and Young, R.A. (1998). Temporal regulation of RNA polymerase II by *Srb10* and *Kin28* cyclin-dependent kinases. *Molecular cell* *2*, 43-53.
- Hengartner, C.J., Thompson, C.M., Zhang, J., Chao, D.M., Liao, S.M., Koleske, A.J., Okamura, S., and Young, R.A. (1995). Association of an activator with an RNA polymerase II holoenzyme. *Genes & development* *9*, 897-910.
- Hereford, L.M., Osley, M.A., Ludwig, T.R., 2nd, and McLaughlin, C.S. (1981). Cell-cycle regulation of yeast histone mRNA. *Cell* *24*, 367-375.
- Hiraoka, Y., Toda, T., and Yanagida, M. (1984). The *NDA3* gene of fission yeast encodes beta-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* *39*, 349-358.
- Hoepfner, S., Baumli, S., and Cramer, P. (2005). Structure of the mediator subunit cyclin C and its implications for CDK8 function. *Journal of molecular biology* *350*, 833-842.
- Huang, S., Holzel, M., Knijnenburg, T., Schlicker, A., Roepman, P., McDermott, U., Garnett, M., Grenrum, W., Sun, C., Prahallad, A., *et al.*

- (2012). MED12 controls the response to multiple cancer drugs through regulation of TGF-beta receptor signaling. *Cell* *151*, 937-950.
- Hurwitz, J., and Leis, J.P. (1972). RNA-Dependent DNA Polymerase Activity of RNA Tumor Viruses I. Directing Influence of DNA in the Reaction. *Journal of Virology* *9*, 116-129.
- Kampjarvi, K., Kim, N.H., Keskitalo, S., Clark, A.D., von Nandelstadh, P., Turunen, M., Heikkinen, T., Park, M.J., Makinen, N., Kivinummi, K., *et al.* (2015). Somatic MED12 mutations in prostate cancer and uterine leiomyomas promote tumorigenesis through distinct mechanisms. *The Prostate*.
- Kampjarvi, K., Makinen, N., Kilpivaara, O., Arola, J., Heinonen, H.R., Bohm, J., Abdel-Wahab, O., Lehtonen, H.J., Peltari, L.M., Mehine, M., *et al.* (2012). Somatic MED12 mutations in uterine leiomyosarcoma and colorectal cancer. *British journal of cancer* *107*, 1761-1765.
- Kampjarvi, K., Park, M.J., Mehine, M., Kim, N.H., Clark, A.D., Butzow, R., Bohling, T., Bohm, J., Mecklin, J.P., Jarvinen, H., *et al.* (2014). Mutations in Exon 1 highlight the role of MED12 in uterine leiomyomas. *Human mutation* *35*, 1136-1141.
- Kapoor, A., Goldberg, M.S., Cumberland, L.K., Ratnakumar, K., Segura, M.F., Emanuel, P.O., Menendez, S., Vardabasso, C., Leroy, G., Vidal, C.I., *et al.* (2010). The histone variant macroH2A suppresses melanoma progression through regulation of CDK8. *Nature* *468*, 1105-1109.
- Katahira, J. (2015). Nuclear export of messenger RNA. *Genes* *6*, 163-184.
- Kim, S., Xu, X., Hecht, A., and Boyer, T.G. (2006). Mediator is a transducer of Wnt/beta-catenin signaling. *The Journal of biological chemistry* *281*, 14066-14075.
- Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. (1994). A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* *77*, 599-608.
- Knuesel, M.T., Meyer, K.D., Bernecky, C., and Taatjes, D.J. (2009). The human CDK8 subcomplex is a molecular switch that controls Mediator coactivator function. *Genes & development* *23*, 439-451.
- Koh, S.S., Ansari, A.Z., Ptashne, M., and Young, R.A. (1998). An activator target in the RNA polymerase II holoenzyme. *Molecular cell* *1*, 895-904.
- Landry, J.J.M., Pyl, P.T., Rausch, T., Zichner, T., Tekkedil, M.M., Stütz, A.M., Jauch, A., Aiyar, R.S., Pau, G., Delhomme, N., *et al.* (2013). The Genomic and Transcriptomic Landscape of a HeLa Cell Line. *G3: Genes|Genomes|Genetics* *3*, 1213-1224.
- Lee, M.G., and Nurse, P. (1987). Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. *Nature* *327*, 31-35.
- Leopold, P., and O'Farrell, P.H. (1991). An evolutionarily conserved cyclin homolog from *Drosophila* rescues yeast deficient in G1 cyclins. *Cell* *66*, 1207-1216.

- Li, H., Lahti, J.M., Valentine, M., Saito, M., Reed, S.I., Look, A.T., and Kidd, V.J. (1996). Molecular cloning and chromosomal localization of the human cyclin C (CCNC) and cyclin E (CCNE) genes: deletion of the CCNC gene in human tumors. *Genomics* 32, 253-259.
- Li, N., Fassl, A., Chick, J., Inuzuka, H., Li, X., Mansour, M.R., Liu, L., Wang, H., King, B., Shaik, S., *et al.* (2014). Cyclin C is a haploinsufficient tumour suppressor. *Nature cell biology* 16, 1080-1091.
- Lim, J., Lee, O.K., Hsu, Y.C., Singh, A., and Choi, K.W. (2007). Drosophila TRAP230/240 are essential coactivators for Atonal in retinal neurogenesis. *Developmental biology* 308, 322-330.
- Lim, W.K., Ong, C.K., Tan, J., Thike, A.A., Ng, C.C., Rajasegaran, V., Myint, S.S., Nagarajan, S., Nasir, N.D., McPherson, J.R., *et al.* (2014). Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. *Nature genetics* 46, 877-880.
- Linder, T., Rasmussen, N.N., Samuelsen, C.O., Chatzidaki, E., Baraznenok, V., Beve, J., Henriksen, P., Gustafsson, C.M., and Holmberg, S. (2008). Two conserved modules of *Schizosaccharomyces pombe* Mediator regulate distinct cellular pathways. *Nucleic acids research* 36, 2489-2504.
- Liu, Y., Kung, C., Fishburn, J., Ansari, A.Z., Shokat, K.M., and Hahn, S. (2004). Two cyclin-dependent kinases promote RNA polymerase II transcription and formation of the scaffold complex. *Molecular and cellular biology* 24, 1721-1735.
- Liu, Z.J., Ueda, T., Miyazaki, T., Tanaka, N., Mine, S., Tanaka, Y., Taniguchi, T., Yamamura, H., and Minami, Y. (1998). A critical role for cyclin C in promotion of the hematopoietic cell cycle by cooperation with c-Myc. *Molecular and cellular biology* 18, 3445-3454.
- Loncle, N., Boube, M., Joulia, L., Boschiero, C., Werner, M., Cribbs, D.L., and Bourbon, H.M. (2007). Distinct roles for Mediator Cdk8 module subunits in *Drosophila* development. *The EMBO journal* 26, 1045-1054.
- Lu, H., Flores, O., Weinmann, R., and Reinberg, D. (1991). The nonphosphorylated form of RNA polymerase II preferentially associates with the preinitiation complex. *Proceedings of the National Academy of Sciences of the United States of America* 88, 10004-10008.
- Luche, D.D., and Forsburg, S.L. (2009). Cell-cycle synchrony for analysis of *S. pombe* DNA replication. *Methods in molecular biology* (Clifton, NJ) 521, 437-448.
- Ma, D., Olave, I., Merino, A., and Reinberg, D. (1996). Separation of the transcriptional coactivator and antirepression functions of transcription factor IIA. *Proceedings of the National Academy of Sciences of the United States of America* 93, 6583-6588.
- Maillard, I., and Pear, W.S. (2003). Notch and cancer: best to avoid the ups and downs. *Cancer cell* 3, 203-205.
- McInerney, C.J. (2004). Cell cycle-regulated transcription in fission yeast. *Biochemical Society transactions* 32, 967-972.

- Meinhart, A., Kamenski, T., Hoepfner, S., Baumli, S., and Cramer, P. (2005). A structural perspective of CTD function. *Genes & development* *19*, 1401-1415.
- Monni, O., Barlund, M., Mousses, S., Kononen, J., Sauter, G., Heiskanen, M., Paavola, P., Avela, K., Chen, Y., Bittner, M.L., *et al.* (2001). Comprehensive copy number and gene expression profiling of the 17q23 amplicon in human breast cancer. *Proceedings of the National Academy of Sciences of the United States of America* *98*, 5711-5716.
- Morgan, D.O. (2007). *The Cell Cycle: Principles of Control* (OUP/New Science Press).
- Nelson, C., Goto, S., Lund, K., Hung, W., and Sadowski, I. (2003). Srb10/Cdk8 regulates yeast filamentous growth by phosphorylating the transcription factor Ste12. *Nature* *421*, 187-190.
- Oelgeschlager, T., Tao, Y., Kang, Y.K., and Roeder, R.G. (1998). Transcription activation via enhanced preinitiation complex assembly in a human cell-free system lacking TAFIIIs. *Molecular cell* *1*, 925-931.
- Orlando, D.A., Lin, C.Y., Bernard, A., Wang, J.Y., Socolar, J.E.S., Iversen, E.S., Hartemink, A.J., and Haase, S.B. (2008). Global control of cell cycle transcription by coupled CDK and network oscillators. *Nature* *453*, 944-947.
- Ozer, J., Moore, P.A., Bolden, A.H., Lee, A., Rosen, C.A., and Lieberman, P.M. (1994). Molecular cloning of the small (gamma) subunit of human TFIIA reveals functions critical for activated transcription. *Genes & development* *8*, 2324-2335.
- Payne, J.M., Laybourn, P.J., and Dahmus, M.E. (1989). The transition of RNA polymerase II from initiation to elongation is associated with phosphorylation of the carboxyl-terminal domain of subunit I $\alpha$ . *The Journal of biological chemistry* *264*, 19621-19629.
- Porter, D.C., Farmaki, E., Altiglia, S., Schools, G.P., West, D.K., Chen, M., Chang, B.D., Puzyrev, A.T., Lim, C., Rokow-Kittell, R., *et al.* (2012). Cyclin-dependent kinase 8 mediates chemotherapy-induced tumor-promoting paracrine activities. *Proceedings of the National Academy of Sciences of the United States of America* *109*, 13799-13804.
- Prenzel, T., Kramer, F., Bedi, U., Nagarajan, S., Beissbarth, T., and Johnsen, S.A. (2012). Cohesin is required for expression of the estrogen receptor-alpha (ESR1) gene. *Epigenetics & chromatin* *5*, 13.
- Raithatha, S., Su, T.C., Lourenco, P., Goto, S., and Sadowski, I. (2012). Cdk8 regulates stability of the transcription factor Phd1 to control pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Molecular and cellular biology* *32*, 664-674.
- Risheg, H., Graham, J.M., Jr., Clark, R.D., Rogers, R.C., Opitz, J.M., Moeschler, J.B., Peiffer, A.P., May, M., Joseph, S.M., Jones, J.R., *et al.* (2007). A recurrent mutation in MED12 leading to R961W causes Opitz-Kaveggia syndrome. *Nature genetics* *39*, 451-453.

- Roeder, R.G. (1996). The role of general initiation factors in transcription by RNA polymerase II. *Trends in biochemical sciences* 21, 327-335.
- Rustici, G., Mata, J., Kivinen, K., Lio, P., Penkett, C.J., Burns, G., Hayles, J., Brazma, A., Nurse, P., and Bahler, J. (2004). Periodic gene expression program of the fission yeast cell cycle. *Nature genetics* 36, 809-817.
- Schneider, E.V., Bottcher, J., Blaesse, M., Neumann, L., Huber, R., and Maskos, K. (2011). The structure of CDK8/CycC implicates specificity in the CDK/cyclin family and reveals interaction with a deep pocket binder. *Journal of molecular biology* 412, 251-266.
- Schwartz, C.E., Tarpey, P.S., Lubs, H.A., Verloes, A., May, M.M., Risheg, H., Friez, M.J., Futreal, P.A., Edkins, S., Teague, J., *et al.* (2007). The original Lujan syndrome family has a novel missense mutation (p.N1007S) in the MED12 gene. *Journal of medical genetics* 44, 472-477.
- Sipiczki, M. (2000). Where does fission yeast sit on the tree of life? *Genome Biology* 1, reviews1011.1011-1014.
- Speck, C., Chen, Z., Li, H., and Stillman, B. (2005). ATPase-dependent cooperative binding of ORC and Cdc6 to origin DNA. *Nature structural & molecular biology* 12, 965-971.
- Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D., and Futcher, B. (1998). Comprehensive Identification of Cell Cycle-regulated Genes of the Yeast *Saccharomyces cerevisiae* by Microarray Hybridization. *Molecular Biology of the Cell* 9, 3273-3297.
- Takagi, Y., Calero, G., Komori, H., Brown, J.A., Ehrensberger, A.H., Hudmon, A., Asturias, F., and Kornberg, R.D. (2006). Head module control of mediator interactions. *Molecular cell* 23, 355-364.
- Trickey, M., Fujimitsu, K., and Yamano, H. (2013). Anaphase-promoting complex/cyclosome-mediated proteolysis of Ams2 in the G1 phase ensures the coupling of histone gene expression to DNA replication in fission yeast. *The Journal of biological chemistry* 288, 928-937.
- Umesono, K., Hiraoka, Y., Toda, T., and Yanagida, M. (1983). Visualization of chromosomes in mitotically arrested cells of the fission yeast *Schizosaccharomyces pombe*. *Current genetics* 7, 123-128.
- Vannini, A., and Cramer, P. (2012). Conservation between the RNA polymerase I, II, and III transcription initiation machineries. *Molecular cell* 45, 439-446.
- Vulto-van Silfhout, A.T., de Vries, B.B., van Bon, B.W., Hoischen, A., Ruitkamp-Versteeg, M., Gilissen, C., Gao, F., van Zwam, M., Harteveld, C.L., van Essen, A.J., *et al.* (2013). Mutations in MED12 cause X-linked Ohdo syndrome. *American journal of human genetics* 92, 401-406.
- Weil, P.A., Segall, J., Harris, B., Ng, S.Y., and Roeder, R.G. (1979). Faithful transcription of eukaryotic genes by RNA polymerase III in systems

- reconstituted with purified DNA templates. *The Journal of biological chemistry* 254, 6163-6173.
- Wood, V., Gwilliam, R., Rajandream, M.A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., *et al.* (2002). The genome sequence of *Schizosaccharomyces pombe*. *Nature* 415, 871-880.
- Wu, S.Y., de Borsetti, N.H., Bain, E.J., Bulow, C.R., and Gamse, J.T. (2014). Mediator subunit 12 coordinates intrinsic and extrinsic control of epithalamic development. *Developmental biology* 385, 13-22.
- Wu, S.Y., and McLeod, M. (1995). The *sak1+* gene of *Schizosaccharomyces pombe* encodes an RFX family DNA-binding protein that positively regulates cyclic AMP-dependent protein kinase-mediated exit from the mitotic cell cycle. *Molecular and cellular biology* 15, 1479-1488.
- Yang, C., Bolotin, E., Jiang, T., Sladek, F.M., and Martinez, E. (2007). Prevalence of the Initiator over the TATA box in human and yeast genes and identification of DNA motifs enriched in human TATA-less core promoters. *Gene* 389, 52-65.
- Yoshida, K., Toki, T., Okuno, Y., Kanezaki, R., Shiraishi, Y., Sato-Otsubo, A., Sanada, M., Park, M.J., Terui, K., Suzuki, H., *et al.* (2013). The landscape of somatic mutations in Down syndrome-related myeloid disorders. *Nature genetics* 45, 1293-1299.
- Zhao, J., Ramos, R., and Demma, M. (2013). CDK8 regulates E2F1 transcriptional activity through S375 phosphorylation. *Oncogene* 32, 3520-3530.