Mediator and its role in non-coding RNA and chromatin regulation

Jonas Carlsten

Department of Medical Biochemistry and Cell Biology
Institute of Biomedicine
Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2014

Cover illustration: “Mediator - the predator” by Jonas Carlsten

Mediator and its role in non-coding RNA and chromatin regulation

jonas.carlsten@gu.se

ISBN: 978-91-628-9063-6 (Electronic)
ISBN: 978-91-628-9052-0 (Printed)

Printed in Gothenburg, Sweden 2014
Aidla Trading AB / Kompendiet

Dedicated to my beloved father, you are deeply missed.

Mediator and its role in non-coding RNA and chromatin regulation

Jonas Carlsten

Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine
Sahlgrenska Academy at University of Gothenburg
Gothenburg, Sweden

ABSTRACT

Mediator is a multiprotein complex required for the regulation of RNA Polymerase II (Pol II) transcription. Mediator transmits regulatory signals from activators and repressors to the Pol II machinery at the promoter, but the complex has also many other functions related to control of gene transcription. This thesis aims to expand our knowledge of Mediator's involvement in regulation of the specialized chromatin structures found at telomeres and centromeres as well as its role in regulation of non-coding RNA transcription.

A fine-tuned balance between the histone deacetylase Sir2 and the histone acetyltransferase Sas2 determines the location of the boundary between active and inactive chromatin at budding yeast telomeres. In our work, we demonstrate that Mediator interacts with heterochromatin at telomeres and directs the position of this boundary. Mutations in Mediator subunits cause a depletion of the complex from heterochromatin, which changes the balance between Sir2 and Sas2, and ultimately results in desilencing of subtelomeric regions. Telomeres are important regulators of replicative life span, which is reduced as a consequence of mutations in the Mediator complex.

The *Schizosaccharomcyes pombe* centromeres are also characterized by silent heterochromatin, which is assembled and maintained through a complex multifactorial system. In our work, we find that Mediator is involved in formation of these heterochromatin structures. Loss of the Mediator subunit Med20 causes disruption of heterochromatin and leads to increased transcriptional activity at the centromere. The *med20*Δ mutant also causes reduced levels of CENP-A^{Cnp1}, a centromere specific form of histone H3 found at centromeres, and chromosome instability during cell division. Previous data have demonstrated that pericentromeric transcription may

contribute to heterochromatin formation at pericentromeres via two parallel mechanisms, one depending on the exosome RNA degradation complex and one dependent on the RNAi machinery. In our work, we find that inactivation of the exosome can reverse the increased levels of pericentromeric transcription observed in *med20*Δ cells, but that it fails to alleviate the chromosome segregation defects. Furthermore, loss of Med20 leads to a changed pattern of siRNA products, which is not further affected in the *med20*Δ*/rrp6*Δ strain. Our results therefore suggest that Mediator and the exosome act in partially independent pathways to influence centromere function.

We also demonstrate that Mediator influences RNA polymerase III (Pol III) transcription. Deletion of *med20*$^{+}$ results in increased transcription of ribosomal protein genes, but also affects Pol III transcription causing an accumulation of aberrant tRNA transcripts with evidence of incorrect transcription termination. The aberrant transcripts are polyadenylated and targeted for degradation by the exosome. The effects of Mediator on Pol III transcription are distinct from those involving Maf1, the classical repressor of Pol III activity. Based on our findings we suggest that fission yeast Mediator takes part in a pathway that coordinates expression of ribosomal protein genes with Pol III transcription.

Work in this thesis demonstrates that Mediator regulates the chromatin structure of several regions characterized by silenced chromatin. Mediator mutations cause loss of heterochromatin at both telomeres and centromeres, which has implications for replicative aging and cell division. Our observation of chromosome segregation defects in *med20*Δ cells may also have more general implications. Chromosomal instability is a driving force in tumorigenesis and mutations in genes encoding Mediator subunits have been linked to the development of several forms of cancer. The thesis also introduces the unexpected finding that Mediator influences Pol III transcription. All together, our results support the view that Mediator does not only mediate signals from gene specific transcription factors to the Pol II transcription machinery. Instead Mediator is a multifaceted protein complex involved in many processes connected to transcription.

Keywords: Mediator, transcription, heterochromatin, centromere, telomere, tRNA, exosome

ISBN: 978-91-628-9063-6

SAMMANFATTNING PÅ SVENSKA

Den genetiska informationen är lagrad i form av DNA i stora makromolekylära komplex kallade kromosomer. För att producera proteiner behöver de gener som återfinns i DNA läsas av och kopieras till budbärar-RNA. Denna process kallas transkription och utförs av RNA polymeras II (Pol II). I våra celler finns ett stort antal faktorer, som styr transkriptionsprocessen, så att gener kommer till uttryck i rätt celltyp och vid rätt tidpunkt. Mediatorn är ett stort proteinkomplex som har som huvuduppgift att förmedla regulatoriska signaler från omgivningen till Pol II maskineriet. Mediatorn är evolutionärt konserverad från jäst till människa och består av 20 - 30 proteiner. Komplexet indelas traditionellt i fyra moduler: svans-, mitt-, huvud- och CDK8-modulen. Faktorer som kan stimulera transkription av en viss gen, s.k. aktivatorer, interagerar i första hand med svansmodulen medan huvudmodulen binder till Pol II. Konformationsförändringar i Mediatorn kan sedan överföra signaler från aktivatorer till Pol II-maskineriet.

Det är viktigt att DNA inte skadas, då strängbrott och mutationer kan leda till sjukdom. För att skyddas, viras DNA runt ett proteinkomplex, innehållande åtta histonproteiner och formar på där med nukleosomer. Nukleosom-packning styr även uttrycket av gener. Det komplex som bildas av protein och DNA i cellkärnan kalls kromatin, där eukromatin är en lös sammansättning av nukleosomer, ofta associerad med hög transkriptionsaktivitet. Motsatsen, heterokromatin, är istället tätt packat och transkriptionellt tyst.

På ändarna av kromosomerna finns telomerer som skyddar kromosomändarna från nedbrytning. I anslutning till telomererna finns s.k. subtelomera regioner, som är täckta av en transkriptionellt tyst heterokromatin-liknande struktur. Gränsen mellan heterokromatin och eukromatin måste bevaras för att inte ändra transkriptionsmönstret i de närliggande regionerna. Enzymerna Sir2 och Sas2 har motverkande aktiviteter och balansen mellan dessa bestämmer denna kromatingräns. I vår studie upptäckte vi att Mediator är involverad i regleringen av Sir2/Sas2 balansen (Artikel I). Förlust av Mediatorsubenheten Med5 resulterade i ökad dominans av Sas2 vilket i sin tur ledde till ökad transkriptionell aktivitet i de subtelomera regionerna. Som en konsekvens av detta, minskade antalet livscykler som cellen totalt kunde genomgå.

I centrum av kromosomen återfinns centromeren, fästpunkten för det maskineri, som reglerar kromosomsegregation under celldelning. Stora delar av centromeren är täckt av heterokromatin som är avgörande för dess

funktion. Många faktorer är involverade i bildningen av detta heterokromatin och vi har funnit att Mediatorkomplexet är en viktig faktor i denna process (Artikel II). Utan Med20, en subenhet i Mediatorns huvudmodul, luckras heterokromatinet upp, vilket leder till ökad transkription i centromeren. Dessutom försvinner den centromerspecifika histonvarianten CENP-A^{Cnp1} från centromeren. Dessa förändringar stör normal kromosomsegregation under celldelning. Tidigare studier har visat att mutationer i ett maskineri, som behövs för s.k. RNA interferens (RNAi) ofta orsakar liknande kromosominstabilitet. Störningar i den RNAi-beroende mekanismen leder dock inte till förlust av CENP-A ^{Cnp1} och Mediatorn fungerar därför troligen inte via denna process.

Nivåerna av RNA i cellen styrs av balansen mellan bildning (främst transkription) och nedbrytning. Exosomen är en central del av RNA-degradationsmaskineriet och detta enzymkomplex bryter ner RNA molekyler som är felaktiga eller inte längre behövs. Exosomen är bland annat involverad i degradation av RNA som bildas vid transkription av centromerer. Vi studerade därför vilken effekt förlust av exosomsubenheten Rrp6 har på nivåerna av de centromera transkript, som bildas vid förlust av Med20 (Artikel III). Våra studier visade att förlust av Rrp6 delvis kunde återställa den ökning av dessa transkript, som vi observerat när *med20*$^{+}$ genen slogs ut. Genom att slå ut *rrp6*$^{+}$ genen kunde vi även rädda den nedgång i heterokromatin vi hade observerat vid förlust av Med20. Trots dessa effekter på transkription och kromatin, påverkade Rrp6 inte den defekta kromosomsegregation vi observerade i avsaknad av Med20.

Exosomen är också inblandad vid nedbrytning av felaktiga tRNA transkript, producerade av RNA polymeras III (Pol III) maskineriet. Vi fann en kraftig ökning av felaktiga tRNA molkyler i *med20*Δ*/rrp6*Δ, en jäststam som saknade generna för såväl Med20 som Rrp6 (Artikel IV). Resultatet var överraskande eftersom Mediatorn endast är känd som en regulator av Pol II-beroende gener. Vidare undersökningar visade även på förhöjda nivåer av defekta 5S rRNA, snRNA och snoRNA transkript, även dessa producerade av Pol III. En noggrann analys av dessa RNA molekyler indikerade att förlust av Med20 orsakar defekt av transkriptionsterminering vid Pol III-beroende gener. De felaktiga transkripten hade dessutom en poly(A)-svans, vilket visar att de är märkta för Exosom-beroende degradation. I avsaknad av Rrp6 stabiliseras dock dessa transkript, vilket gjorde det möjligt att observera effekter av Med20. Hur Mediatorn utövar sin effekt på Pol III-beroende transkription är fortfarande oklart, men vi har identifierat en oväntad funktion hos Mediator, som åter visar på hur funktionellt mångfacetterat detta viktiga komplex är.

LIST OF PAPERS

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

I. **Mediator influences telomeric silencing and cellular life span.**
Zhu X, Liu B, **Carlsten JO**, Beve J, Nyström T, Myers LC, Gustafsson CM.
Mol Cell Biol. 2011 Jun; 31(12): 2413-21.

II. **Mediator promotes CENP-A incorporation at fission yeast centromeres.**
Carlsten JO, Szilagyi Z, Liu B, Lopez MD, Szászi E, Djupedal I, Nyström T, Ekwall K, Gustafsson CM, Zhu X.
Mol Cell Biol. 2012 Oct; 32(19): 4035-43.

III. **Mediator effects on centromere function are not dependent on the exosome subunit Rrp6.**
Carlsten JO, Zhu X, Lopez MD, Gustafsson CM.
Manuscript

IV. **Loss of the Mediator subunit Med20 causes an increase of aberrant RNA polymerase III transcripts in fission yeast.**
Carlsten JO, Zhu X, Lopez MD, Samuelsson T, Gustafsson CM.
Manuscript

Related publication:

The multitalented Mediator complex.
Carlsten JO, Zhu X, Gustafsson CM.
Trends Biochem Sci. 2013 Nov; 38(11): 531-7.

CONTENT

ABBREVIATIONS

ABD	Activator binding domain
ARE	AU-rich element
BRE	TFIIB recognition element
CDK	Cyclin dependent kinase
cnt	Core centromere
CPSF	Cleavage and polyadenylation specific factor
CstF	Cleavage-stimulating factor
CTD	C-terminal domain of Pol II subunit Rbp1
DNA	Deoxyribonucleic acid
DSIF	DRB sensitivity inducing factor
eIF	Eukaryotic translation initiation factor
EM	Electron microscopy
ES	Embryonic stem cells
GTF	General transcription factor
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
IE	Intermediate element
imr	Inner most repeats
Inr	Initiator
mRNA	Messenger RNA
ncRNA	Non-coding RNA
NELF	Negative elongation factor
NPC	Nuclear pore complex
otr	Outer repeats region
P-TEFb	Positive transcription elongation factor
PAZ	Piwi Argonaute Zwille
PIC	Pre-initiation complex

Pol II/III	RNA Polymerase II/III
POM	Postnatal-onset microcephaly
RDRP	RNA dependent RNA polymerase
REST	RE1 silencing transcription factor
RISC	RNA-induced silencing complex
RITS	RNA-induced transcription silencing
RNA	Ribonucleic acid
RNAi	RNA interference
RNP	RNA-protein complex
SEC	Super elongation complex
siRNA	Small interfering RNA
SKI	Super killer
snoRNA	Small nucleolar RNA
snRNA	Small nuclear RNA
ssRNA	Single-stranded RNA
TAD	Transactivation domain
TAF	TBP associated factor
TAS	Telomere-associated sequence
TBP	TATA binding protein
TF	Transcription factor
TGV	Transposition of the great vessels
TRAMP	Trimeric Trf4/Trf5-Air1/Air2-Mtr4 polyadenylation complex
tRNA	Transfer RNA
rRNA	Ribosomal RNA
TSS	Transcription start site
UTR	Untranslated region
XLMR	X-linked mental retardation

DEFINITIONS IN SHORT

Activator Protein that increases transcription of a gene.

Centromere Central chromosome region needed for chromosome separation during cell division.

Chromatin loop A loop that allows for two separate chromosome regions to come into close proximity of each other.

Elongation Transcriptional phase were RNA is synthesized.

Endonuclease Enzyme that cleaves inside a transcript or DNA.

Exonuclease Enzyme that digest transcript or DNA from the end.

Initiation Transcriptional phase were PIC is assembled and RNA polymerase initiate transcription.

Kinetochore Multiprotein complex, which connects the centromere and microtubules during cell division.

Mediator Multiprotein complex that regulates transcription.

Mitosis Cell cycle phase were the cell divides.

Polyadenylation Process that adds a poly(A)-tail to transcripts.

Promoter proximal stalling Pausing of Pol II after promoter clearance.

Repressor Protein that decreases transcription of a gene.

RNAi Machinery that digests RNA with the help of small RNA molecules.

Splicing Removal of introns from pre-RNA transcripts

Telomeres Structures at chromosome ends.

Termination Transcriptional phase where the transcript is released from Pol II, which dissociates from the template.

The exosome A RNA degradation complex.

Transcription Process that copies DNA into RNA molecules.

Translation Process that reads the RNA and produces proteins.

1 INTRODUCTION

DNA is the genetic blueprint for our cells. RNA polymerases copy DNA sequences into RNA through a process called transcription. Transcripts of protein-coding genes are denoted messenger RNA (mRNA) and they carry the genetic information to the ribosome where protein production (translation) takes place. In most eukaryotes, nuclear transcription depends on three distinct polymerases: RNA polymerase I (Pol I) produces ribosomal RNA (rRNA) used as parts of the ribosome (1), RNA polymerases II (Pol II) is responsible for production of mRNA and non-coding RNA (ncRNA) molecules (2), whereas RNA polymerase III (Pol III) primarily produces transfer RNA (tRNA) and some rRNA (3).

1.1 Basic transcription

The functional unit of inheritance is the gene. A gene corresponds to a DNA sequence that provides information for RNA synthesis. Apart from the actual transcribed sequence there are a number of regulatory elements that control gene transcription. The promoter defines where the RNA polymerase should initiate transcription. There is considerable variation in promoter sequences, also between genes transcribed by the same polymerase.

A classical DNA sequence element found in Pol II dependent promoters is the TATA-box (Figure 1), which is located about 30 bp upstream of the transcription start site (TSS) at about one fifth of all promoters (4).

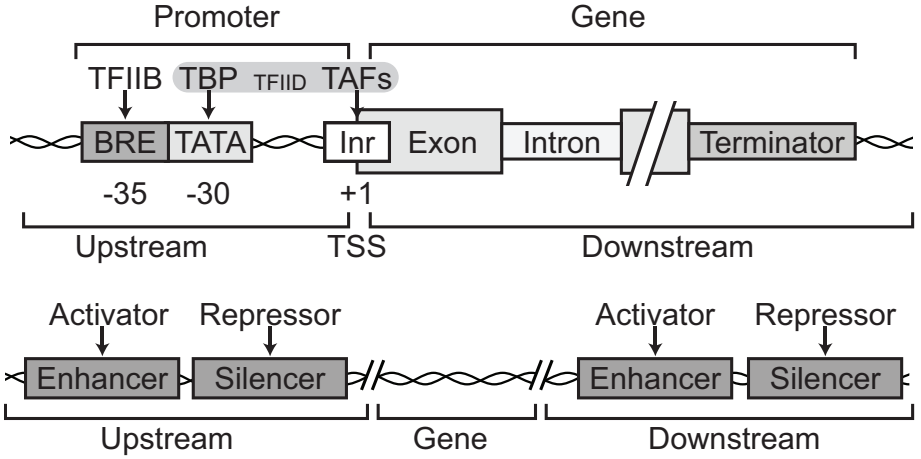

Figure 1. Schematic presentation of a Pol II dependent gene structure.
The different sequence elements are discussed in the text.

At some promoters, the TATA-box is flanked by the TFIIB recognition element (BRE), which may have either a positive or a negative impact on transcription of the associated gene (5). The most commonly occurring promoter motif is the initiator (Inr), which centers on the TSS (4).

Pol II cannot recognize promoter elements by itself, but needs the help of a set of additional general transcription factors (GTFs: TFIIA, B, D, E, F, and H). These factors assemble together with Pol II on the promoter and form the pre-initiation complex (PIC) (Figure 2)(6, 7).

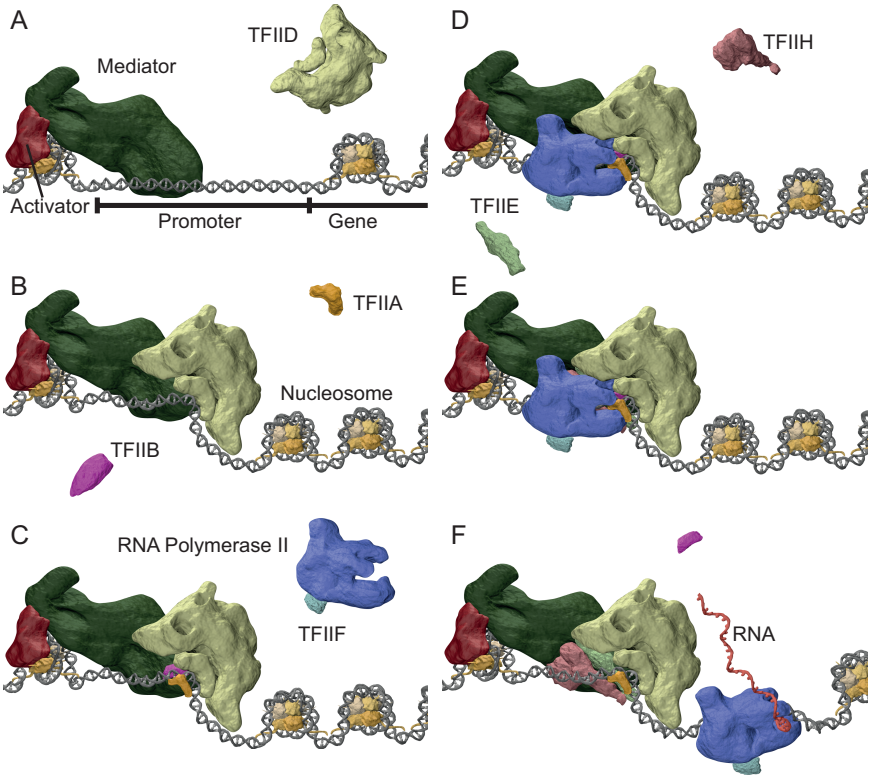

Figure 2. Initiation of Pol II transcription

A model describing the different steps in Pol II dependent initiation. A) Mediator (see 1.3) is initially recruited to the promoter via activator interactions. B) Mediator helps to recruit TFIID to the TATA-box and Inr. TBP creates a sharp bend in the DNA. C) TFIIA stabilizes the TFIID-DNA interaction. Subsequently, TFIIB enters the complex and interacts with BRE. D) Pol II is recruited in complex with TFIIF. E) TFIIE and TFIIH enter and complete PIC formation. TFIIH unwinds the promoter and allows access for Pol II to the template. F) Pol II is released in complex with TFIIF. Mediator, TFIID, TFIIA, TFIIE, and TFIIH remain bound at the promoter and facilitate re-initiation and further rounds of transcription.

According to the classical model of Pol II transcription initiation, the process is initiated by TFIID, which consists of the TATA-binding protein (TBP) in complex with a set of TBP-associated factors (TAFs). TBP recognizes the TATA-box whereas the TAFs interact with the Inr. TBP binding introduces a sharp bend in the promoter DNA, which enables TFIIA to bind upstream of TFIID. In the next step, TFIIB can interact with both TBP and DNA surrounding TATA-box, for instance the BRE, and thereby stabilizing the TBP-TFIIB-TFIIA-DNA complex (8, 9). In turn TFIIB recruits Pol II in association with TFIIF (10). Finally TFIIE and TFIIH are recruited which enables transcriptional initiation (11).

After release from the promoter Pol II transcribes the gene, which in most cases contains both protein-coding sequences called exons and non-protein-coding sequences termed introns (12). At a terminator site, transcription ends by cleavage of the nascent RNA and subsequent release of Pol II (13).

Genes are present in the context of chromatin. Chromatin is the state of DNA packaging in the nucleus and it is formed by protein-DNA units called nucleosomes (14). They consist of a core of histone proteins wrapped by DNA. While the nucleosomes can protect the genetic material it may also prevent Pol II from accessing DNA and initiate transcription (15). A transcription factor's affinity for its binding site is dependent on the precise nucleosome configuration (16). The chromatin structure is regulated by histone modifications (e.g. acetylation or methylation) or changes in the composition and position of nucleosomes by chromatin remodeling enzymes (17).

Regulatory proteins, referred to as activators and repressors, associate with specific DNA elements (enhancers resp. silencers) and control the frequency of transcription at specific genes. Activators can help recruit the transcription machinery and stimulate assembly of PIC (18). These regulatory proteins can also affect transcriptional activity by modifying the chromatin structure. In most cases, interactions between activators and the general transcription factors are not direct, but instead mediated by a large multiprotein complex called the Mediator (19). This thesis explores the role of Mediator in chromatin formation and transcriptional regulation of non-coding RNAs.

1.2 RNA Polymerase II

1.2.1 Structure

At its core, Pol II consists of 12 subunits, denoted Rpb1 to Rpb12 (20). The subunits are highly conserved and their sizes usually vary between 6 and 200

kDa. Rpb1 is the largest subunit and together with Rpb2 it forms the opposite sides of a “cleft” through which the DNA template can travel to reach the active site (21). Rpb1 includes a mobile “clamp”, which shifts from an open to a closed state when Pol II transitions into elongation (22). When closed, the clamp locks the RNA-DNA hybrid in place during transcription and at the bottom of the cleft is a channel, which enables new nucleotides to enter and the nascent RNA to exit.

Rpb1 also contains a C-Terminal Domain (CTD), which consists of tandem repeats of the heptapeptide sequence YSPTSPS (23). The CTD functions as a binding site for a number of other factors required during transcription and for correct processing of the primary transcript (24-27). The phosphorylation status of the CTD governs interactions with these factors and thereby helps to coordinate the different steps in mRNA formation, including transcription initiation, elongation, termination and post-transcriptional processing. The CTD heptapeptide repeats are modified at different sites. For instance, serine 2 and 5 phosphorylation (Ser2-P, Ser5-P) regulate transition from initiation to elongation, but also have further functions (27).

The other Pol II subunits are needed for structural and regulatory purposes. Rpb3 together with Rpb5 connects to a majority of the other subunits and constitutes the structural core of Pol II (28). During Pol II assembly Rpb3 together with Rpb11 forms a platform, which enables Rpb1 and Rpb2 to assemble (29). Rpb4 and Rpb7 differ from the other Pol II subunits, since they form a sub-complex not always associated with the core complex (30). Interestingly, deletion of the *RPB4* gene in yeast results in a viable but temperature sensitive phenotype. The Rpb4/Rpb7 dimer has mostly been linked to regulation of stress responses, but more recent findings connect it to processes such as DNA repair, mRNA export, mRNA decay, and translation (30). Rpb6 is found close to the ”clamp” and is believed to influence the positioning of this structure (31).

Four of the Pol II subunits, Rpb5, Rpb6, Rpb8, and Rpb10, can also be found in Pol I and Pol III, suggesting that these subunits are involved in mechanisms common to all three polymerases (31, 32).

1.2.2 Initiation

After the assembly of PIC has been completed the two strands of the promoter DNA needs to be separated in order for Pol II to access the template sequence. The ATPase/helicase activity of TFIIH melts the DNA close to the TSS (33). An initial transcription bubble is formed between base -9 and +2 relative to TSS (34). The bubble is stabilized by TFIIB and TFIIE (35).

Access to the single stranded template enables Pol II to begin transcribing a short stretch of RNA. The initial transcription bubble is unstable and after a short progression of Pol II, the upstream bubble collapses (36). At this point the polymerase has cleared the promoter and it no longer requires the helicase activity of TFIIH to progress. A part of TFIIB can enter the channel in which the nascent RNA exits and block the active site. Promoter escape, i.e. the transition to the elongation phase, can thus be regulated by TFIIB.

During promoter escape, Ser5 of the CTD is phosphorylated by TFIIH (37). Before Pol II can engage in subsequent rounds of transcription the CTD needs to be dephosphorylated (38). After the transcription of the first four nucleotides, conformational changes occur in Pol II that commit the polymerase for further elongation (39). During promoter escape TFIIB, TFIIF, and Pol II dissociate from the rest of the PIC (40). The other factors, TFIIA, TFIID, TFIIE, TFIIH, and Mediator, still remain at the promoter and help to facilitate re-initiation of additional rounds of transcription.

1.2.3 Elongation

Pol II activity is also regulated after it has cleared the promoter. Pol II often stalls about 50 nucleotides from the TSS (41). Pausing of Pol II is a rate-limiting event during transcription, which allows for more precise expression timing and regulation of transcript levels. For example, promoter-proximal stalling is observed at many genes involved in differentiation and genes that require signal stimulation (42). The molecular mechanisms for stalling of Pol II are still unclear. However there are indications that part of the capping machinery (see 1.6.2) is involved (43). In fact, at many mRNA genes the polymerase is paused at the capping checkpoint (44, 45). The checkpoint coincides with the 5′-end of the nascent RNA emerging from the Pol II exit channel. At this point a 7-methyl guanosine is added to the 5′-end of the RNA via a 5′-5′ triphosphate bridge (46).

Pausing is promoted by two factors, DSIF (DRB sensitivity inducing factor) and NELF (Negative elongation factor) (Figure 3) (47). Gdown1, a substochiometric subunit of Pol II, can further enhance NELF/DSIF induced stalling (48). Pol II remains paused until P-TEFb (Positive transcription elongation factor) phosphorylates several targets, including Ser2 of the CTD, as well as NELF and DSIF (49). Paused Pol II is then released and phosphorylation also causes DSIF to dissociate from NELF and instead accompany Pol II during elongation.

One major obstacle for Pol II to overcome during transcription is the nucleosome. As Pol II progresses through the gene it needs to disassemble

the nucleosomes in front of it to access the DNA template. Several elongation factors are required for progression through the nucleosome environment (45). ATP-dependent chromatin remodeler factors can modify the nucleosome-DNA interaction and thus promote elongation (50). For instance, the histone chaperone complex FACT facilitates Pol II access to DNA (15). Another example is the histone chaperone Spt6 that binds to phosphorylated Ser2 of the CTD and promotes elongation (51). To maintain the chromatin structure at the transcribed gene, nucleosomes need to be reassembled behind the elongating polymerase. As Pol II progresses through the gene, it continuously bends the template 90°. It has been proposed that this bend brings the upstream and downstream DNA into close proximity, which in turn, enables the polymerase to transfer the nucleosome to its original position (52).

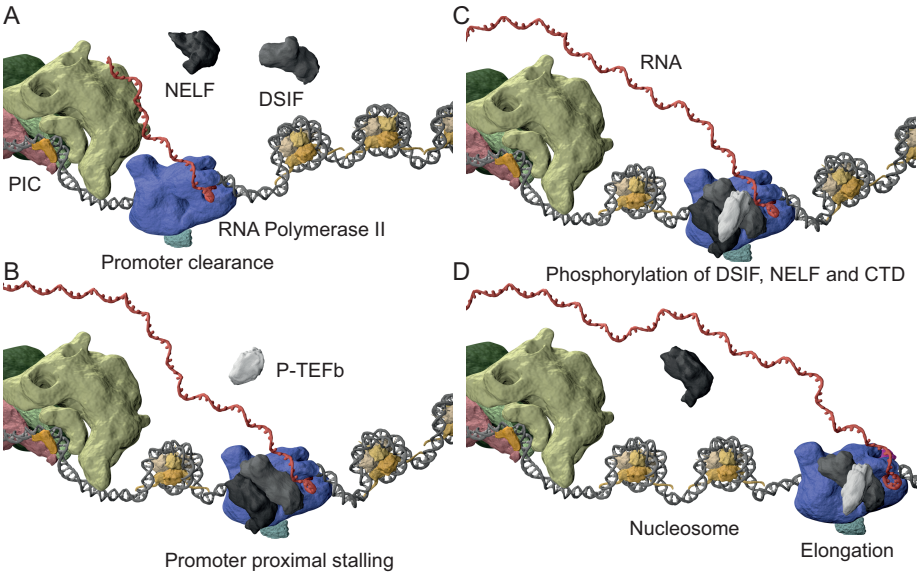

Figure 3. Promoter proximal stalling and transcriptional elongation

A) Pol II transcription is initiated. B) NELF and DSIF are recruited and Pol II stalls after synthesis of ~50 nt of RNA. C) P-TEFb phosphorylates NELF, DSIF, and CTD. D) Pol II escapes from stalling and continues elongation in complex with P-TEFb and DSIF, whereas NELF leave the template.

1.2.4 Termination

Termination is one of the least understood processes of Pol II transcription (13). Each transcribed gene needs a clear termination signal, indicating where the RNA polymerase should end transcription. For most mRNA genes the

termination signal is a stretch of A's at the 3′ end of a gene (13). The consensus sequence AAUAAA together with a G/U-rich downstream element on the nascent RNA constitutes the termination signal. Current theories suggest that Pol II pauses upon reaching the AAUAAA signal, which is recognized by the CPSF (cleavage and polyadenylation specific factor) subunit CPSF160 (Figure 4) (53). The cleavage-stimulating factor (CstF) is recruited to the G/U rich downstream domain, and binds to the CTD and CPSF, thus disrupting the interaction between CPSF and the Pol II body (54). The complex of CstF, CPSF and $CFII_m$ (Cleavage Factor II) instead associates with the CTD and allows the CPSF subunit CPSF73 to cleave the nascent transcript at a CA-sequence (53, 54). The termination process at histone genes differs from other protein-coding genes in that the nascent RNA at the end of the gene forms a stem loop that is recognized by the U7 small nuclear ribonucleoprotein (snRNP) (55). This enables CPSF73 mediated cleavage of the RNA and rapid release of Pol II from the histone gene. At non-histone mRNA genes, Pol II can instead progress several kilobases downstream of the cleavage site before dissociation from DNA (56).

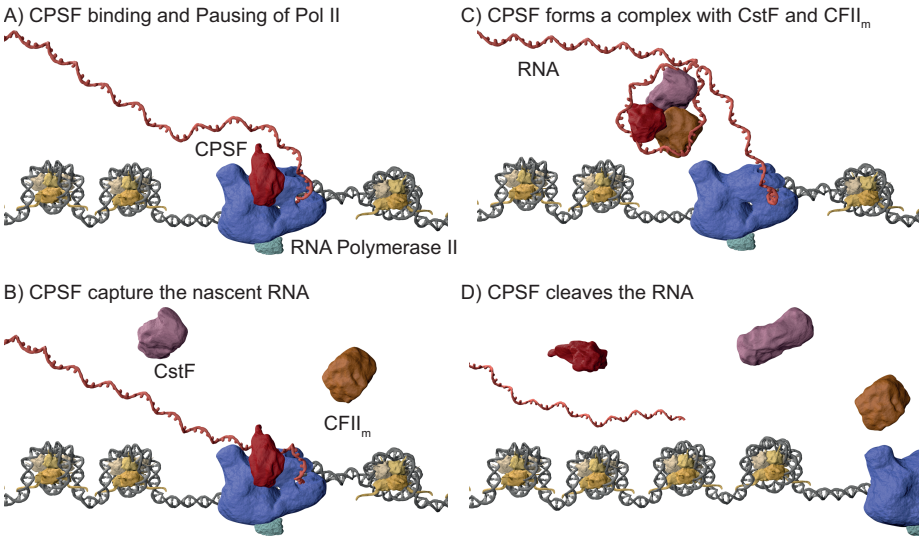

Figure 4. Transcriptional termination at Pol II dependent genes.
A) CPSF binds to elongating Pol II. B) CPSF captures the transcript by binding to the AAUAAA signal on the nascent RNA. C) CstF is recruited to downstream G/U rich element. CstF also interacts with CPSF and disrupts CPSF's interaction with the Pol II body. Instead CPSF, CstF and CFIIm form a complex that interacts with CTD. D) CPSF cleaves the nascent RNA and Pol II continues to transcribe for a short distance before it falls off the template DNA.

Exactly how Pol II is released from the DNA template is still not clear, but there are observations that clarify parts of the process. First, the Pol II elongation complex slows down or pauses at the end of the gene (57). This enables the exchange of Pol II associated elongation factors for transcription termination factors (58). Another release mechanism involves destabilization of the RNA-DNA hybrid, thus facilitating dissociation of Pol II from the template. In *E. Coli,* the termination factor Rho invades the main channel and either uses its helicase activity or sterically interferes with the polymerase to cause melting of the RNA/DNA hybrid (59). Although there are no known Rho homologues in eukaryotes, the 5′-3′ exonuclease, Rat1 and the Sen1 (essential super family I helicase) proteins in *Saccharomyces cerevisiae*, act as termination factors and have been suggested to work through a Rho-like mechanism (60). These two factors have been shown to cooperate and induce transcriptional termination (61).

Termination of transcription at non-coding genes differs from that of protein-coding genes. In *S. cerevisiae* for instance, the termination sequence at snoRNA genes includes the two motifs UCUU and GUA(A/G) (62). Two proteins bind to these motifs, Nab3p (nuclear poly(A) RNA binding 3) and Nrd1 (nuclear pre-mRNA down regulation 1) respectively. Nrd1, Nab3, and Sen1 form a complex, which can bind to Ser5-P and Ser2-P of the CTD (63). Sen1 is believed to utilize its ATP-dependent helicase activity to disrupt the elongation complex and thus induce termination (64). The Nrd1/Nab3/Sen1 complex can physically interact with the TRAMP-exosome complex (see 1.6.1) to enable further 3′-processing.

1.3 The Mediator Complex

The Mediator complex is a multiprotein complex involved in a wide variety of transcriptional processes. Its main function is to transfer regulatory signals from activators and repressors to the Pol II machinery. Originally, it was discovered as an activity that mediates activator dependent regulation of Pol II in *S. cerevisiae* (65, 66). Before the discovery of Mediator it was believed that activators and repressors primarily functioned via direct interactions with the Pol II machinery (67, 68). However, in a reconstituted Pol II *in vitro* transcription system with partially purified proteins, these activators were not able to stimulate transcription unless a fraction containing a so-called mediator of activation was added (65). In parallel, four subunits (Srb2, Srb4, Srb5, Srb6) of a larger complex were discovered to suppress Pol II CTD activity and were thus named the suppressor of RNA polymerase B (Srb) (69). It was later discovered that they in fact were subunits of the Mediator complex (70).

Mediator was subsequently purified to homogeneity and demonstrated to be part of a holoenzyme, made up of the core polymerase and the Mediator complex (70). Many of the Mediator subunits are encoded by genes that had previously been identified in genetics screens as factors involved in activation and repression of transcription (71). The connection to these previous genetic studies demonstrated the relevance of Mediator *in vivo*. Later, structural analysis showed that there were several contact points through which Mediator could influence the activity of Pol II (72-76).

1.3.1 Structure

The Mediator complex is conserved from yeast to humans (77). The number of subunits differs between species, from about 20 to 30. Mediator is perhaps best characterized in *S. cerevisiae* where it contains 25 subunits (78). The budding yeast Mediator is subdivided into four submodules termed the tail, middle, head, and CDK8 module (Figure 5). The structure of Mediator has been extensively studied, but its size, flexibility and heterogeneity have made it difficult to obtain a high-resolution structure of the entire complex. There is, however, low-resolution electron microscopy (EM) 3D structure data available for the entire Mediator complex (75, 79). In addition, several subunits have been studied by X-ray crystallography, either alone or in subcomplexes with other Mediator components (80-83). The Pol II interacting head module is the best characterized while the structure of the tail module is still poorly understood (78). Many of the subcomplexes are functional units that are connected to Mediator through flexible linkers (84, 85).

Tail module

Many activators and repressors interact with Mediator via the tail module. The proteins comprising this module in *S. cerevisiae* are Med2, Med3, Med5, Med14, Med15, and Med16 (86). The genes encoding these proteins are non-essential, but when they are deleted in combination lead to a lethal phenotype. The Med16 protein functions as the bridge between the tail module and the Mediator middle module. Interestingly, in cells lacking Med16, the tail module can assemble and exert its function as a separate entity and for instance be recruited by the Gcn4 activator protein to promoters (87). Interestingly, even if loss of Med16 prevents recruitment of the middle and head modules of Mediator, the presence of the isolated tail module is enough to attract TBP and Pol II, and to stimulate transcription of the *ARG1* gene. In fact, recruitment of the tail module on its own resulted in higher activation of *ARG1* gene transcription than recruitment of an intact Mediator.

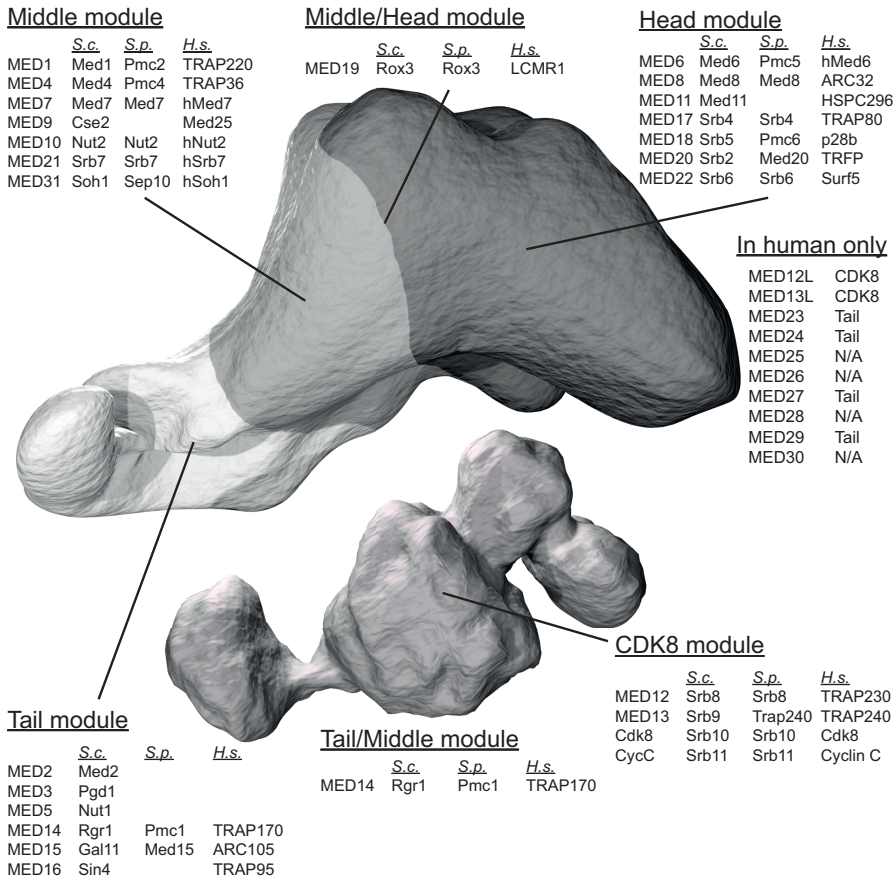

Figure 5. The Mediator modules
Schematic representation of Mediator, showing the different modules with the corresponding subunits in *S. cerevisiae*, *S. pombe,* and human cells. N/A indicates subunits with unassigned module affiliation.

The tail module subunits contain several activator-binding domains (ABDs), which interact with the transactivation domains (TAD) of activators (88-91). ABDs are found in many different Mediator subunits and individual subunits may contain more than one ABD. Although each individual ABD-TAD interaction can be weak, many cooperative-binding surfaces stabilize the Mediator-activator interaction (82, 89). For instance, the activator Gcn4p interacts with Med2, Med3, Med15, and Med16 (87, 92). The involvement of multiple ABDs creates very diverse and complex interaction surface between Mediator and activators. Even if the structures of the different ABDs are diverse, the same TAD can bind to more than one ABD. Studies have

demonstrated that in their free states, the TADs may be unstructured. However, when a TAD comes into contact with an ABD it adjusts its structure to fit the interacting domain (89). The flexibility of TAD structures allows for different interactions with Mediator. The absence of a single TAD-ABD conformation has led others to term the interaction between the activator Gal4 and Mediator subunits for a 'fuzzy' complex, to reflect the flexibility of the TAD-ABD interaction (82).

The binding of an activator to a Mediator subunit results in conformational changes in the entire complex. The conformational changes differ depending on the type of activator-Mediator interaction (93, 94). Mediator can thus assume a conformational state specific for the activator and the gene it regulates, which may contribute to Mediator's ability to regulate a very diverse set of genes.

Middle module

In *S. cerevisiae*, the middle module contains four essential subunits (Med4, Med7, Med10 and Med21) and three non-essential subunits (Med1, Med9 and Med31) (95). Due to the flexible nature of the middle module it has been difficult to obtain a detailed 3D-structure. However, structures of several subcomplexes have been reported (81, 84, 95). The C-terminal part of Med7 interacts with Med21 and forms a flexible hinge (81). Modeling of the Med4/Med9 heterodimer led to the conclusion that this complex may harbor a similar hinge and together with the Med7/Med21 dimer form the backbone of the middle module (95). The Med7/Med21 and Med4/Med9 hinges could play an important role in the transfer of activator induced conformational changes to the rest of the Mediator complex (81).

The non-essential Med1 subunit has been implicated in regulation of the CDK8 module (96). This module contains a cyclin dependent kinase-cyclin pair (Cdk8 - CycC), which interacts with Med1 and Med4 (97, 98). In humans MED1 interacts with a number of activators, including nuclear hormone receptors, and assists in the recruitment of Mediator to promoters under their control (99, 100). Med19 has been placed at the boundary between the middle and head module (101). Interestingly, loss of Med19 results in loss of the middle module and leads to the formation of a stable Mediator complex containing only the tail and head module. Loss of the middle module does however remove Mediator's ability to regulate transcription through activator-Mediator-Pol II interaction.

Head module

The head module harbors the conserved subunits Med6, Med8, Med11, Med17, Med18, Med20, and Med22. The structure of this module has been

carefully studied with X-ray crystallography (102, 103). A comparison between the *Schizosaccharomcyes pombe* and *S. cerevisiae* head modules demonstrated high structural conservation despite only 15 % sequence conservation (103).

Med17 is the largest of the head module subunits (103). It spans a sizeable portion of the module and interacts with the majority of the subunits. The non-essential subunits Med18 and Med20 form a mobile subcomplex with Med20 being the most peripheral subunit. In fission yeast, loss of Med18 results in dissociation of Med20 from the rest of the complex, while deletion of the *med20*$^{+}$ gene does not have an impact on Med18-Mediator interaction (104). Med8p tethers the Med18p/Med20p complex to the head module (103). The head module has been described as a jaw-like structure, with mainly Med17 constituting a fixed upper jaw and Med18/Med20 functioning as a flexible lower jaw (103).

TBP appears to interact with several subunits of the head module (105). The jaw of the head module is closed when Mediator is not associated with TBP. After TBP contacts the head module the jaws opens, allowing for interaction with Pol II. The Pol II subcomplex Rpb4/Rpb7 binds between the two jaws in the open conformation and can interact with Med18 and Med20. The head module also connects to Pol II via a direct physical interaction between Rpb3 and Med17 (76).

The structure of the head module has been determined in complex with the CTD, which primarily interacts with Med6, Med8, and Med17 (102). The Mediator head module specifically associates with dephosphorylated CTD. During transition from transcriptional initiation into elongation the CTD is phosphorylated at Ser2 and Ser5 (37, 49). Phosphorylation of these residues significantly reduces the affinity of CTD for the head module (102). This can explain how Pol II can separate from the Mediator complex when Pol II transitions into the elongation phase. The head module not only binds to the polymerase but can also interact with the general transcription factors TFIIB, TFIIH and TBP, further demonstrating the central role of this Mediator region (80, 97, 106).

CDK8 module

The CDK8 module differs from the head, middle and tail module in that it is only intermittently associated with the core complex. The module is composed of Med12, Med13, CycC, and Cdk8 (107). The CDK8 module has been described as a repressor of Mediator-Pol II interaction. The actual mechanism of how this occurs is still under debate and could even differ between species. Studies of human Mediator suggest that the binding of the

CDK8 module to core Mediator results in conformational changes that disrupt Mediator-Pol II interactions (108). In contrast, the CDK8 module in *S. pombe* competes with Pol II for access to the core Mediator (109). Med12 and Med13 are two of the biggest subunits of the Mediator complex. They are the basis for the assembly and stability of the CDK8 module (73, 107). Med13 contains a 'hook' domain that connects to a 'hook' structure of the Mediator tail module (73). Med12 constitutes the central bulk of the module and connects with both Med13 and CycC. In turn, CycC bridges between Med12 and Cdk8. The Med13 domain that binds to Med12 is flexible and provides freedom of movement for the rest of the CDK8 module (107).

Cdk8 may also block transcription via alternative mechanisms. A recent report demonstrated that the CDK8 module could regulate the stability of the Mediator tail module by phosphorylating Med3 (110). The phosphorylation in turn triggers ubiquitylation and subsequent degradation of the Med3 protein. This mechanism would allow Cdk8 to repress transcription by simply removing subunits interacting with activators, from the Mediator complex.

Even though the CDK8 module has primarily been associated with repression of transcription, the module also plays a role in gene activation. CDK8 module binding to the Mediator tail module results in an open conformation of Mediator and induction of holoenzyme formation (107). The flexibility of the Mediator-CDK8 module interaction may allow the CDK8 module to associate with the back of the Mediator complex (73). Even in this case the dominant connecting subunit is Med13, which tethers the CDK8 module to the tail while Cdk8 interacts with the back of the middle module. It appears that Cdk8 association with the middle module can block a binding site for the CTD and thereby interfere with Pol II recruitment.

1.3.2 Mediator and transcriptional initiation

Mediator has been shown to regulate most Pol II transcribed genes in *S. cerevisiae*. It can function in both an activating and a repressing capacity (Figure 6) (19, 111). The role of Mediator has been the target of extensive research, but the complexity of transcriptional initiation leaves much more to be discovered. According to the general model, activators bind to enhancer regions and then recruit Mediator (112, 113). In the next step, the complex helps to recruit and assemble the GTFs and Pol II at the promoter, leading to initiation of transcription (19). Evidence that Mediator is recruited before the GTFs has been obtained in *Drosophila melanogaster*. Upon heat shock, the activator HSF and Mediator are recruited without the GTFs or Pol II (114).

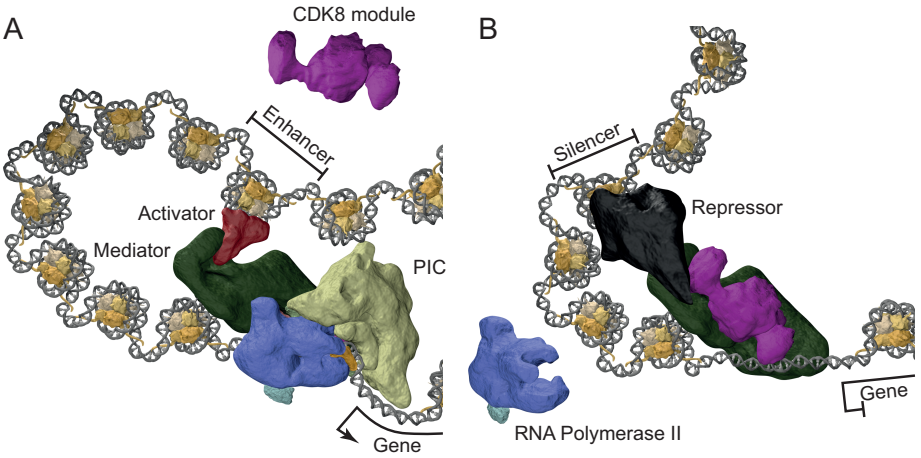

Figure 6. Mediator transduces signals from activators and repressors to the basal Pol II transcription machinery at the promoter.
A) Mediator is recruited by activators bound at enhancer elements and stimulates assembly of the Pol II transcription machinery. B) Repressors bind to silencer elements and inhibit assembly of the transcription machinery. This effect can for instance be due to a stabilization of CDK8 module interactions with the rest of Mediator.

In *S. cerevisiae*, Mediator is recruited to the *HO* promoter at the end of mitosis with the help of transcription factor SBF (115). Pol II and GTF however are not recruited until late in G1-phase.

Mediator interaction with general transcription factors

Mediator helps to stabilize the GTFs at the promoter and physically interacts with e.g. TBP, TFIIE and TFIIH, which stimulates transcription initiation and facilitates reinitiation of transcription (80, 116-118). Mediator stimulates TFIIB recruitment to promoters *in vitro* and higher TFIIB concentrations are required for transcription initiation in the absence of Mediator (119). Mediator also helps to regulate the enzymatic activity of the GTFs. In support of this notion, the Mediator subunit Med11 is important for the recruitment of TFIIH (116). Depending on the specific *MED11* mutation, the outcome may be very different. While some mutations inhibited TFIIH recruitment, others resulted in normal recruitment of TFIIH, but reduced Pol II recruitment. Yet another *MED11* mutation impaired the function of a TFIIH submodule TFIIK, which is responsible for phosphorylation of CTD. In conclusion, Mediator does not only affect the recruitment of TFIIH, but it also regulates its enzymatic activity.

Mediator as an activator

Many activators interact with the Mediator tail module and in *S. cerevisiae* the Med2, Med3, and Med15 proteins play a central role for these

interactions (120). Loss of these subunits cripples the direct interaction between Mediator and the activator proteins. Further evidence for tail module dependent recruitment comes from studies of a *med16Δ* mutant strain. As noted above (see 1.3.1), loss of this subunit leads to the formation of a free tail module, which can be recruited by activators as a separate entity (87). *MED15* is an important point of interaction for activators in both human and *Caenorhabditis elegans* (121, 122). In metazoan cells, MED29 and MED27 replace Med2 and Med3, but the function of these proteins for activator interactions is still unclear (77).

Activators that function via the tail module often regulate inducible genes, whereas constitutively active genes often are tail module independent (123, 124). While the tail module dependent genes often have a TATA-box element, the independent genes instead require TFIID (124). This is however not a general rule, since there are many examples of activators that interact with the head or middle module that regulate inducible genes. In metazoans, for instance, MED1 of the middle module is required for activation of nuclear hormone (NR) receptor genes (125, 126). MED1 contains an NR recognition motif (LxxLL) that interacts with the AF2 domain of the NR, which in turn leads to recruitment of the entire Mediator complex. (127).

Most studies link the CDK8 module to transcriptional repression and transcriptional inhibition of activator dependent genes (128). In mammals, CDK8 associates with inactive transcription complexes and disengages once a gene is activated (129). As another example, in *S. cerevisiae* Cdk8 dependent phosphorylation of Med2 causes repression at specific genes (128). There are however many examples of the opposite. In yeast, the CDK8 module is needed for Mediator dependent activation of the *GAL1* gene (130). At the *GAL1* promoter CDK8 module helps to facilitate TBP association. Furthermore, CDK8 in human cells is essential for activation of thyroid hormone-dependent transcription and for recruitment of Pol II to the promoters of the regulated genes (131).

1.3.3 Mediator and transcriptional elongation

Mediator may interact not only with the promoter region, but also with the gene body (132). The role of Mediator at these positions is not fully understood. As noted above (see 1.2.3), Pol II is paused at a promoter proximal position in *D. melanogaster* heat shock genes (114). Upon heat chock, Mediator is recruited to these genes, leading to Pol II release. A role for Mediator in Pol II release is further supported by studies in mural embryonic stem (ES) cells, were deletion of *Med23* abolished Mediator recruitment to the *Egr1* gene and prevented Pol II release from its stalled

position (133). Mediator may in part function via the NELF/DSIF enhancing protein, Gdown1. This protein functionally interacts with Mediator, which can alleviate Gdown1 induced Pol II inhibition *in vitro* (48, 134, 135).

Yet another link to transcription elongation was provided by the observation that Mediator helps to recruit the super elongation complex (SEC) (136). SEC contains a number of Pol II transcription elongation factors, including P-TEFb, a cyclin dependent kinase that can phosphorylate DSIF, NELF and CTD, and thereby stimulate the transition from promoter proximal pausing to productive Pol II transcription (see 1.2.3) (49). Components of SEC can copurify with MED26 due to the interaction between the MED26 N-terminal domain (NTD) and the SEC component EAF (136). Interestingly, domains that are similar to MED26-NTD are found in other elongation factors, including TFIIS, Elongin A and IWS1 (137). Apart from interacting with SEC, MED26 also interacts with TFIID suggesting that this Mediator subunit is important for the transition from transcription initiation to elongation (136).

Another Mediator subunit that has been linked to transcription elongation is mammalian CDK8. Interestingly, CDK8 and MED26 are not present in the Mediator complex simultaneously (138). These two mutually exclusive factors appear to affect transcription elongation in distinct ways. CDK8 interacts with P-TEFb and is important for P-TEFb recruitment to genes with paused Pol II (139). Knockdown of CDK8 did not however affect the release of paused Pol II. This would implicate CDK8 involvement in the effective localization of P-TEFb to genes with paused Pol II, but not P-TEFb dependent Pol II release. Other examples of genes where CDK8 has been suggested to regulate transcription elongation are the thyroid receptor induced genes and hypoxia response genes (131, 140).

Mediator can, apart from recruiting elongation factors, influence elongation by affecting chromatin structure. One obstacle for Pol II during transition from initiation to elongation is the +1 nucleosome. Mediator has been shown to collaborate with TFIIS to induce changes in the catalytic properties of Pol II, in order to facilitate polymerase clearance of the +1 nucleosome (141).

1.3.4 Mediator and transcriptional termination

Recent evidence has implicated Mediator in regulation of transcription termination (142). Mediator has been found at the 3′-end of genes (132, 143). In *S. cerevisiae,* Mediator helps to recruit the termination factors CF1 (cleavage factor 1) and CPF (cleavage and polyadenylation factor) to the 3′-end of the *INO1* and *CHA1* genes (142, 144). Loss of the Mediator head

module subunit, Med18, results in loss of CF1 and CPF from the termination site. As a consequence transcription termination fails and Pol II reads through the termination site.

In order to maintain high frequency transcription of certain genes, there is a need for efficient re-initiation. To facilitate re-initiation, the promoter and termination sites can connect through the formation of a chromatin loop (144). At the *INO1* and *CHA1* genes, TFIIB, promotes gene looping and juxtapositioning of the promoter and termination sites (144). Mutations in TFIIB cause disassociation of the promoter and termination sites, and abolish Mediator localization to the end of the transcribed region. Similarly if *MED18* is deleted the connection between promoter and termination regions is lost. TFIIB mutations and loss of Med18 both cause a depletion of CF1 and CPF at the 3′-end of genes. These observations suggest that the head module of Mediator is crucial for the communication between the promoter and termination sites. Since the head module also interacts with Pol II, it is possible that Mediator dependent association between the promoter and termination sites helps to reinitiate Pol II after transcriptional termination (145). This mechanism would ensure fast and reliable re-initiation of transcription, allowing Pol II to transcribe the same gene multiple times.

1.3.5 Mediator and Chromatin

The composition and organization of chromatin is of utmost importance for transcriptional activity. Mediator has been shown to influence a large number of chromatin related processes, including the composition and modification of single nucleosomes as well as the 3D organization and positioning of chromosomes. Mediator occupancy also correlates with specific histone modifications and the complex can interact directly with nucleosomes (146, 147).

Chromatin organization

In the nucleus, the 3D-organization of genomic DNA is very important for the control of gene expression. Mediator has been found to influence many levels of this organization (144, 148, 149). As previously mentioned, Mediator can help to juxtapose the promoter and transcription termination site of a gene (see 1.3.4)(144). This is accomplished by the formation of a gene loop that brings two distant regions into close proximity. In order for Mediator to transduce the signal of an activator to the Pol II machinery it needs to close the gap between these factors. Enhancer regions that activators bind to can be at a considerable distance from the promoter of the regulated gene (150). By forming a gene loop, Mediator bridges the vast distance between the enhancer and the promoter. For example, MED1 is required for

thyroid hormone receptor dependent juxtapositioning of enhancer and promoter elements at the *Crabp1* gene (99). To stabilize gene loops, Mediator can be assisted by cohesin (151, 152). This four-subunit complex forms a ring around two DNA strands and holds them in place (153). An important function of cohesin is to hold the two sister chromatids together during metaphase. Mediator can bind to both cohesin and the cohesin-loading factor Nipbl (152). In mouse ES cells, Mediator and cohesion co-localize to both the promoter and the enhancer of the *Nanog* gene, and the resulting gene loop is dependent on both MED12 and the cohesin subunit SMC1a.

Interestingly, Mediator seems to not only promote enhancer/promoter proximity but the complex also affects higher levels of chromatin organization. Mediator together with cohesin is required for the formation of dynamic, cell-type specific chromatin loops shorter than 100 kb (149). Independently of cohesin, Mediator also influences the formation of very short chromatin loops (600 – 1000 bp). Given the close connection between Mediator and cohesin, it is tempting to speculate that Mediator could utilize enhancer regions from a sister chromosome, or even an entirely different chromosome, to influence genes expression (145).

An interesting new mechanism by which Mediator may regulate gene looping and gene activation has recently emerged. Long non-coding RNA species, called non-coding RNA-activating (ncRNA-a), associate with the CDK8 module (154). The other end of the ncRNA-a can associate with an enhancer region several megabases from the regulated gene using sequence complementarity. The combination of ncRNA-a and Mediator can thus bring regulatory regions into proximity of a target gene, similar to the situation with transcriptional activators and gene looping.

Chromatin remodeling

Apart from being involved in recruitment of chromatin remodeling factors, Mediator can also directly modify histones. CDK8 can phosphorylate histone 3 serine 10 (H3S10), which in turn stimulates the histone acetyltransferase GCN5L to acetylate lysine 14 (H3K14) on the same histone (155). Together, GCN5L and CDK8 interact with the transformation/transcription domain associated protein (TRRAP). This scaffold protein is known to cooperate with several chromatin-modifying complexes (156). CDK8 may promote transcriptional activity at genes by creating a permissive chromatin environment. In support of this notion, ncRNA-a binds to CDK8 and helps it to localize to a specific gene, which in turn leads to H3S10 phosphorylation and transcriptional activation of the gene (154).

Chromatin structure is not only important for gene activation, but also for suppression of gene transcription. For instance, MED12 has been found to suppress neuron specific genes in non-neuron cells (157). The protein connects the histone methyltransferase G9a with the RE1 silencing transcription factor (REST). G9a can then silence REST targeted genes through dimethylation of histone 3 lysine 9 (H3K9me2). Mutations in *MED12* that inhibit binding of ncRNA-a to the Mediator complex also cause disassociation between Mediator and REST (154, 157).

In *S. pombe*, the CDK8 module containing form of Mediator can associate with the chromatin-remodeling factor Hrp1 (homologue of Chd1 in *S. Cerevisiae*) (20622008). Together, Mediator and Hrp1 seem to have a positive impact on transcription of a subset of genes. In mammals, Mediator is also needed for CHD1 recruitment to genes (158). Here, CHD1 binds to trimethylated histone 3 lysine 4 (H3K4me3), a marker known to associate with active genes. Both Mediator and CHD1 can promote the formation of a PIC.

Recent findings have demonstrated that Mediator also is crucial for controlling heterochromatin formation at different regions. As discussed in Paper I of this thesis, as well as the findings from other groups, Mediator is needed for transcriptional silencing of the telomere (159). The tail module of Mediator helps to control the boundary of silent chromatin at *S. cerevisiae* telomeres (160). The boundary is decided by the competitive interplay between the histone acetyltransferase Sas2 and the histone deacetylase Sir2, which both regulate the acetylation status of histone 4 lysine 16 (H4K16). Mediator binds to this region and influences Sir2 activity. In fact, deacetylation of H4K16 is essential for Mediator binding to the nucleosome (161). Mutations in *MED5* of the tail module allow Sas2 to dominate and increase the spread of H4K16Ac with results in desilencing of the region (160). In support of these findings, genetic studies have shown that mutations in Mediator subunits can cause shortening of telomeres in *S. cerevisiae* (162).

Also discussed in this thesis (Paper II) is the finding that Mediator helps regulate chromatin structure of the centromere (163-165). The transcriptionally silent heterochromatin of the pericentromeric region is needed for proper chromosome segregation during mitosis. Disruption of the *S. pombe* Mediator head module subunits Med18 and Med20 causes loss of heterochromatin and desilencing of the region. The levels of H3K9me, which is a characteristic feature of heterochromatin, drop significantly in these mutants. In addition, the centromere specific H3 variant CENP-A is lost from the core centromere when *med20*$^{+}$ is deleted (164).

1.3.6 Mediator and human disease

Mediator is involved in almost all Pol II dependent transcription in humans and it is therefore not surprising that mutations affecting this complex can cause a multitude of diseases. Among known diseases caused by impaired Mediator function are cardiovascular disease, cancer, as well as neurodevelopmental and behavioral disorders (166).

Cardiovascular diseases

MED13L, a paralogue of *MED13,* is highly expressed in heart and brain (167). Mutations in *MED13L* cause transposition of the great vessels (TGV) as well as congenital heart defects (167-169). Mutations in *MED1* and *MED30* cause lethal cardiomyopathy and mitochondrial defects (170, 171).

Neurodevelopment disorders

The hereditary motor and sensory neuropathy, Charcot-Marie-Tooth disease, has been associated with a point mutation in *MED25* (172). The pathological pathway is still unknown, but it has been suggested that mutated *MED25* gene causes molecular lesions in Schwann cells that form the myelin sheaths surrounding the neuronal axons.

Postnatal-onset microcephaly (POM) is a disorder characterized by decelerated head growth after birth, leading to a low cranial circumference, mental retardation, and seizures (173). Mutation affecting the essential Mediator subunit MED17 has been identified as the cause for this disease in a subset of patients (174). The corresponding point mutation in *S. cerevisiae* disrupts a crucial domain of the protein.

MED12 has been connected to X-linked mental retardation (XLMR), or more specifically the FG and Lujan syndromes (154, 175). The MED12 protein helps to regulate Wnt, Notch and Sonic Hedgehog signaling, all of which are important in brain development pathways (176-178). MED12 is also important for epigenetic repression of neuronal gene expression in non-neural cells (157). In wild type cells, Mediator contribute to the establishment of repressive H3K9 dimethylation at specific genes and disease causing mutations in *MED12* impair this function. Desilencing of these genes in turn causes pathological phenotypes.

Behavioral disorders

Schizophrenia is a chronic and severe mental disorder, characterized by delusions, hallucinations and paranoia. The disorder has hereditary tendencies, but the genetic background is complex, with influences from several different genes (179). A 12 bp insertion and a 15 bp deletion in the

MED12 gene have been identified as disease inducing mutations (180). These mutations are distinct from those responsible for the FG and Lujan syndromes (175, 181). The exact mechanism for MED12 mediated development of Schizophrenia is not known. However, there are data demonstrating that MED12 is crucial during development of dopaminergic neurons and deregulated dopamine signaling is a known risk factor in the development of schizophrenia (182, 183).

Cancer

Many studies have connected mutations in Mediator subunits to the development of cancer (184). The first reported example was the role of *MED1* in breast cancer (185). The estrogen-receptor depends on MED1 to activate specific genes and mutations in *MED1* cause a reduced response to estrogen (186). Estrogen is a risk factor for development of breast cancer and *MED1* is overexpressed in many tumors (187, 188). MED1 levels strongly correlate with the amount of human epidermal growth factor receptor 2 (HER2) (189). Increased *HER2* expression is an important step in the development of breast cancer and it causes resistance to hormonal treatment. MED1 has also been implicated in melanoma, prostate cancer, and lung cancer (190-192).

MED28 has also been connected to the development of breast cancer, but the mechanism is distinct from that of MED1 (193). High expression of *MED28* causes increased proliferation, a common property of cancer. MED28 levels are elevated in several different tumor types and can be used as a prognostic marker, with higher levels indicating poor prognosis (193, 194).

Overexpression of *CDK8* is common in several forms of colon cancers (195). CDK8 phosphorylates the transcription factor β–catenin, which in turn triggers increased expression of genes vital for colon cancer proliferation. In melanomas, CDK8 levels are also elevated. Expression of *CDK8* in normal cells is repressed by the histone H2A isoform macroH2A (mH2A) (196). The levels of mH2A are reduced in malignant cells and as a consequence the CDK8 levels rise. Interestingly, *MED1* expression is decreased in highly malignant melanoma cancers (192). This is an inverse situation compared to the high levels of *MED1* expression observed in breast cancer. These contrasting observations demonstrate how complex and diverse the role of Mediator may be in different types of cancers.

1.4 RNA Polymerase III

While Pol II transcribes all the protein coding genes in the cell, Pol III transcribes many housekeeping non-coding RNAs. For instance, tRNA and

5S rRNA are transcribed from Pol III dependent genes (197). Pol III also transcribes snRNA (required for splicing) and snoRNA (guide RNA-modifications) genes. Pol III is structurally and functionally distinct from Pol II, using different means for regulation of initiation and termination.

1.4.1 Structure

The core structure of Pol I, Pol II and Pol III is very similar. In yeast, twelve subunits of the different polymerases are homologues or identical to each other (198). Pol III also contains five additional subunits not found in the other polymerases. The pre-initiation complex of Pol III consists of the three factors: TFIIIA, TFIIIB, and TFIIIC (199). TFIIIB includes the TATA-binding protein TBP and binds to upstream elements of Pol III promoters. TFIIIA and TFIIIC instead bind to intragenic promoter elements.

The five distinct subunits of Pol III form two subcomplexes, Rpc31/Rpc34/Rpc82 and Rpc53/Rpc37, which are situated on either side of the Pol III 'cleft' (see 1.2.1) (200). The subcomplex Rpc31/Rpc34/Rpc82, which binds near the 'clamp' of Pol III, is needed for promoter-dependent initiation and interactions with TFIIIB (201). The subcomplex also interacts with the Rpc17/Rpc25 subcomplex (homologues to Rpb4/Rpb7 in Pol II) that increases interactions with TFIIIB during Pol III initiation (202).

The Rpc53/Rpc37 subcomplex associates with Rpc11 and is involved in termination and reinitiation of Pol III transcription (203). This subcomplex slows down the polymerase when it approaches the end of the gene to facilitate termination of transcription. The presence of Rpc11 in the subcomplex facilitates reinitiation of Pol III allowing for several rounds of transcription. Rpc53/Rpc37 mimics the role of TFIIF in Pol II as a stabilizer of the initiation complex (204).

1.4.2 Gene structure and Pol III initiation

The promoter elements of Pol III dependent genes differ from Pol II dependent genes. In fact, Pol III transcription factors primarily associate with promoter elements inside the gene body (197). There are four internal promoter elements: the A-, B-, C-boxes, and the intermediate element (IE). Based on primary promoter element compositions, three main types of promoters may be distinguished. Type 1 is used for expression of 5S rRNA and utilizes the A-box, C-box, and the IE. TFIIIA binds to the IE and C-box element and in turn recruits TFIIIC to the A-box resulting in subsequent recruitment of Pol III. Type 2 promoters that include the A-box and B-box regulate tRNA genes. In this case only TFIIIC is required to bind the promoter elements. Interestingly, flanking the mating type region in *S. pombe*

are inverted repeat (IR) boundary elements, which contain multiple B-boxes (205). It has been shown that TFIIIC binds to these B-boxes and inhibits heterochromatin spread. In addition, TFIIIC has been implicated in organization of the genome by tethering these IR boundary elements to the nuclear periphery.

In higher eukaryotes there is a third type of promoter that lacks both the A-box and B-box. Instead, the promoter consists of a TATA-box and a proximal sequence element (PSE) upstream of the TSS (206). SNAPc (snRNA activating protein complex) recognizes the PSE and interacts with the TBP subunit of TFIIIB that recognizes the TATA-box, which in turn leads to Pol III recruitment (207).

There is an increasing number of genes that are attributed to Pol III transcription (208). For instance, snoRNAs have previously been described as Pol II dependent. However in *S. cerevisiae*, transcription of the *SNR52* gene (snoRNA 52) has been shown to be Pol III dependent (209). The *SNR52* gene utilizes a type 2-like promoter upstream of the mature snoRNA52 sequence (210). snoRNAs guide chemical modifications of other RNAs such

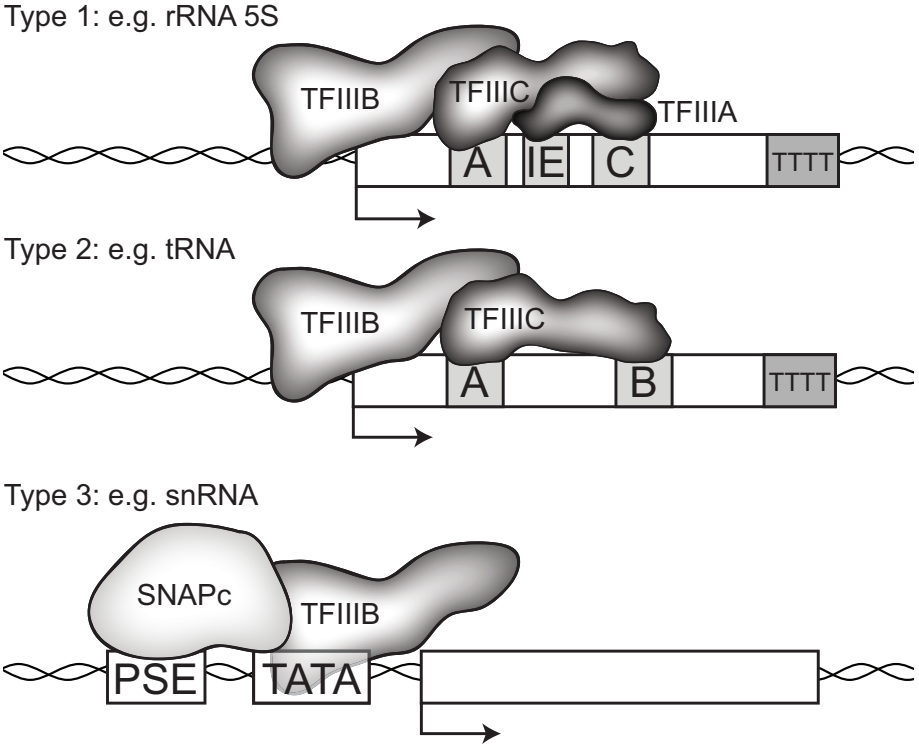

Figure 7. The three main promoter types of Pol III genes.
The different sequence elements are discussed in the text.

as rRNA, tRNA, and snRNA (211). snoRNA have been identified as Pol III dependent also in other species such as *Arabidopsis thaliana, C. elegans and D. melanogaster* (212-214).

1.4.3 Termination

Termination of Pol III transcription is directed by a short poly(T) stretch on the non-template strand (197). Longer thymine stretches function as a stronger termination signal than shorter ones. However, some genes like *SNR52* in *S. cerevisiae* include a stretch of 6 thymines and avoid premature termination by a termination-weakening signal surrounding this poly(T) sequence (215). The actual mechanism for Pol III termination is not yet known. However, transcription termination is directed by the newly synthesized transcript (216) and the proximity of Rpc53/Rpc37 to the exiting nascent RNA suggests that this subcomplex is involved in recognition of the poly(T) signal (poly(U) in RNA) (217). High elongation rates inhibit termination and Rpc53/Rpc37 has been suggested to function as a brake for the polymerase (218). Disruption of these subunits results in defective termination and read-through transcription. The poly(T) stretch causes pausing and backtracking of the polymerase. Pol III backtracks a short distance until it encounters a secondary structure, e.g. a hairpin, on the nascent RNA, which stimulates Pol III release. During this process, Rpc11 mediates Pol III dependent 3′-cleavage of the nascent RNA (203). Rpc11 includes an Rpb9-like domain that prevents read-through past the termination site, and a TFIIS-like domain needed for RNA 3′-cleavage (219). As mentioned above (see 1.4.1), the Rpc53/Rpc37/Rpc11 complex couples termination to reinitiation, enabling Pol III to rapidly switch from termination to a new round of transcription.

1.5 Post-transcriptional Processing

After a gene has been transcribed the resulting RNA needs further processing to become a mature transcript. Post-transcriptional modifications are diverse and include processes such as addition of a poly(A)-tail, splicing, and modification of specific nucleosides.

1.5.1 Polyadenylation

After termination, a stretch of adenines is added to the 3′-end of most mRNAs. The poly(A)-tail regulates the degradation rate of the transcript and assists in the export of mRNA from the nucleus (220). The process of polyadenylation is tightly coupled to transcription termination (221). In eukaryotes, adenines are added by the Poly(A) polymerase (PAP), which is

tethered to the nascent RNA by CPSF during the transcription termination event (222). PAP is further anchored to the nascent RNA by the poly(A)-binding protein nuclear 1 (PABPN1) that binds to the poly(A)-tail after the addition of 11 adenines. The formation of the PAP-PAPBN1-CPSF complex switches PAP from a distributive to a processive mode, leading to the formation of longer poly(A) tails. The average poly(A) length is 250 nucleotides in humans and between 40 and 70 nucleotides in yeast (223). How poly(A)-length is regulated is still unclear, but current models suggest that during poly(A) elongation PABPN1 forms a globular complex with the poly(A)-tail. The complex cannot accommodate further nucleotides when the poly(A) reaches 250 nucleotides. At this point CPSF is forced to dissociate from PAP, which causes reduced processivity by PAP. Although the mechanism is supposed to be very similar in yeast and human cells, *S. cerevisiae* does not contain a PABPN1 orthologue. Instead the PAP homologue Pap1 utilizes Nab2 (nuclear polyadenylated RNA binding 2) to regulate the poly(A) length and nuclear export (224).

Once the poly(A)-tail has been produced, PABPN1 transports the nascent RNA to the nuclear pore complexes (NPC), which export the mRNA molecule over the nuclear membrane (223). After transport to the cytoplasm, the poly(A)-tail is instead bound by PABPC1 in order to protect the mRNA. The mature mRNA also contains a 5′-cap structure that associates with eukaryotic translation initiation factors (eIFs) (225). The eIFs and PABP interact with each other to form a closed mRNA loop, which both protects the mRNA and enables it to initiate translation (226). Many processes in the cytoplasm modify poly(A) length. For instance, PUF-family proteins recognize a 3′ untranslated region (UTR) on the transcript and recruit deadenylases causing shortening of the poly(A)-tail (227). Similarly, ARE-binding proteins bind to the UTR ARE (AU-rich element) and recruit deadenylases, such as the poly(A) ribonuclease (PARN) (228). The poly(A)-tail is not only shortened but can also be elongated in the cytoplasm (229). The cytoplasmic polyadenylation element binding protein (CPEB) binds to the poly(A)-tail and can recruit both a deadenylase and the cytoplasmic PAP GLD2 simultaneously. Although the deadenylase has higher efficiency, resulting in net shortening of the poly(A)-tail, phosphorylation of CPEB causes dissociation of the deadenylase, enabling GLD2 to elongate the poly(A)-tail. When the poly(A)-tail has been substantially deadenylated PABP detaches from the poly(A)-tail causing dissociation of the closed loop (226). As a consequence the ability of the mRNA to be translated is reduced and it becomes targeted for RNA degradation.

Apart from the canonical nuclear PAP there are several non-canonical poly(A) polymerases (ncPAP) as for example the aforementioned GLD2

(230). The *S. pombe* ncPAP Cid1 adds a short uridylated tail on polyadenylated mRNAs. Uridylation in turn stimulates decapping of the 5′-cap, thus inducing RNA-decay (see 1.6.2) (231).

Cid14 is another ncPAP in *S. pombe* (Trf4/Trf5 in *S. cerevisiae*) and this enzyme is a part of the TRAMP complex (the trimeric Trf4/Trf5-Air1/Air2-Mtr4 polyadenylation complex) (232). TRAMP targets a large variety of transcripts for degradation e.g. aberrant mRNA, pre-rRNA, snRNA, snoRNA, defective tRNA, and spliced introns (233). In addition, TRAMP is also involved in gene silencing and heterochromatin formation at the centromere (234). Cid14 differs from canonical PAPs in that it has a preference for incorporating purines and not only adenine, and can thus add both adenine and guanosines into the poly(A)-tail (235). The length of the poly(A) tail is regulated by the TRAMP helicase Mtr4p (236). The nucleotide composition together with the length of the poly(A)-tail distinguishes these transcripts from those with a canonical poly(A)-tail (235, 236). This could explain why TRAMP polyadenylated transcripts are targeted for rapid degradation while a normal poly(A)-tail protects the associated transcript.

1.5.2 Splicing

The human genome have about 20 0000 - 25 000 protein-coding genes (237). However, there are many more functionally distinct proteins in the cell. The gene body consists of protein-coding sequences (exons) spaced by non-protein-coding sequences (introns) (12). After transcription, the pre-mRNA includes both intron and exon sequences. The process of splicing removes the introns and may also exclude some exons from the mature mRNA. By combining different exons a large variety of protein products can be produced, a process termed alternative splicing (238). These protein variants can for instance enable tissue specific protein functions.

The spliceosome is a megadalton RNA-protein complex, which may vary in composition, thus enabling the complex to regulate a large variety of targets (12). The classical U2-spliceosome contains the snRNAs U1, U2, U4, U5 and U6, which are each associated with seven Sm proteins (B/B′, D1, D2, D3, E, F, G), except for U4/U6 that are in the same snRNP. There are three active regions in an intron, the 5′- splice site (ss), the 3′-ss and the branch site (BS). Initially, the U1 snRNP is translocated to the 5′-ss and non-snRNP factors are attached to BS. Subsequently, U2 snRNP also binds to the BS, which forms the pre-spliceosome (A complex). In the next step, the U5 snRNP/U4/U6 snRNP complex is recruited to the splice site (the pre-catalytic B complex). Activation occurs when U1 and U4 snRNP are dissociated from the

assembled spliceosome. Destabilization of the U2 snRNP component enables the spliceosome to cleave the 5′-ss (239). The 5′-end of the intron is then bound to the BS (C complex) causing consecutive cleavage at the 3′-ss (12). Finally the two processed exon ends are ligated together.

Not only mRNA contains introns. A subset of human (6%) and *S. cerevisiae* (21%) tRNA genes also have an intron (240). In yeast, the introns are removed by the tRNA-splicing endonuclease (TSEN) that cleaves both the 5′-ss and 3′-ss resulting in an excised intron and two tRNA-exon molecules, which are fused together by tRNA ligase to form an intron-free pre-tRNA (241).

1.5.3 tRNA processing

The function of tRNA, rRNA, snRNA, and snoRNA is dependent on their secondary structures. The tRNA gene transcripts do not only need to be folded correctly but they must also be processed at the 3′- and 5′-ends as well as spliced and modified at certain nucleosides. tRNA genes are transcribed in the nucleolus, forming a pre-tRNA transcript (242). The pre-tRNA contains a 5′-leader and a 3′-trailer sequence that need to be removed. Usually, the endonuclease RNase P cleaves the 5′-leader before the 3′-trailer is removed (243). In these cases, the pre-tRNA is then exported to the nucleoplasm where a La protein (Lhp1 in *S. cerevisiae*) binds to the oligo(U) stretch at the 3′-end (242). The RNase Z endonuclease (Trz1 in *S. cerevisiae*) then cleaves and removes the 3′-trailer (244). However, Lhp1 competes with the 3′-5′ exonuclease Rex1 for binding to the oligo(U) stretch (245). While Lhp1 prefers a length of more than three uridines, Rex1 predominantly binds to sequences containing only one or two uridines (244). For the pre-tRNAs that are processed by Rex1, the digestion of the 3′-trailer precedes the cleavage of the 5′-leader by RNase P.

Not only Lhp1 and Rex1 compete for access to the 3′-trailer. The TRAMP complex (see 1.5.1) can also bind to a single stranded 3′-trailer with the length of at least three nucleotides (244). As a result the transcript is polyadenylated and thus marked for degradation by the exosome (see 1.6.1). Pre-tRNAs that are associated with Lhp1 are protected from the activity of TRAMP until RNase Z has processed the 3′-end, leaving only a single nucleotide overhang, which precludes TRAMP binding. In contrast, 3′-trimming by Rex1 occasionally leads to the formation of aberrant transcripts that are targeted by TRAMP to mark them for degradation (245). Defective

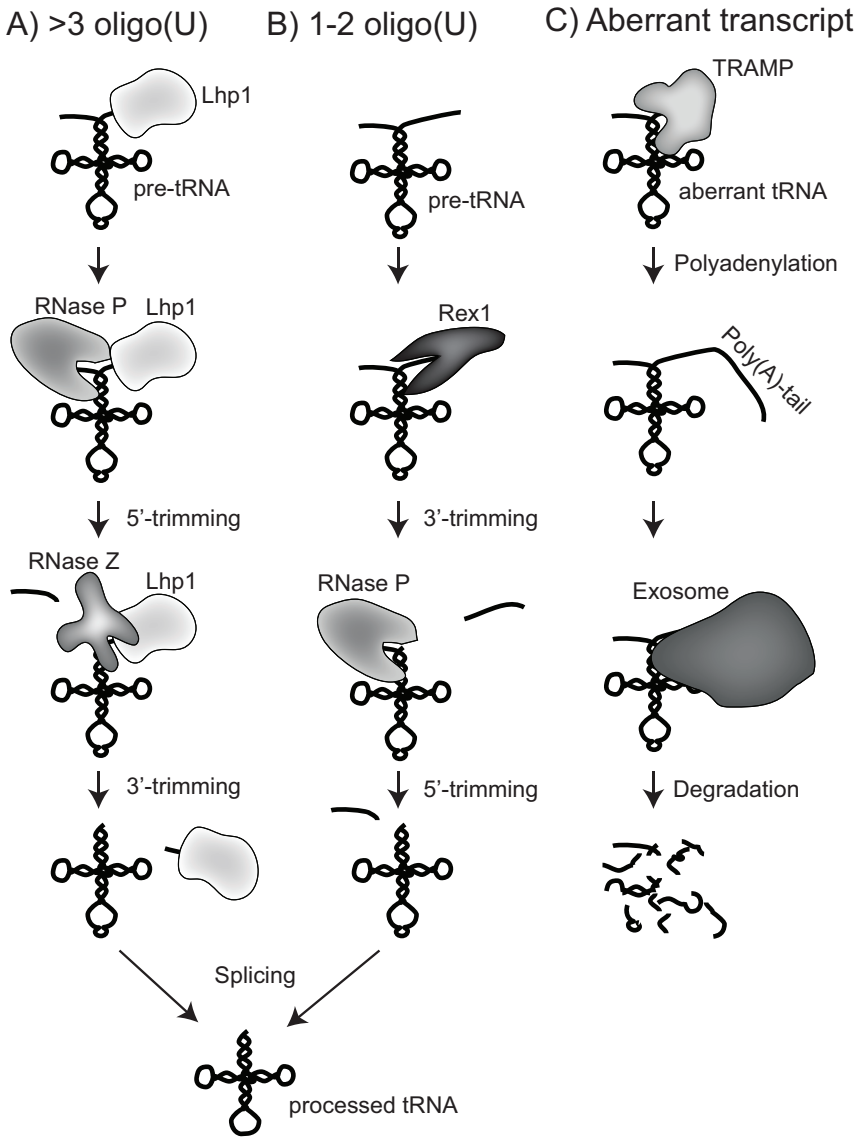

Figure 8. Processing of pre-tRNA
Maturation of pre-tRNA can take two pathways depending on the length of the oligo(U) termination signal. A) pre-tRNAs with 1-2 Us are recognized by Rex1 that cleaves the 3′- trailer. The 5′- leader is then removed by RNase P. B) Transcripts with more then 3 Us are bound by Lhp1. RNase P then removes the 5′- leader followed by RNase Z mediated digestion of the 3′-trailer. C) Aberrant pre-tRNA transcripts in the Rex1 pathway are polyadenylated by TRAMP complex and consequativly degrade by the exosome subunit Rrp6. D) After trimming of 3′- and 5′-*end intron containing pre-tRNA are spliced to produce mature tRNA.*

tRNAs are then degraded by the exosome, while mature tRNA turnover is controlled by Xrn1 of the decapping machinery (242, 244).

A sequence of CCA at the 3′-end of the tRNA is needed for loading of the amino acid (246). After removal of the 3′-trailer, the yeast tRNA nucleotidyltransferase Cca1 adds CCA to the 3′-end.

The location of tRNA introns is always one base downstream from the anticodon site (242) and the splicing process is needed for certain nucleoside modifications at this position (247).

In yeast, the tRNA splicing endonuclease removes the intron leaving a 2′-3′ cyclic phosphate group on the 3′-end of the first exon (242). The ligase Trl1 phosphorylates the 5′-end of the second exon, and thus enables ligation. Splicing of tRNA occurs in the nucleus in humans, but in yeast it instead occurs on the outer surface of mitochondria (248, 249).

Nucleosides are heavily modified in tRNA and over 85 different modifications have been identified (250). There is a large number of enzymes involved in tRNA modification processes and many of them have been characterized. Modifications in the vicinity of the anticodon are needed for translation and cellular growth (251, 252) while the stability and folding of tRNA is usually dependent on modifications in the tRNA body (251-254). Finally, certain modifications help to identify a specific tRNA ((255, 256)).

1.6 RNA degradation

The balance between mRNA production and degradation decides the steady-state levels of specific transcripts. The degradation pathways are also used for RNA quality control by rapid degradation of aberrant RNA species occasionally produced by the different RNA polymerases. As discussed above (see 1.5.1), poly(A)-tails influence RNA stability (257). Shortening of the poly(A)-tail triggers mRNA degradation by the 3′-5′ exoribonuclease exosome complex or the 5′-3′ exoribonuclease decapping complex (258, 259). Polyadenylation of aberrant transcripts by the TRAMP complex has the opposite effect and leads to rapid degradation by the exosome (260). Transcripts are also protected from degradation by RNA-binding proteins in RNP complexes. Defective mRNAs have reduced affinity for these proteins, leading to increased access for degrading enzymes (261). A stable secondary structure in the RNA can also interfere with the degradation process (262).

1.6.1 The exosome

The 3′-5′ RNA degradation pathways are functionally conserved from bacteria to humans (258). There are three major 3′-5′ exoribonucleases in eukaryotes: the main catalytic subunit of the exosome Rrp44 (Dis3 in *S. pombe*), the nucleus specific exosome subunit Rrp6, and the mitochondrial PNPase. (263). In the cytoplasm, the exosome complex contains nine core subunits (Exo9) and Rrp44 (Exo10). In addition to the Exo10 subunits, the exosome present in the nucleus also contains Rrp6, thus forming Exo11. A third type of exosome has been hypothesized in the nucleolus, which only includes Exo9 and Rrp6 but not Rrp44 (264). The Exo9 core regulates the accessibility of RNA to Rrp44 and Rrp6 and modulates the activity of these enzymatic subunits (265).

The eukaryotic core exosome consists of nine unique subunits (263). Three subunit pairs, Rrp41-Rrp45, Rrp46-Rrp43 and Mtr3-Rrp42 form a hexamer ring just large enough to enable single stranded RNA to enter the central channel (266). Capping the hexamer ring are the three subunits Csl4, Rrp4, and Rrp40. At the bottom of the channel is Rrp44 that digests the entering single stranded RNA. The second enzymatic subunit, Rrp6, is instead located at the top of the complex.

In addition to its exoribonuclease activity, Rrp44 is also an endoribonuclease (267). Whereas the exoribonuclease domain of Rrp44 degrades linear 5′-phosphorylated ssRNA, the endonuclease domain processes other RNA species including circular RNA (267). The targeted RNA needs an at least 9 nucleotide long single stranded overhang to be directly processed by the exoribonuclease domain of Rrp44 (268). However, Rrp44 can unwind double-stranded structures if a single-stranded overhang of at least 4-8 nucleotides is exposed. Interestingly, the unwinding activity of Rrp44 does not seem to be ATP driven like conventional helicases. Instead, the complex retains the force produced by four consecutive nucleotide cleavages of the single stranded overhang. The narrow exosome channel causes steric occlusion of double stranded RNA structures until the accumulated force separates the two strands in a burst (262). Cooperation between the endoribonuclease and exoribonuclease domains enables a second mechanism for Rrp44 to degrade structured RNAs. The endoribonuclease cleaves the RNA at the loop of a hairpin structure, which facilitates separation of the two strands and further processing by the exoribonuclease (269).

The nucleus specific exosome subunit Rrp6 functions in the mRNA surveillance pathways (270). Rrp6 has also been implicated in processing of many small, structured RNAs (271). As discussed in Paper IV of this thesis,

these targets include tRNA, rRNA, snoRNA, and snRNA. Rrp6 seems primarily to be involved in degradation and quality control of these transcripts. For instance, Rrp6 degrades pre-tRNAs that fail the quality control (244). Yeast cells lacking Rrp6 are viable but display accumulation of polyadenylated transcripts in the nucleolus, which is the location of tRNA genes (272, 273). Evidence has suggested that Rrp6 and Rrp44 can cooperate in the degradation process of at least some small, structured RNAs (271). There are also reports of Rrp6 functioning independently of the core exosome (274).

Co-factors, such as the TRAMP (see 1.5.1) and SKI (super killer), help the exosome to achieve high target specificity (260, 275). Other examples are Rrp47, a cofactor that interacts with Rrp6 and helps it in the maturation process of 5.8S rRNA (276), and Mpp6, a RNA binding protein that is needed for surveillance of pre-rRNA, pre-tRNA, and cryptic ncRNA (277). Sequence elements in transcripts can also regulate exosome activity. The AU-rich element (ARE) is found in highly unstable transcripts such as cytokines and other inflammatory transcripts (228, 278). ARE is located in untranslated regions of the transcript and is bound by AUBP (the ARE-binding protein). The exosome can interact with AUBPs in order to rapidly degrade transcripts (278). For instance, the RNA helicase RHAU recognizes specific AUBPs and in turn recruits the poly(A)-specific ribonuclease (PARN) and the exosome, resulting in RNA decay (279).

The nuclear exosome associates with the non-canonical polyadenylation complex TRAMP (see 1.5.1) to promote exosome dependent degradation of aberrant transcripts (260). However, both the exosome and TRAMP is also involved in normal 3′-processing. For example, Nrd1 recruits the exosome and TRAMP to the Nrd1-Nab3-Sen1 binding site of snoRNA genes where it trims the 3′-end of nascent transcripts (280). The transcript needs to be associated with a protective RNA-binding protein in order to avoid that the exosome digests the entire RNA. Nrd1 also connects Pol II transcribed RNA with TRAMP and the exosome (281).

In the cytoplasm, the exosome interacts with the SKI complex (super killer) (275). Together they are involved in several different RNA decay pathways. For instance, RNA that has been cleaved by the RNA interference (RNAi) machinery (see 1.6.3) is completely degraded by the exosome (282). Furthermore, three different pathways are used to degrade defective mRNA. First, the nonsense mediated decay (NMD) pathway removes mRNAs with premature termination signals (283). Secondly, the non-stop decay (NSD) pathway processes transcripts lacking a termination sequence (284). Finally,

transcripts that are stalled in the ribosome are degraded by the no-go decay (NGD) pathway (285).

1.6.2 The Decapping complex

The second major RNA decay pathway operates through the decapping complex that removes the 5′-cap of the transcript and enables 5′-3′ exoribonuclease RNA decay (286). Upon exiting active translation, the mRNA can be deadenylated, which results in recruitment of the decapping complex to the transcript. The decapping complex subunits Dcp1 and Dcp2 along with additional factors and the mRNA, form the decapping P-body unit. In yeast, the decapping complex subunit Dcp2, with the support of Dcp1, removes the RNA 5′-cap, a protective cap formed by a N7 methylated guanosine triphosphate (m7Gppp) linked to the 5′-end of mature mRNA via a 5′-5′ triphosphate bridge (287). This enables the 5′-3′ exoribonuclease Xrn1 to digest the targeted RNA (288). Deadenylation of the mRNA is needed prior to decapping since the poly(A)-binding protein Pab1 inhibits the decapping process (259). The cap-binding complex eIF4E associates with the 5′-cap of RNA in yeast, and inhibits the activity of the decapping complex (289). The RNA-binding proteins Edc1 and Edc2 can however alleviate the inhibitory effect of eIF4E, allowing the decapping complex to remove the 5′-cap.

1.6.3 RNAi

RNA interference (RNAi) can influence gene expression at many different levels, including transcription, mRNA turnover, translation, and regulation of chromatin structure (290). RNAi utilizes several species of small RNAs to exert these functions. The small interfering RNA (siRNA) molecules are completely complementary to a specific RNA target (291). Hybridization between the target and the siRNA leads to degradation of the transcript by the RNAi machinery. Another group of regulatory RNA molecules is micro RNA (miRNA), which are partially complementary to the target RNA (292). The rest of the miRNA forms a hairpin structure that for instance can interfere with translation. Yet another type of RNA with regulatory potential is PIWI interacting RNA (piRNA), which silences transcription of transposons genes (293).

The biogenic pathways of the different small RNA species differ from each other. miRNAs are originally transcribed as long primary miRNAs (pri-miRNA) (294), which are processed by the microprocessing complex into 70 nt long stem-loop structures called pre-miRNAs (295). These precursors are then transported into the cytoplasm where the hairpin loops are removed by the endoribonuclease Dicer, thus creating 21 bp long complementary double

strand RNAs (296). A helicase separates the two strands, allowing one of them, designated the guiding strand, to associate with the complementary target. The 3′ region of the miRNA is not complementary with the targeted RNA and instead forms a hairpin that interferes with translation of the target mRNA.

Double stranded pre-siRNA is produced in four different fashions: convergent transcription, complementary sense and antisense transcription, structured loci, or RNA dependent RNA complex (RDRC) produced dsRNA from a single stranded transcript (290). The dsRNA is then digested by Dicer to produce 21 bp long fragments. One strand is discarded allowing the other strand to bind to its completely complementary target. The siRNA promotes cleavage of the target RNA with the help of the RNA-induced silencing complex (RISC) (297).

In *D. melanogaster,* pre-piRNA is transcribed from gene clusters that encode antisense sequences complementary to transposon RNA (293). The pre-piRNA is then exported to the cytoplasm where it is cleaved into piRNA fragments by an endonuclease, followed by loading onto either Piwi protein or Aubergine (Aub). The piRNA is further trimmed and modified, and finally binds to the targeted transposon RNA, which is cleaved by Aub.

Argonaute proteins bind all the different classes of RNAi associated small RNA molecules and help to guide them to the correct target (298). In this process, the double stranded small RNA first binds to the Argonaute protein, after which one of the strands (the passenger strand) is discarded while the guiding strand remains (298). The 5′-end of the guiding strand has low affinity to the 3′-end of the passenger strand and recognition of this instability allows the Argonaute protein to decide which strand to keep (299). The guiding strand of either the miRNA or the siRNA binds to Argonaute proteins in RISC and guides the complex to the targeted mRNA (300). Some Argonaute proteins include an endonuclease ‘slicer’ domain that can cleave complementary target RNA (301). In humans there are four Argonaute proteins, but only AGO2 has a ‘slicer’ domain (302). The Argonaute proteins promote post-transcriptional gene silencing (PTGS) also via other processes, for instance by influencing the deadenylation process, decapping activity, inhibition of translation and association with the degrading P-body (303). The Argonaute proteins include a PAZ (Piwi Argonaute Zwille) domain that recognizes and binds the ssRNA or dsRNA with a 2 nucleotide 3′-overhang, while the MID domain recognizes the phosphorylated 5′-end of the small RNA (298, 304). In fission yeast Ago1 is the only Argonaute protein and the protein includes an endonuclease ‘slicer’ domain (305).

The Dicer family of class 3 RNase III enzymes controls the production of small RNA from double stranded precursor RNA (306). The pre-RNA binds to the Dicer double stranded RNA binding domain (dsRBD). The PAZ domain of Dicer can then decide the correct length of the small RNA to be produced, usually between 21 and 28 nucleotides (307). Dicer cleaves the associated double stranded RNA by hydrolysis of phosphodiester bonds (308). The enzyme can process a long dsRNA using its helicase domain and ATP to move along the substrate and digest the entire length (309). Dicer can also target ssRNA loops for small RNA production as in the case of pre-miRNA processing (310).

RNA dependent RNA polymerase (RDRP) is a vital component of the RNAi machinery (311). It converts ssRNA into dsRNA, which is then processed by Dicer to produce siRNA. This mechanism is for instance used in the defense against viral RNA species and in the regulation of chromatin structure at centromeres. In plants, Dicer digests viral dsRNA into siRNA that in turn guides RDRP to other viral ssRNAs to in order to convert them to dsRNA (312). Dicer can then digest and neutralize the double stranded viral RNA, and as a byproduct produce more antiviral siRNA. There is no RDRP counterpart yet identified in humans. However, Pol II has been reported to have RDRP activity (313).

In fission yeast, the RNA induced transcriptional silencing (RITS) complex is guided to centromeric transcripts by siRNA associated with Ago1 (314). In turn, RITS recruits the RDRP complex to the centromere that converts the centromeric transcripts into dsRNA. This enables Dicer to produce more siRNA leading to further recruitment of RITS to the centromere. This process leads to heterochromatin formation at the centromere, a major topic in Paper II of this thesis.

1.7 Chromatin

Packaging of the genetic material into chromatin helps to regulate both genomic organization and gene expression.

1.7.1 Heterochromatin, Euchromatin and the Nucleosome

The nucleosome protein core consists of two copies each of the four subunits H2A, H2B, H3 and H4 (315). DNA (147 bp) tightly wraps around the histones with the aid of many DNA-protein interactions (316). One histone H1 subunit (linker histone) binds to DNA on the outside of the nucleosome,

leading to the formation of the chromatosome (317). The spacing between nucleosomes differs depending on the genomic location (318).

Protruding from the nucleosome are the histone tails containing residues commonly targeted by histone modifiers to control epigenetic regulation, chromatin structure, and gene expression (14). Examples of modifications to these residues are methylation, acetylation, and phosphorylation. For instance, active genes are commonly marked by histone modifications such as H3 serine 4 trimethylation (H3K4me3) and acetylation of H3 serine 9 (H3K9ac) (319). Nucleosome function can also be regulated by the exchange of canonical histones to specific histone variants, such as CENP-A (centromeric H3 variant) and H2A.Z (320, 321).

Transcriptionally repressed and nucleosome dense heterochromatin regions are marked by hypoacetylation and methylation of H3 serine 9 (H3K9me) (322). A precise boundary between heterochromatin and euchromatin is vital for avoiding unwanted activation or repression of bordering genes.

1.7.2 Centromere

The centromere is the genomic region where the kinetochore assembles during mitosis. Microtubules attach to the kinetochore and the centrosome, enabling separation of the chromosomes during cell division (323). A dysfunctional centromere leads to chromosome instability and loss of genetic information during mitosis. The size of the centromere varies between 125 bp in *S. cerevisiae*, 40-100 kb in *S. pombe,* and several megabases in humans (324).

The core centromere is distinguished by the H3 variant CENP-A (Centromere protein A) (325). Overexpression of CENP-A in *D. melanogaster* causes incorporation of CENP-A in non-centromeric locations resulting in assembly of a functional ectopic kinetochore (326). Centromeric core chromatin is distinct from both euchromatin and heterochromatin (327). Interspersed between CENP-A containing nucleosomes are nucleosomes with canonical H3 dimethylated at the serine 4 position (328). CENP-A is removed during replication and later reintroduced to enable kinetochore assembly (329). Newly synthesized CENP-A is incorporated into centromeric nucleosomes with the help of HJURP (Holliday junction recognizing protein) and the Mis18 complex.

CENP-A is the key kinetochore-assembly factor. There are over 80 proteins that constitute the kinetochore (330). Kinetochore is divided into an outer plate that connects to the microtubule, and an inner plate containing CENP-A and several other CENP proteins, forming a constitutive centromere

associated-network (CCAN). While CCAN is present during most of the cell cycle, the other factors of the kinetochore are assembled at different stages between late G2 and the end of mitosis. The general structure of the kinetochore is conserved in eukaryotes (330). However, the number of kinetochores occupying each centromere differs between species. For instance, *S. cerevisiae* have point centromeres covered by a single kinetochore while the regional centromeres of *S. pombe* and humans are bound by many kinetochores (331).

The function of the core centromere (*cnt*) in *S. pombe* is dependent on the surrounding chromatin structure. The flanking pericentromeric region is covered by heterochromatin and is primarily transcriptionally silent (332). Bordering the *cnt* on both sides is the inner most repeat (*imr*), proceeded by the outer repeat region (*otr*), which is further subdivided into the *dg* and *dh* elements. The sequence of the repeat region on one side is almost perfectly mirrored on the other side of *cnt* (333). In *S. pombe*, there is little size and sequence variation between the *cnt* regions of the individual chromosomes (332). The size of *imr* does not differ between the chromosomes either, but the sequence partially differs, enabling identification of the chromosome. Even though the sequence of *otr* is conserved between chromosomes, the copy number and orientation of the *dg* and *dh* elements differ.

Although there are no protein-coding genes in the centromere, there are ncRNA and tRNA genes in *otr* and *imr* (334). Transcription of pericentromeric genes is restricted to a short window associated with the replication events of S-phase (335). Dicer (Dcr1), the ribonuclease of the RNAi machinery, rapidly processes centromeric ncRNA transcripts into siRNA (336). The siRNAs are then loaded onto Ago1 of the RITS complex (RNA-induced transcriptional silencing), and guide RITS to actively transcribed centromeric genes (Figure 9) (314). Association of RITS to the nascent RNA recruits RDRC, including the RNA-directed RNA polymerase (Rdp1) that converts the transcript into double stranded RNA. The double-stranded RNA is then processed by Dicer to produce more siRNA, creating a positive feedback loop.

RITS association with nascent transcripts also recruits the Clr4 methyltransferase complex (ClrC) that methylates H3K9, a marker of heterochromatin (337). The RITS subunit Chp1 binds to H3K9me and thus further stabilizes RITS's association with the centromere. *De novo* establishment of heterochromatin is initiated by methylation of H3K9 at two nucleation sites in *dg* associated with ncRNA genes (338). In order for Clr4

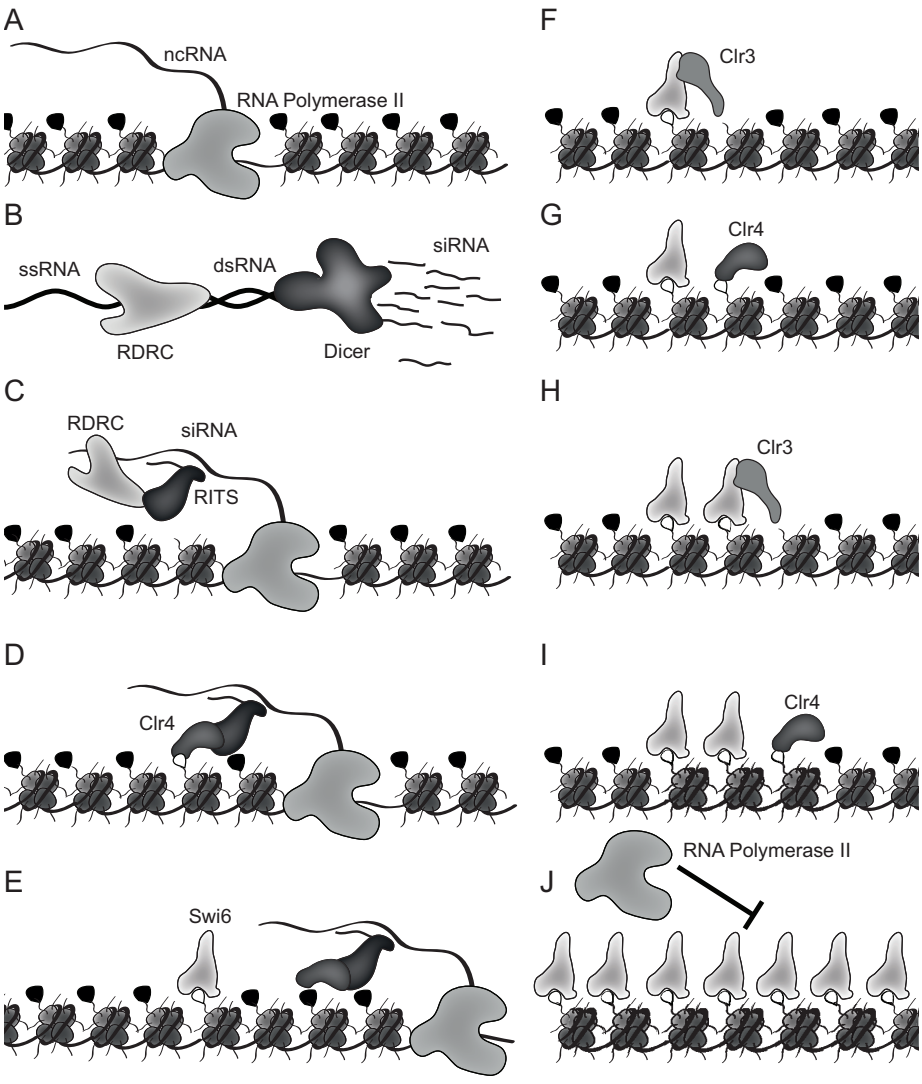

Figure 9. RNAi-dependent heterochromatin formation at the pericentromeres
A) Pol II transcribes ncRNA in the pericentromeric region. B) RDRC copies the ncRNA and creates dsRNA that is used as substrate for Dcr1 to produce siRNA. C) The siRNA associates with the Ago1 subunit in the RITS complex. The siRNA helps to recruit RITS to the nascent ncRNA. RITS stimulates recruitment of RDRC for further production of siRNA. D) RITS recruits Clr4, which methylates H3K9 in the pericentromeric region. E) Swi6 binds to H3K9me. F) Swi6 recruits Clr3, which together with Sir2 deacetylates H3K9. G and H) Clr3, Clr4, and Swi6 cooperate to stimulate spread of H3K9me and heterochromatin. H) Pol II activity is repressed by heterochromatin formation. Reduced transcription limits the nucleosome turn over and further facilitates spreading of silent chromatin.

to methylate H3K9, it must be deacetylated by Sir2 or Clr3. The H3K9me binding chromodomain proteins Swi6 and Chp2 recruit these histone deacetylases (HDACs) to the centromere. As previously mentioned (see 1.7.1), H3K9ac is associated with active promoters and enables Pol II transcription (339). The activity of Pol II can counteract methylation of H3K9 by Clr4. Thus, deacetylation of H3K9 at the centromeric promoter inhibits Pol II activity and leads to the stable establishment of H3K9me (338). Once stable H3K9me is established at the nucleation sites, the heterochromatin can start to spread outwards.

The tRNA genes located inside *imr* and flanking the outer perimeter of the pericentromere, keep the heterochromatin from spreading into the core centromere and the flanking euchromatin (332, 340). The Pol III general transcription factor TFIIIC contributes to the establishment of chromatin boundaries in fission yeast (205). However, at *imr* tDNA, TFIIIC alone does not prevent the spread of heterochromatin (340). Instead, recruitment of the entire Pol III machinery is needed to stop heterochromatin spreading into *cnt*.

1.7.3 Telomere

In eukaryotes the chromosomes are linear with vulnerable ends, which are protected by telomeres. The DNA repair machinery can interpret these ends as double stranded DNA breaks (341). During replication, the DNA polymerase cannot replicate from the very end of the chromosome, due to the requirement of a RNA primer (342). Instead a short fraction of the telomere is lost. This results in shortening of the telomeres at each cell division, the so-called end-replication problem. If the cells did not compensate for this problem there would eventually be a loss of genetic information and finally cell death. To circumvent the end-replication problem the reverse transcriptase telomerase increase the length of the chromosome ends. While shortening of the telomeres leads to reduced life span, overexpression of telomerase can establish immortalized cell lines (343).

The telomere consists of a cap followed by the telomere-associated sequences (TAS) (344). In vertebrates the cap is composed of TTAGGG repeats ending in a single stranded 3′-overhang (345). In yeast this region is nucleosome free but covered with other DNA binding complexes. In contrast, the vertebrate cap does contain nucleosomes. The chromatin structure of the telomere resembles heterochromatin and is characterized by silencing and heterochromatin specific histone modifications. In *S. cerevisiae*, the subtelomere is divided into the two TASs, X and Y′ (346). The X region is found in all chromosomes and has a low nucleosome density. In contrast, the Y′ region has high nucleosome density and is only present in some

chromosomes with varying copy number. Interestingly, although Y′ has a higher nucleosome density it has intermittent transcriptional activity, while X is always silent and is associated with the silent information regulator (Sir) proteins.

After replication, a single stranded 3′-overhang is added to the telomere end (347). In humans, the POT1 protein binds the overhang, which is then folded into an upstream telomere sequence, forming a T-loop (348). The invading overhang separates the two strands of the upstream sequence and then binds to it, forming a D-loop structure. The TRF proteins of the shelterin complex facilitate T-loop formation by bending the telomeric DNA (349). In *S. pombe* there are homologues for the shelterin complex, but in *S. cerevisiae* there are no orthologues to the TRF proteins (350). In human and *S. pombe* the repressor-activator protein (RAP1), binds to the telomere through association with TRF2. Since *S. cerevisiae* lacks TRF proteins Rap1 instead binds to telomere DNA directly. Several shelterin complexes can bind to long telomeres, while short telomeres contain fewer complexes (351). High abundance of shelterin at the telomere inhibits telomerase by POT1 binding to the single stranded overhang. This system enables a mechanism for regulation of telomere length.

In *S. cerevisiae*, Rap1 can recruit Sir4 into the subtelomeric region, which in turn leads to recruitment of Sir2 and Sir3 (352). Binding of the Sir proteins causes silencing of the subtelomeric region (353). While Sir3 and Sir4 are structural proteins Sir2 is a HDAC that deacetylates H4K16. The deacetylation of H4K16 enables Sir3 and Sir4 to bind to H3 and H4 tails (354, 355). This results in a positive feedback loop that spreads the Sir-proteins over subtelomeric region. The deacetylase activity of Sir2 is counteracted by the histone acetyltransferase (HAT) Sas2 (356). Hyperacetylation caused by Sas2 inhibits Sir3 and Sir4 binding and creates a subtelomeric boundary. Mediator is involved in regulating the balance between Sir2 and Sas2, which is the main focus of Paper I.

2 AIMS OF THIS THESIS

The Mediator complex was initially believed to only function as a bridge between gene specific transcription factors and the transcription machinery at the promoter. Over time, many other functions have been attributed to the complex. Among these is the ability of the complex to regulate chromatin structure by promoting histone modifications, both directly and indirectly (155, 157, 357). In this thesis, we set out to investigate if Mediator is involved in regulation of heterochromatin structure.

We first decided to study how Mediator affects heterochromatin formation at telomeres (Paper I). To this end we opted to use *S. cerevisiae*, since previous work in this model organism had identified Mediator mutants that caused telomere length alternations and influenced telomere silencing (358, 359).

We also investigated the effect of Mediator on heterochromatin formation at centromeres. For this work, we used *S. pombe,* which, compared to *S. cerevisiae*, has a centromere organization more similar to that found in mammalian cells (360). Tight regulation of centromere transcription is crucial for maintaining a correct chromatin structure at the centromere and as a regulator of Pol II, Mediator was a prime candidate for regulation of centromeric transcription. In addition, Mediator had previously been connected to the chromodomain-remodeling factor Hrp1, which is involved in loading of CENP-A at centromeres (357, 361). These observations prompted us to investigate Mediator's role in centromeric function (Paper II).

In Paper II we discovered that Rrp6 and Med20 together influenced transcript levels at the core centromere. To follow up this observation, we performed RNA sequencing in a mutant *med20*Δ/*rrp6*Δ strain. We wanted to study if Med20 and Rrp6 had similar effects on transcription as those we had seen in the core centromere. Further more we wanted to find out if deletion of both *med20*$^{+}$ and *rrp6*$^{+}$ had more severe centromeric defects then the single mutants alone (Paper III).

In addition to the findings presented in Paper III, the RNA sequencing analysis uncovered an unexpected result, a significant increase in transcripts mapping to tRNA genes. The observation was surprising since Mediator is known to specifically regulate Pol II genes, but not Pol III dependent genes. However, the observed effect was very strong and we therefore set out to further elucidate Mediator's effect on tRNA transcription (Paper IV).

3 RESULTS AND DISCUSSION

3.1 Paper I

In *S. cerevisiae,* the subtelomeric region flanks the telomere and consists of the Y′ and X elements that are characterized by their chromatin state. Y′ region is highly enriched in nucleosomes and display high levels of H4K16 acetylation. In contrast, X element lacks a defined nucleosomal structure, is bound by Sir proteins, and has very low levels of H4K16 acetylation. Sir proteins that insure transcriptional silencing of the X element maintain the hypoacetylated state. The histone deacetylase Sir2 removes acetyl group from H4K16 and this activity is counteracted by the histone acetyltransferase Sas2. The interplay between these two enzymes decides the boundary between silenced and transcriptionally active chromatin. Impaired balance of histone modification in the X element is found in aging cells, implicating the importance of the X element and its chromatin states for the life span of the cell.

Mediator had previously been shown to affect chromatin silencing of telomeres (162). Our, initial investigations uncovered that deletion of *MED16* caused a specific transcriptional increase at genes close to telomeres (Figure 1A). However, the transcriptional activity at the subtelomeres in *MED16* deletion mutant was fluctuating between a silenced and a desilenced state (Figure 1B). In contrast, a *med5*Δ strain demonstrated a stable desilencing effect resulting in increased Pol II binding to the subtelomeric region (Figure 1D). Mediator localizes to the bordering regions of the X element, and deletion of *MED5* caused significant decrease of Mediator occupancy at both these locations (Figure 2B and C).

The balance between Sir2 and Sas2 was also disrupted in *med5*Δ (Figure 10 of this thesis). While Sas2 binding increased significantly in the X element, the opposite was observed for Sir2 (Figure 3A-D). As a consequence, the H4K16ac levels were increased in the X element, demonstrating the functional significance of Med5 for maintaining a proper boundary between hypo- and hyperacetylated regions. In addition, deletion of *MED5* caused a 20 % decrease in replicative life span of the *S. cerevisiae* cells.

Our investigation demonstrated that several Mediator subunits influence transcriptional activity at the subtelomeres (e.g. Med5, Med7, Med16). How Mediator exerts this control is still unclear. The observation that Mediator binds to the bordering regions, but not the X element itself raised the question

how Mediator's regulatory function is transmitted into the X element. We found that the Mediator and Sir3 compete for binding to mononucleosomes *in vitro* (Figure 5B). Furthermore, we had previously shown that the association between Mediator and the histone tail is inhibited by acetylation of H4K16. It is possible that Mediator impedes access of Sas2 to H4K16 and therefore inhibits Sas2 dependent spread of H4K16ac. Deletion of *MED5* could cause reduced Mediator binding at the border of X element and thus allow Sas2 activity to continue further into the X-element. In fact, it has later been shown that Med5 physically interacts with the H4-tail (161). This correlates with our finding that without Med5, Mediator is lost from the subtelomeric regions and provides a mechanistic explanation for Mediator's role in subtelomeric transcriptional regulation.

Mediator is involved in regulating the silenced state of several genetic regions. For instance, the fluctuating chromatin state observed at the telomere in the *med16*Δ mutant also affected mating type loci. During mating in *S. cerevisiae*, the cells secrete pheromones to induce structural projections called shmoo. The cells' response to α cell mating pheromone (α factor) is dependent on the chromatin state of the mating type loci (362). Deletion of *MED16* resulted in less response to the α factor caused by a metastable chromatin phenotype. Our results indicate that Mediator may not only affect telomeres but also other silenced regions. Indeed, further research revealed

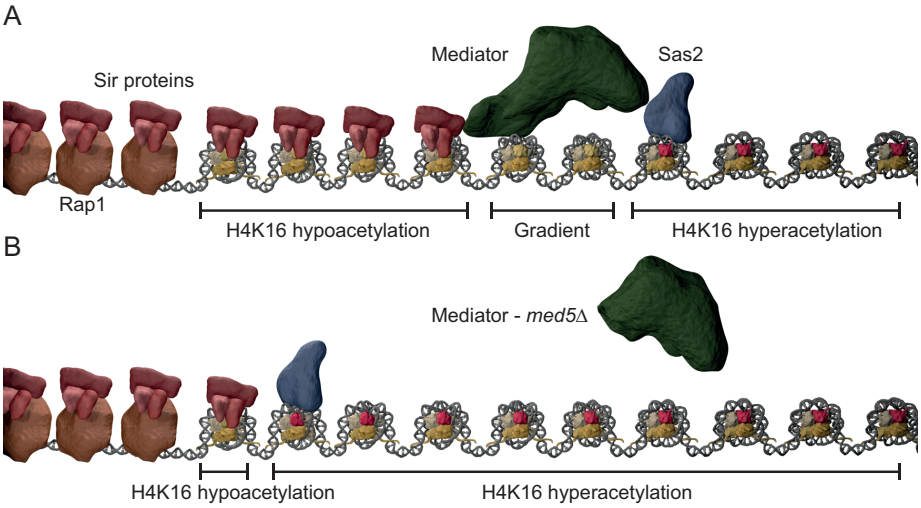

Figure 10. Mediator influences the boundary between euchromatin and heterochromatin at telomeres.
A) Mediator binds to deacetylated H4K16 and blocks Sas2 ability to acetylate H4K16. B) Loss of Med5 impairs Mediator's ability to interact with H4K16, allowing Sas2 to acetylate H4K16, which in turn inhibits binding of Sir proteins.

that Mediator also play a role in the maintenance of heterochromatin at *S. pombe* centromeres (Paper II).

3.2 Paper II

We set out to investigate if Mediator, apart from its effect at telomeres, also could influence the chromatin state at centromeres. We decided to use *S. pombe* as our model systems, since its centromeres resemble those found in human cells (331). The microtubule destabilizing drug thiabendazole (TBZ) is commonly used for investigating centromeric defects in *S. pombe*. Genetic screening identified a number of Mediator head-module mutants, including *med18Δ* and *med20Δ*, which were especially sensitive to TBZ (Figure 1A). Med18 and Med20 form a flexible subcomplex of the head module. Med20 is the most external of the two subunits and deletion of *med18*$^{+}$ causes disassociation of Med20 from the Mediator complex, whereas deletion of *med20*$^{+}$ does not affect Med18 (104). We decided to focus our investigation on Med20's role in centromere function.

Mutations in the RNAi machinery cause defects in centromere function, since it is required to produce siRNA molecules used for heterochromatin assembly. In our experiments we used the endonuclease Dcr1, which is an integral part of the RNAi pathway, as a positive control. By DAPI-staining DNA and immunostaining tubulin, we could monitor the effects of *med20*$^{+}$ deletion on centromere function. We found that the *med20*Δ mutant caused the same elevated levels of chromosome segregations defects as those observed in the *dcr1*Δ strain (Figure 1C-D).

The heterochromatin structure of the pericentromeric regions is essential for proper centromeric function. We found that the levels of the heterochromatin marker H3K9me and the Swi6 protein were reduced in the *med20*Δ mutant strain. Since the formation of heterochromatin is dependent on siRNA species, we also wanted to see if their levels were changed. Indeed, the siRNA levels were reduced in the *med20*$^{+}$ deletion strain, even if the reduction was less severe than that observed in the *dcr1*Δ mutant strain.

Heterochromatin formation causes gene silencing in the pericentromeric regions and disruption of this chromatin structure should therefore cause increased transcriptional activity. Using chromatin immunoprecipitation followed by deep sequencing we found that Pol II binding was increased over the pericentromeric regions in *med20*Δ mutant strain, but not in the neighboring euchromatin regions (Figure 2A). Transcription of the centromere is normally restricted to the S-phase of the cell cycle and we therefore performed a time curve experiment of Pol II binding in

synchronized cells. The increase of Pol II binding was indeed specific for the S-phase (Figure 2D). Investigation of Mediator binding also revealed a cell cycle dependent association with the pericentromeres (Figure 2C). However, deletion of *med20*$^{+}$ did not cause any change in Mediator binding compared to wt.

Our studies also revealed that transcripts from *imr* and *otr* were increased during the S-phase (Figure 2F-G). However, these transcripts continued to be higher than those observed in wt cells, also during the rest of the cell cycle. A possible explanation for this discrepancy could be that loss of Med20 disrupts the connection between the pericentromeric transcripts and the RNAi machinery and thus decreases the degradation rate of ncRNA into siRNA. Therefore the transcripts remain until the next S-phase. This model could also explain why deletion of *med20*$^{+}$ causes increased ncRNA transcription but reduced siRNA levels.

To investigate Mediator's effects on silencing of the pericentromere in more detail, we performed a tiling array assay. The *med20*Δ mutant caused increased expression of the pericentromeric transcripts compared to wt (Figure 3E-F). Although *dcr1*Δ causes a much higher transcriptional increase than *med20*Δ alone, deletion of these two genes in combination, causes an additive effect. This would suggest that Med20 does not work specifically in the RNAi pathway. It is more likely that the Mediator works upstream of RNAi pathway and contribute to increased Pol II transcription. Perhaps the increased transcript levels get distributed between the RNAi-dependent pathway and the exosome pathway.

Desilencing of the pericentromeric region was further demonstrated by analysis of an *ura4*$^{+}$-marker placed in *otr*, *imr*, and *cnt* (Figure 3A). These markers were not expressed in wt cells and deletion of *dcr1*$^{+}$ only caused a desilencing of the *otr* and *imr* regions, but did not affect *cnt*. In contrast, the *med20*Δ mutant caused increased transcriptional activity in *otr*, *imr,* as well as in *cnt*. We found this observation interesting, since it distinguished the role of Med20 in centromere regulation from that of the RNAi machinery. The second RNA degradation pathway involved in centromere regulation utilizes the exosome. We therefore wanted to see if Med20 was involved in the RNAi-independent pathway at the core centromere. The expression of core centromere transcripts has only been described in a small number of publications (363). One reason for this is that the base level of transcription is very low and difficult to detect. We deleted *rrp6*Δ in combination with *med20*Δ and could detect *cnt* transcripts (Figure 3G). This result suggested to us that the RNAi-independent RNA degradation pathway is needed to regulate core centromere transcripts.

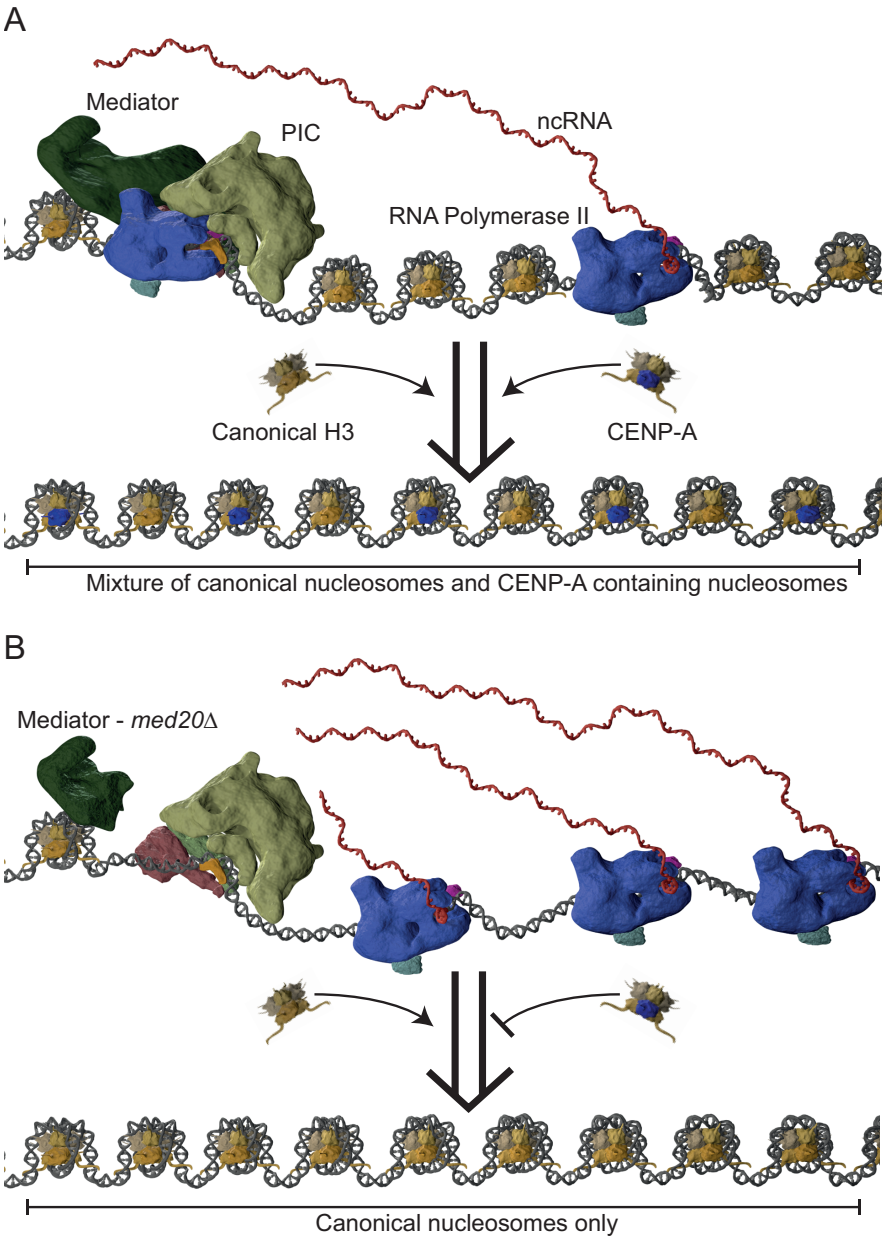

Figure 11. A potential mechanism for Mediator dependent regulation of core centromere function.

A) Mediator inhibits Pol II activity at *cnt,* allowing CENP-A to be reincorporated into centromeric nucleosomes after DNA replication. B) Loss of Med20 from the Mediator leads to increased Pol II transcription and higher nucleosome turn over at the centromere. As a consequence stable association of CENP-A with the centromere is impaired, leading to centromere dysfunction and chromosome segregation defects.

The chromatin structure at the core centromere is characterized by a unique nucleosome composition including the centromere specific H3 variant CENP-A, which replaces the canonical H3 at many of the nucleosomes (328). Since loss of Med20 caused desilencing of *cnt*, we wanted to see if it also affected CENP-A levels. Indeed, centromeric CENP-A levels were decreased in the *med20*$^{+}$ deletion mutant (Figure 4A-B). In fact, the total amount of CENP-A in the cell was significantly reduced in the *med20Δ* strain (Figure 4C), even if the transcript levels encoding for CENP-A remained unchanged (Figure 4D).

We could conclude that expression of the *cnp1*$^{+}$ gene was unaffected, but without proper centromere incorporation, the translated CENP-A protein is rapidly degraded. During replication, CENP-A is removed from the core centromere and the DNA is exposed, enabling transcription of the region (329). We hypothesized that deletion of *med20*$^{+}$ caused increased Pol II activity at the core centromere, which could inhibit CENP-A incorporation. By treating the cells with the RNA polymerase inhibitor Actinomycin D we hoped to restore CENP-A levels at the centromere. Immunostaining experiments did indeed demonstrate that inhibition by Actinomycin D could partly restore CENP-A incorporation at the core centromere (Figure 4E).

The combined data of the study prompted us to suggest a model in which Med20 acts to repress Pol II activity at the centromere (Figure 11 in this thesis). Without Med20 present, the activity of Pol II will increase, which in turn will interfere with CENP-A incorporation, causing kinetochore destabilization and chromosome segregation defects.

3.3 Paper III

In fission yeast, packaging of pericentromeric regions into heterochromatin is required for proper kinetochore assembly and chromosome separation. Heterochromatin formation is a very complicated, multistep process. Parts of the pericentromeric regions are transcribed during S phase and these transcripts are used to direct heterochromatin assembly via the RNAi pathway. Recently, an alternative regulatory pathway has been suggested, which involves the exosome RNA degradation machinery and functions in parallel to the RNAi pathway (364). In support of this idea, the simultaneous loss of both the exosome component Rrp6 and the RNA helicase required for siRNA formation, Dcr1, leads to additive effects with a strong increase in pericentromeric transcription levels.

We wanted to better understand how loss of Med20 could cause the defects in centromere function described in Paper II. Especially, we wanted to find out

if Med20 functioned via the exosome pathway, since in Paper II, we had observed that loss of Med20 in combination with Rrp6 caused increased levels of *cnt* transcripts. We now used strand-specific RNA sequencing and analyzed how loss of Med20 affects centromeric transcription. We found that the levels of pericentromeric transcription were very low and not notably affected by loss of Rrp6 (Figure 1A and B). In *med20Δ* we observed a ~20-40 fold increase in pericentromeric transcript, which could be reduced by the simultaneous deletion of *rrp6*$^{+}$. We also used RT-PCR to quantify transcripts from the *dh* region and obtained supporting results, i.e. that loss of Med20 causes upregulation of *dh* transcripts, which can be partially reversed when *rrp6*$^{+}$ is deleted (Figure 2A).

tRNA genes play an important role in the formation of chromatin boarders between the pericentromeric *imr* and the core centromere. Since loss of Med20 impairs tRNA gene transcription (Paper IV), we wondered if changes in centromeric tRNA transcription patters could explain the observed increase in pericentromeric transcript levels. Based on our findings in a reporter system with a centromere elements and an *ura4*$^{+}$ gene, we rejected this idea (Figure 2B). Deletion of Med20 did not seem to affect boundary function of the centromeric tRNA gene.

Production of siRNA from pericentromeric transcripts guides the assembly of heterochromatin at the pericentromere and we wondered if Rrp6 could influence this process in *med20*Δ cells. Especially since the increased transcript levels that we now observed in the pericentromeric region upon loss of Med20 overlapped with regions of siRNA production (Figure 2C). To address this possibility, we isolated small RNA from wild type and mutant cells and performed strand-specific small RNA sequencing. As expected from our previous publication (Paper II), we observed a reduction of siRNA production in the *med20*Δ cells. However, this effect was not reversed in the *med20*Δ*/rrp6*Δ. We therefore concluded that even if loss of Rrp6 decreases pericentromeric transcripts levels associated with loss of Med20, Rrp6 did not affect the changes in siRNA production observed in *med20Δ* cells.

Next, we monitored if Rrp6 could influence the chromosome segregation defects seen in *med20Δ*. To this end we used the same approach as in Paper II and monitored chromosome segregation following tubulin and DAPI staining. There were no clear effects on chromosome segregation in the *rrp6Δ* strain and loss of Rrp6 did not affect the defects in chromosome segregation observed in *med20Δ* (Figure 4A and B). Therefore, even if loss of Rrp6 can reduce the increased pericentromeric transcript levels seen in *med20Δ*, the exosome component did not affect the defects in chromosome segregation.

Based on our results we could conclude that even if the exosome is involved in regulating the stability of Mediator dependent transcripts, it is not required for the chromosome segregation effects observed in the *med20*$^{+}$ deletion strains. The effects of Mediator and exosome at the centromere is however not completely independent. When we analyzed if Rrp6 could affect the changes centromeric histone modifications caused by *med20Δ* we noted that H3K9me, a prominent marker for heterochromatin formation in the pericentromeric region was decreased *med20*Δ cells (as reported in paper II) and that the H3K9me levels was restored to near wt levels in the *med20/rrp6*Δ strain (Figure 5C).

3.4 Paper IV

In paper II we found that loss of Med20 caused increased core centromere transcription. These transcripts were rapidly degraded by the exosome and to study them, we had to inactivate the exosome by deleting the *rrp6*$^{+}$ gene. We wondered if exosome degradation could mask effects of *med20*Δ also on other transcripts. We therefore sequenced polyadenylated RNA isolated from wild type and mutant strains. Our analysis revealed a strong increase of Pol III dependent transcripts, including tRNA, snRNA, and snoRNA in *med20*Δ*/rrp6*Δ cells. This finding was unexpected, since Mediator is a regulator of specifically Pol II transcription and Pol III transcripts are not normally polyadenylated.

Defective tRNA may be degraded in a process, which involves polyadenylation by the TRAMP complex, which targets the tRNA for destruction by the exosome. We therefore hypothesized that loss of Med20 leads to the formation of aberrant tRNA transcripts, which are polyadenylated by TRAMP. However, since *rrp6*$^{+}$ is deleted, these aberrant transcripts accumulate in the *med20*Δ*/rrp6*Δ cells. Indeed, we noted that the polyadenylated tRNA were much longer than expected, about 200-250 nt compared to an expected length of 70-100 nt (Figure 2A). To follow up this observation, we mapped transcripts at a number of individual tRNA genes. Whereas the 5′ was identical to the normal tRNA, mapping of the 3′ ends revealed that the existence of long, aberrant transcripts. Based on our observations, we concluded that deletion of *med20*$^{+}$ might lead to transcription termination read-through at tRNA gene loci. We could support this conclusion using quantitative PCR analysis and demonstrate that the long transcripts were rapidly degraded when transcription was inhibited with the drug 1,10-phenantroline (Figure 3B). We could also observe the read-through transcript using Northern blot analysis (Figure 3C).

An interesting characteristic of the TRAMP complex is that during poly(A) synthesis it introduces a rather high frequency of bases other than A (Figure 4A and B). To address if the TRAMP complex was responsible for the poly(A) tails on tRNA, we analyzed reads with respect to poly(A) content. Indeed, poly(A)-containing reads that could be mapped to the tRNA gene *GLN01* contained high proportion of bases other than A, strongly supporting the idea that polyadenylation of tRNA transcripts is performed by Cid14, the atypical poly(A) polymerase present in the TRAMP complex.

We also observed that other Pol III-dependent transcripts were affected in the *med20Δ/rrp6Δ* strain. Analyzing 5S rRNA genes, we observed on average a 9-fold increase in the mutant compared to wild type cells (Figure 5A). We also observed a very strong increase in polyadenylated snoRNA and snRNA transcripts (Figure 5B-D). In addition, the Pol III transcripts coding for RNase P and RNase MRP RNAs were also increased in *med20Δ/rrp6Δ*.

In budding yeast *MED20* interacts genetically with *MAF1*, which encodes an evolutionary conserved repressor of Pol III transcription. We therefore addressed if Maf1 could mediate Med20's effect on Pol III transcripts. We used reverse transcription followed by quantitative PCR, to analyze the production of long read-through tRNA transcripts, but the levels of these were not significantly affected by the loss of Maf1 (Figure 6A). Maf1 is activated by dephosphorylation and we also analyzed if Med20 could affect this process. We found that the ratio of dephosphorylated relative phosphorylated Maf1 was slightly higher in the *med20Δ* cells (Figure 6C). Therefore, if anything, Maf1 counteracted the effect of Med20 on Pol III transcription. Since there is so far no evidence to support a physical connection between Mediator and the exosome, we currently believe that the effects of Med20 and Rrp6 on aberrant transcript levels are additive, i.e. Mediator regulates the transcriptional activity and the exosome is needed to degrade the aberrant transcripts.

As demonstrated in our report, mutations in fission yeast Mediator influences both transcription of Pol II and Pol III genes required for translation. These observations are somewhat related to findings in budding yeast, which has demonstrated that Med20 is required for transcription of ribosomal proteins genes. Based on our studies we therefore suggest that Med20 takes part in a pathway that coordinates expression of ribosomal protein genes with Pol III transcription. Unexpectedly, our study suggests that Med20 is involved in repressing certain activities of Pol III. The most likely explanation is that changes in Pol III transcription is due to indirect effects, probably via Pol II dependent genes involved in Pol III function, but we can still not completely

rule out a direct effect of mutant Mediator on the Pol III transcription machinery.

4 CONCLUSION

The Mediator complex is a diverse and multi-functional complex (145). In this thesis we aim to expand our knowledge of Mediator's involvement in ncRNA and chromatin regulation. We show that Mediator is important for the maintenance of silenced chromatin regions such as telomeres, mating type loci, and centromeres. At *S. cerevisiae* telomeres, Mediator is involved in regulating the exact border between silenced and transcriptionally active regions. Mutations affecting Mediator subunits disturb this function and have consequences for replicative ageing in yeast cell. If these findings are of relevance for telomere maintenance and replicative ageing also in higher cells remains to be investigated.

Our findings also demonstrate that Mediator dependent regulation of centromeric transcription is important for proper chromosome segregation. Mutations affecting the Mediator head module cause defects in heterochromatin formation and reduce CENP-A incorporation at fission yeast centromeres, which in turn results in chromosome segregation defects. Mutations in genes encoding Mediator subunits have been linked to the development of several forms of cancer (184) and we believe that our findings may be of relevance for understanding the oncogenic potential of some of these mutations. Chromosomal instability is a driving force in tumorigenesis and CENP-A has for instance been suggested as a prognostic marker in breast cancer (365). In future work, we intend to analyze if Mediator can influence chromosome stability in mammalian cells.

In the thesis, we have also presented data demonstrating that Mediator can influence transcription of ribosomal protein genes as well as Pol III dependent genes. The Mediator-dependent regulatory pathway characterized here is parallel to the repressive pathway involving the classical repressor of Pol III transcription, Maf1. Since Mediator is at the crossroad of many different signaling pathways and in a position to sense the nutritional status of the cell, Med20 could be involved in coordinating Pol II responses to cellular stress with regulation of Pol III activity. However, the underlying mechanisms of this coordination are still obscure and more work is clearly needed to establish the relevance of these ideas.

In conclusion, our work reinforces the idea that Mediator is more then a mere mediator of signals from activator proteins to the basal Pol II transcription machinery at the promoter. Mediator influences vital cellular processes such as cell division, replicative ageing and even Pol III transcription. The complexity of Mediator function is still largely uncharted and the future will bring many new exciting discoveries about this multi-talented complex.

ACKNOWLEDGEMENT

My time as a PhD student has been very exciting and memorable. I have many people to thank for making this experience so great and rewarding.

The most important reasons that this thesis came to fruition, is my main supervisor **Claes Gustafsson.** We first met a late, dark and cold night on 23rd of December. Demonstrating how dedicated you are to your work. As my supervisor you have inspired me and made me excited about research. One conversation with you could remove the frustration I sometimes felt over the massive inertia that surrounds science. You have really increased my confidence as a scientist and I always felt that you trusted my judgment and have given me free reins to pursue the direction of research that I wanted.

Secondly I would like to extend my deepest gratitude to my supervisor, **Xuefeng Zhu**. Without you I wouldn't have been able to succeed in most of my experiments. Your advice and help has been invaluable and I find our many scientific discussions to be stimulating and inspirational. The kindness and patience you have shown me has been endless. You have been great supervisor and I've had a lot of fun with you. The only thing I regret is that we didn't play more table tennis ☺.

To my third supervisor, **Per Elias**, I would like to say that your scientific insight and knowledge is very impressive. You're calm and sharp way of reasoning and analyzing scientific conundrums makes you a very good scientific role model.

Maria Falkenberg, I've always been very impressed with your skill as experimental scientist and even though I have not work with you that much so far, I look forward to do many experiments with you in the future.

A special thank to my partner in crime, **Elisabeth Jemt**. Thank you for all the great moments and endless discussions we've had. I've always liked your energy and curiosity. Hearing you sing in the corridor and calling out 'I love this song', always put a smile on my face. Thanks for learning climbing with me. Even though my interest didn't last too long, I always appreciated our climbing sessions. And don't you worry; Mr and Mrs Overanalyze will strike again!!! Muhahahaha…….

Jennifer Uhler, I always get so happy when I see you. You're always smiling and laughing. Even though I have invaded your lab bench and stolen your equipment far to many times, you have always met me with a smile. The grammatical excellence of this thesis is attributed to your help ☺. Thank you!

I want to extend my appreciation for the stamina of my officemate **Bertil Macao**, who always answered my questions even after I've endlessly

interrupted his work. Whenever I smell a can of mackerel in tomato sauce, I will always think of you.

Zsolt Szilagyi thanks for all your help and practical advice over the years. You are a true Pombe guru. Thank you, **Gabor Banyai,** for many interesting conversations and sharing late hours at the lab. We yeast people have to stick together against the overwhelming horde of the mitochondria cult. So to the Hungarian mafia on floor three I raise my glass and say: Egészségedre!!!!

I also want to thank, **Marcela Davila López**, for the opportunity to work with you. You have opened my eyes for the fascinating and limitless world of bioinformatics. With a smile, you have always helped me and taught me all the things I know about next generation sequencing.

Tore Samuelsson, it has been exciting to explore the world of tRNA with you. You sit in your office silently but your door is always open. There is always a joke, a laugh or a smile lurking behind that quiet façade.

Dr. **Ka-Wei Tang**, thank you for all your initiatives and crazy ideas. I'm glad you have been able to stand next to me through these years, even though you think I smell bad.

Emily Hoberg, what would I do without you? You are the order to my chaos. You have always helped me to find my way. **Örjan Persson,** I've always liked your sense of humor, and you have always been nice to me even when I insulted your girlfriend over and over again. **Triinu Kriisk**, you crazy dog lover. I'm going to miss your good mood and wonderful laugh. **Majda Mehmedovic,** it's so much fun to talk to you. You have always had so many good things to say. **Viktor Posse,** it's always a pleasure to see you work, the calm and the focus. Keep up the good work. **Anna-Karin Berglund**, good luck with your new baby. I'm sure we'll run into each other in Majorna, and then I promise I will join you for lunch ☺. **Yazh Muthukumar,** I would really love to try more of your food. I just hope my taste buds survive. **Yuan-Bo Zhao** it's been exciting to see you experience Sweden for the first time. I really wish you the best of luck when you return to China.

And let's not forget our very own trigger happy Chinese, **Yonghong Shi**. You always make me laugh. I just hope that you don't get arrested for stealing someone else's baby.

Commandant **Monica Olsson** you're the first person to make me feel like the real Hulk. Keep chopping wood. **Isabella Muylaert,** thank you for your kindness. I'm going to miss the competition between you and Hong to hold other peoples babies. **Zhiyuan Zhao**, I've appreciated the interest you show in other people and the fun conversations we've had. Good luck with your PhD. **Erik Larsson**, thank you for teaching me Linux and for letting me abuse your server. You always take time to help me even when you have a thousand things to do.

Tack, **Koviljca Petrovic**, för alla intressanta berättelse och glada skratt. Man blir alltid glad när man ser dig komma rullande med din vagn genom korridoren. Jag kommer sakna att saga hej då till dig när jag kommer till jobbet ☺.

Thank you to all the previous members of our group. I've missed you all. **Javier Miralles Fusté** Good luck in the States. The lab is just not quite the same without you. Keep on climbing just don't fall. **Paulina Wanrooij** and **Sjored Wanrooij** I wish you all the best in your future life together. Just don't kill each other ☺. **Stefan Bäckström** I've always admired your thirst for knowledge. It was really contiguous and I hope it will stay with me for a long time.

Frida thank you for always being my friend and for being the warm, kind and caring person you are. You have helped me through so much over the years and I hope I one day can repay you.

Lurk (Martin, Martin, Nils, Mikael, Erik, Jonas, Oscar) thank you for all the great games and intrigues. Let's hope the future holds as many gaming opportunities as the past. Never let the dies stop rolling.

Andreas I miss you so much. I miss our talks and training sessions. You were a great friend and I will always carry you with me. Sist är bäst!!!!

Staffan its always fun to chill with you and together we will let **Alex** drag us to hell and back. **Ramtin**, thank you for all the crazy things we've done. I think its time to do 'räkan' again soon, don't you think ☺. **Jaakko** thank you for all great parties, high kicks and sauna adventures. I'm so happy you found **Janette** and that you both are my friends.

My beloved mother **Inger**, thank you for your support and help over the years. You're the one that taught me to write as well as I can today. **Mattias**, you have my gratitude for your support and scientific insight. **Lisa,** thank you for your love and support. I've always admired your creative talent and your courage. We all miss **Claes** and I know he would be very proud of us all.

Many thanks to my grandmother **Älsi.** You are a superwoman. I've always looked up to you and the warm person you are. Many thoughts also go to my recently departed grandfather **Professor Arne Carlsten.**

And finally, my beautiful **Sofie.** Thank you for being you, for making me so happy and for putting up with all my quirks. Your loyalty, care and sacrifice for others shows what a wonderful person you are. You have been really supportive and helpful during my PhD and now we both are going to shot for the stars together. And as you know it is so nice that it's finally over ☺.

REFERENCES

1. **Goodfellow SJ, Zomerdijk JC.** 2013. Basic mechanisms in RNA polymerase I transcription of the ribosomal RNA genes. Sub-cellular biochemistry **61:**211-236.
2. **Svetlov V, Nudler E.** 2013. Basic mechanism of transcription by RNA polymerase II. Biochimica et biophysica acta **1829:**20-28.
3. **White RJ.** 2011. Transcription by RNA polymerase III: more complex than we thought. Nature reviews. Genetics **12:**459-463.
4. **Kadonaga JT.** 2012. Perspectives on the RNA polymerase II core promoter. Wiley interdisciplinary reviews. Developmental biology **1:**40-51.
5. **Deng W, Roberts SG.** 2005. A core promoter element downstream of the TATA box that is recognized by TFIIB. Genes & development **19:**2418-2423.
6. **Kandiah E, Trowitzsch S, Gupta K, Haffke M, Berger I.** 2014. More pieces to the puzzle: recent structural insights into class II transcription initiation. Current opinion in structural biology **24C:**91-97.
7. **Thomas MC, Chiang CM.** 2006. The general transcription machinery and general cofactors. Critical reviews in biochemistry and molecular biology **41:**105-178.
8. **Tsai FT, Sigler PB.** 2000. Structural basis of preinitiation complex assembly on human pol II promoters. The EMBO journal **19:**25-36.
9. **Lagrange T, Kim TK, Orphanides G, Ebright YW, Ebright RH, Reinberg D.** 1996. High-resolution mapping of nucleoprotein complexes by site-specific protein-DNA photocrosslinking: organization of the human TBP-TFIIA-TFIIB-DNA quaternary complex. Proceedings of the National Academy of Sciences of the United States of America **93:**10620-10625.
10. **Buratowski S, Zhou H.** 1993. Functional domains of transcription factor TFIIB. Proceedings of the National Academy of Sciences of the United States of America **90:**5633-5637.
11. **Ohkuma Y, Roeder RG.** 1994. Regulation of TFIIH ATPase and kinase activities by TFIIE during active initiation complex formation. Nature **368:**160-163.
12. **Will CL, Luhrmann R.** 2011. Spliceosome structure and function. Cold Spring Harbor perspectives in biology **3**.

13. **Kuehner JN, Pearson EL, Moore C.** 2011. Unravelling the means to an end: RNA polymerase II transcription termination. Nature reviews. Molecular cell biology **12:**283-294.
14. **Kouzarides T.** 2007. Chromatin modifications and their function. Cell **128:**693-705.
15. **Winkler DD, Luger K.** 2011. The histone chaperone FACT: structural insights and mechanisms for nucleosome reorganization. The Journal of biological chemistry **286:**18369-18374.
16. **Weghorn D, Lassig M.** 2013. Fitness landscape for nucleosome positioning. Proceedings of the National Academy of Sciences of the United States of America **110:**10988-10993.
17. **Ho L, Crabtree GR.** 2010. Chromatin remodelling during development. Nature **463:**474-484.
18. **Nikolov DB, Burley SK.** 1997. RNA polymerase II transcription initiation: a structural view. Proceedings of the National Academy of Sciences of the United States of America **94:**15-22.
19. **Bjorklund S, Gustafsson CM.** 2005. The yeast Mediator complex and its regulation. Trends in biochemical sciences **30:**240-244.
20. **Myer VE, Young RA.** 1998. RNA polymerase II holoenzymes and subcomplexes. The Journal of biological chemistry **273:**27757-27760.
21. **Chen HT, Warfield L, Hahn S.** 2007. The positions of TFIIF and TFIIE in the RNA polymerase II transcription preinitiation complex. Nature structural & molecular biology **14:**696-703.
22. **Cramer P, Bushnell DA, Fu J, Gnatt AL, Maier-Davis B, Thompson NE, Burgess RR, Edwards AM, David PR, Kornberg RD.** 2000. Architecture of RNA polymerase II and implications for the transcription mechanism. Science **288:**640-649.
23. **Hsin JP, Sheth A, Manley JL.** 2011. RNAP II CTD phosphorylated on threonine-4 is required for histone mRNA 3' end processing. Science **334:**683-686.
24. **Komarnitsky P, Cho EJ, Buratowski S.** 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes & development **14:**2452-2460.
25. **Ahn SH, Kim M, Buratowski S.** 2004. Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3' end processing. Molecular cell **13:**67-76.
26. **Chapman RD, Heidemann M, Albert TK, Mailhammer R, Flatley A, Meisterernst M, Kremmer E, Eick D.** 2007. Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7. Science **318:**1780-1782.

27. **Hsin JP, Manley JL.** 2012. The RNA polymerase II CTD coordinates transcription and RNA processing. Genes & development **26:**2119-2137.
28. **Acker J, de Graaff M, Cheynel I, Khazak V, Kedinger C, Vigneron M.** 1997. Interactions between the human RNA polymerase II subunits. The Journal of biological chemistry **272:**16815-16821.
29. **Durrin LK, Krontiris TG.** 2002. The thymocyte-specific MAR binding protein, SATB1, interacts in vitro with a novel variant of DNA-directed RNA polymerase II, subunit 11. Genomics **79:**809-817.
30. **Sharma N, Kumari R.** 2013. Rpb4 and Rpb7: multifunctional subunits of RNA polymerase II. Critical reviews in microbiology **39:**362-372.
31. **Tan Q, Prysak MH, Woychik NA.** 2003. Loss of the Rpb4/Rpb7 subcomplex in a mutant form of the Rpb6 subunit shared by RNA polymerases I, II, and III. Molecular and cellular biology **23:**3329-3338.
32. **Kang X, Hu Y, Li Y, Guo X, Jiang X, Lai L, Xia B, Jin C.** 2006. Structural, biochemical, and dynamic characterizations of the hRPB8 subunit of human RNA polymerases. The Journal of biological chemistry **281:**18216-18226.
33. **Lin YC, Choi WS, Gralla JD.** 2005. TFIIH XPB mutants suggest a unified bacterial-like mechanism for promoter opening but not escape. Nature structural & molecular biology **12:**603-607.
34. **Holstege FC, Fiedler U, Timmers HT.** 1997. Three transitions in the RNA polymerase II transcription complex during initiation. The EMBO journal **16:**7468-7480.
35. **Kostrewa D, Zeller ME, Armache KJ, Seizl M, Leike K, Thomm M, Cramer P.** 2009. RNA polymerase II-TFIIB structure and mechanism of transcription initiation. Nature **462:**323-330.
36. **Pal M, Ponticelli AS, Luse DS.** 2005. The role of the transcription bubble and TFIIB in promoter clearance by RNA polymerase II. Molecular cell **19:**101-110.
37. **Buratowski S.** 2009. Progression through the RNA polymerase II CTD cycle. Molecular cell **36:**541-546.
38. **Kobor MS, Archambault J, Lester W, Holstege FC, Gileadi O, Jansma DB, Jennings EG, Kouyoumdjian F, Davidson AR, Young RA, Greenblatt J.** 1999. An unusual eukaryotic protein phosphatase required for transcription by RNA polymerase II and CTD dephosphorylation in S. cerevisiae. Molecular cell **4:**55-62.

39. **Kugel JF, Goodrich JA.** 2002. Translocation after synthesis of a four-nucleotide RNA commits RNA polymerase II to promoter escape. Molecular and cellular biology **22:**762-773.
40. **Yudkovsky N, Ranish JA, Hahn S.** 2000. A transcription reinitiation intermediate that is stabilized by activator. Nature **408:**225-229.
41. **Margaritis T, Holstege FC.** 2008. Poised RNA polymerase II gives pause for thought. Cell **133:**581-584.
42. **Nechaev S, Adelman K.** 2008. Promoter-proximal Pol II: when stalling speeds things up. Cell cycle **7:**1539-1544.
43. **Pei Y, Schwer B, Shuman S.** 2003. Interactions between fission yeast Cdk9, its cyclin partner Pch1, and mRNA capping enzyme Pct1 suggest an elongation checkpoint for mRNA quality control. The Journal of biological chemistry **278:**7180-7188.
44. **Orphanides G, Reinberg D.** 2002. A unified theory of gene expression. Cell **108:**439-451.
45. **Sims RJ, 3rd, Belotserkovskaya R, Reinberg D.** 2004. Elongation by RNA polymerase II: the short and long of it. Genes & development **18:**2437-2468.
46. **Ghosh A, Lima CD.** 2010. Enzymology of RNA cap synthesis. Wiley interdisciplinary reviews. RNA **1:**152-172.
47. **Cheng B, Price DH.** 2007. Properties of RNA polymerase II elongation complexes before and after the P-TEFb-mediated transition into productive elongation. The Journal of biological chemistry **282:**21901-21912.
48. **Cheng B, Li T, Rahl PB, Adamson TE, Loudas NB, Guo J, Varzavand K, Cooper JJ, Hu X, Gnatt A, Young RA, Price DH.** 2012. Functional association of Gdown1 with RNA polymerase II poised on human genes. Molecular cell **45:**38-50.
49. **Smith E, Lin C, Shilatifard A.** 2011. The super elongation complex (SEC) and MLL in development and disease. Genes & development **25:**661-672.
50. **Mohrmann L, Verrijzer CP.** 2005. Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochimica et biophysica acta **1681:**59-73.
51. **Sun M, Lariviere L, Dengl S, Mayer A, Cramer P.** 2010. A tandem SH2 domain in transcription elongation factor Spt6 binds the phosphorylated RNA polymerase II C-terminal repeat domain (CTD). The Journal of biological chemistry **285:**41597-41603.
52. **Bintu L, Kopaczynska M, Hodges C, Lubkowska L, Kashlev M, Bustamante C.** 2011. The elongation rate of RNA polymerase determines the fate of transcribed nucleosomes. Nature structural & molecular biology **18:**1394-1399.

53. **Glover-Cutter K, Kim S, Espinosa J, Bentley DL.** 2008. RNA polymerase II pauses and associates with pre-mRNA processing factors at both ends of genes. Nature structural & molecular biology **15:**71-78.
54. **Nag A, Narsinh K, Martinson HG.** 2007. The poly(A)-dependent transcriptional pause is mediated by CPSF acting on the body of the polymerase. Nature structural & molecular biology **14:**662-669.
55. **Marzluff WF, Wagner EJ, Duronio RJ.** 2008. Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail. Nature reviews. Genetics **9:**843-854.
56. **Anamika K, Gyenis A, Tora L.** 2013. How to stop: the mysterious links among RNA polymerase II occupancy 3' of genes, mRNA 3' processing and termination. Transcription **4:**7-12.
57. **Grosso AR, de Almeida SF, Braga J, Carmo-Fonseca M.** 2012. Dynamic transitions in RNA polymerase II density profiles during transcription termination. Genome research **22:**1447-1456.
58. **Kim M, Ahn SH, Krogan NJ, Greenblatt JF, Buratowski S.** 2004. Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. The EMBO journal **23:**354-364.
59. **Epshtein V, Dutta D, Wade J, Nudler E.** 2010. An allosteric mechanism of Rho-dependent transcription termination. Nature **463:**245-249.
60. **Dengl S, Cramer P.** 2009. Torpedo nuclease Rat1 is insufficient to terminate RNA polymerase II in vitro. The Journal of biological chemistry **284:**21270-21279.
61. **Kawauchi J, Mischo H, Braglia P, Rondon A, Proudfoot NJ.** 2008. Budding yeast RNA polymerases I and II employ parallel mechanisms of transcriptional termination. Genes & development **22:**1082-1092.
62. **Carroll KL, Pradhan DA, Granek JA, Clarke ND, Corden JL.** 2004. Identification of cis elements directing termination of yeast nonpolyadenylated snoRNA transcripts. Molecular and cellular biology **24:**6241-6252.
63. **Chinchilla K, Rodriguez-Molina JB, Ursic D, Finkel JS, Ansari AZ, Culbertson MR.** 2012. Interactions of Sen1, Nrd1, and Nab3 with multiple phosphorylated forms of the Rpb1 C-terminal domain in Saccharomyces cerevisiae. Eukaryotic cell **11:**417-429.
64. **Vasiljeva L, Kim M, Mutschler H, Buratowski S, Meinhart A.** 2008. The Nrd1-Nab3-Sen1 termination complex interacts with the Ser5-phosphorylated RNA polymerase II C-terminal domain. Nature structural & molecular biology **15:**795-804.

65. **Kelleher RJ, 3rd, Flanagan PM, Kornberg RD.** 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61:**1209-1215.
66. **Flanagan PM, Kelleher RJ, 3rd, Sayre MH, Tschochner H, Kornberg RD.** 1991. A mediator required for activation of RNA polymerase II transcription in vitro. Nature **350:**436-438.
67. **Stringer KF, Ingles CJ, Greenblatt J.** 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature **345:**783-786.
68. **Brandl CJ, Struhl K.** 1989. Yeast GCN4 transcriptional activator protein interacts with RNA polymerase II in vitro. Proceedings of the National Academy of Sciences of the United States of America **86:**2652-2656.
69. **Thompson CM, Koleske AJ, Chao DM, Young RA.** 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:**1361-1375.
70. **Kim YJ, Bjorklund S, Li Y, Sayre MH, Kornberg RD.** 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:**599-608.
71. **Myers LC, Kornberg RD.** 2000. Mediator of transcriptional regulation. Annual review of biochemistry **69:**729-749.
72. **Cai G, Chaban YL, Imasaki T, Kovacs JA, Calero G, Penczek PA, Takagi Y, Asturias FJ.** 2012. Interaction of the mediator head module with RNA polymerase II. Structure **20:**899-910.
73. **Tsai KL, Sato S, Tomomori-Sato C, Conaway RC, Conaway JW, Asturias FJ.** 2013. A conserved Mediator-CDK8 kinase module association regulates Mediator-RNA polymerase II interaction. Nature structural & molecular biology **20:**611-619.
74. **Asturias FJ, Jiang YW, Myers LC, Gustafsson CM, Kornberg RD.** 1999. Conserved structures of mediator and RNA polymerase II holoenzyme. Science **283:**985-987.
75. **Dotson MR, Yuan CX, Roeder RG, Myers LC, Gustafsson CM, Jiang YW, Li Y, Kornberg RD, Asturias FJ.** 2000. Structural organization of yeast and mammalian mediator complexes. Proceedings of the National Academy of Sciences of the United States of America **97:**14307-14310.
76. **Soutourina J, Wydau S, Ambroise Y, Boschiero C, Werner M.** 2011. Direct interaction of RNA polymerase II and mediator required for transcription in vivo. Science **331:**1451-1454.
77. **Bourbon HM.** 2008. Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional mediator complex. Nucleic acids research **36:**3993-4008.

78. **Lariviere L, Seizl M, Cramer P.** 2012. A structural perspective on Mediator function. Current opinion in cell biology **24:**305-313.
79. **Davis JA, Takagi Y, Kornberg RD, Asturias FA.** 2002. Structure of the yeast RNA polymerase II holoenzyme: Mediator conformation and polymerase interaction. Molecular cell **10:**409-415.
80. **Lariviere L, Geiger S, Hoeppner S, Rother S, Strasser K, Cramer P.** 2006. Structure and TBP binding of the Mediator head subcomplex Med8-Med18-Med20. Nature structural & molecular biology **13:**895-901.
81. **Baumli S, Hoeppner S, 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.
82. **Brzovic PS, Heikaus CC, Kisselev L, Vernon R, Herbig E, Pacheco D, Warfield L, Littlefield P, Baker D, Klevit RE, Hahn S.** 2011. The acidic transcription activator Gcn4 binds the mediator subunit Gal11/Med15 using a simple protein interface forming a fuzzy complex. Molecular cell **44:**942-953.
83. **Hoeppner S, Baumli S, Cramer P.** 2005. Structure of the mediator subunit cyclin C and its implications for CDK8 function. Journal of molecular biology **350:**833-842.
84. **Koschubs T, Seizl M, Lariviere L, Kurth F, Baumli S, Martin DE, Cramer P.** 2009. Identification, structure, and functional requirement of the Mediator submodule Med7N/31. The EMBO journal **28:**69-80.
85. **Lariviere L, Seizl M, van Wageningen S, Rother S, van de Pasch L, Feldmann H, Strasser K, Hahn S, Holstege FC, Cramer P.** 2008. Structure-system correlation identifies a gene regulatory Mediator submodule. Genes & development **22:**872-877.
86. **Larsson M, Uvell H, Sandstrom J, Ryden P, Selth LA, Bjorklund S.** 2013. Functional studies of the yeast med5, med15 and med16 mediator tail subunits. PloS one **8:**e73137.
87. **Zhang F, Sumibcay L, Hinnebusch AG, Swanson MJ.** 2004. A triad of subunits from the Gal11/tail domain of Srb mediator is an in vivo target of transcriptional activator Gcn4p. Molecular and cellular biology **24:**6871-6886.
88. **Jedidi I, Zhang F, Qiu H, Stahl SJ, Palmer I, Kaufman JD, Nadaud PS, Mukherjee S, Wingfield PT, Jaroniec CP, Hinnebusch AG.** 2010. Activator Gcn4 employs multiple segments of Med15/Gal11, including the KIX domain, to recruit mediator to target genes in vivo. The Journal of biological chemistry **285:**2438-2455.
89. **Vojnic E, Mourao A, Seizl M, Simon B, Wenzeck L, Lariviere L, Baumli S, Baumgart K, Meisterernst M, Sattler M, Cramer P.**

2011. Structure and VP16 binding of the Mediator Med25 activator interaction domain. Nature structural & molecular biology **18:**404-409.

90. **Eletsky A, Ruyechan WT, Xiao R, Acton TB, Montelione GT, Szyperski T.** 2011. Solution NMR structure of MED25(391-543) comprising the activator-interacting domain (ACID) of human mediator subunit 25. Journal of structural and functional genomics **12:**159-166.
91. **Warnmark A, Treuter E, Wright AP, Gustafsson JA.** 2003. Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. Molecular endocrinology **17:**1901-1909.
92. **Herbig E, Warfield L, Fish L, Fishburn J, Knutson BA, Moorefield B, Pacheco D, Hahn S.** 2010. Mechanism of Mediator recruitment by tandem Gcn4 activation domains and three Gal11 activator-binding domains. Molecular and cellular biology **30:**2376-2390.
93. **Meyer KD, Lin SC, Bernecky C, Gao Y, Taatjes DJ.** 2010. p53 activates transcription by directing structural shifts in Mediator. Nature structural & molecular biology **17:**753-760.
94. **Ebmeier CC, Taatjes DJ.** 2010. Activator-Mediator binding regulates Mediator-cofactor interactions. Proceedings of the National Academy of Sciences of the United States of America **107:**11283-11288.
95. **Lariviere L, Plaschka C, Seizl M, Petrotchenko EV, Wenzeck L, Borchers CH, Cramer P.** 2013. Model of the Mediator middle module based on protein cross-linking. Nucleic acids research **41:**9266-9273.
96. **Balciunas D, Galman C, Ronne H, Bjorklund S.** 1999. The Med1 subunit of the yeast mediator complex is involved in both transcriptional activation and repression. Proceedings of the National Academy of Sciences of the United States of America **96:**376-381.
97. **Kang JS, Kim SH, Hwang MS, Han SJ, Lee YC, Kim YJ.** 2001. The structural and functional organization of the yeast mediator complex. The Journal of biological chemistry **276:**42003-42010.
98. **Boube M, Joulia L, Cribbs DL, Bourbon HM.** 2002. Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. Cell **110:**143-151.
99. **Park SW, Li G, Lin YP, Barrero MJ, Ge K, Roeder RG, Wei LN.** 2005. Thyroid hormone-induced juxtaposition of regulatory elements/factors and chromatin remodeling of Crabp1 dependent on MED1/TRAP220. Molecular cell **19:**643-653.

100. **Zhang X, Krutchinsky A, Fukuda A, Chen W, Yamamura S, Chait BT, Roeder RG.** 2005. MED1/TRAP220 exists predominantly in a TRAP/ Mediator subpopulation enriched in RNA polymerase II and is required for ER-mediated transcription. Molecular cell **19:**89-100.
101. **Baidoobonso SM, Guidi BW, Myers LC.** 2007. Med19(Rox3) regulates Intermodule interactions in the Saccharomyces cerevisiae mediator complex. The Journal of biological chemistry **282:**5551-5559.
102. **Robinson PJ, Bushnell DA, Trnka MJ, Burlingame AL, Kornberg RD.** 2012. Structure of the mediator head module bound to the carboxy-terminal domain of RNA polymerase II. Proceedings of the National Academy of Sciences of the United States of America **109:**17931-17935.
103. **Lariviere L, Plaschka C, Seizl M, Wenzeck L, Kurth F, Cramer P.** 2012. Structure of the Mediator head module. Nature **492:**448-451.
104. **Linder T, Rasmussen NN, Samuelsen CO, Chatzidaki E, Baraznenok V, Beve J, Henriksen P, Gustafsson CM, Holmberg S.** 2008. Two conserved modules of Schizosaccharomyces pombe Mediator regulate distinct cellular pathways. Nucleic acids research **36:**2489-2504.
105. **Cai G, Imasaki T, Yamada K, Cardelli F, Takagi Y, Asturias FJ.** 2010. Mediator head module structure and functional interactions. Nature structural & molecular biology **17:**273-279.
106. **Seizl M, Lariviere L, Pfaffeneder T, Wenzeck L, Cramer P.** 2011. Mediator head subcomplex Med11/22 contains a common helix bundle building block with a specific function in transcription initiation complex stabilization. Nucleic acids research **39:**6291-6304.
107. **Wang X, Wang J, Ding Z, Ji J, Sun Q, Cai G.** 2013. Structural flexibility and functional interaction of Mediator Cdk8 module. Protein & cell **4:**911-920.
108. **Knuesel MT, Meyer KD, Bernecky C, Taatjes DJ.** 2009. The human CDK8 subcomplex is a molecular switch that controls Mediator coactivator function. Genes & development **23:**439-451.
109. **Elmlund H, Baraznenok V, Lindahl M, Samuelsen CO, Koeck PJ, Holmberg S, Hebert H, Gustafsson CM.** 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.
110. **Gonzalez D, Hamidi N, Del Sol R, Benschop JJ, Nancy T, Li C, Francis L, Tzouros M, Krijgsveld J, Holstege FC, Conlan RS.**

2014. Suppression of Mediator is regulated by Cdk8-dependent Grr1 turnover of the Med3 coactivator. Proceedings of the National Academy of Sciences of the United States of America **111:**2500-2505.

111. **Ansari SA, Morse RH.** 2013. Mechanisms of Mediator complex action in transcriptional activation. Cellular and molecular life sciences : CMLS **70:**2743-2756.
112. **Kuras L, Borggrefe T, Kornberg RD.** 2003. Association of the Mediator complex with enhancers of active genes. Proceedings of the National Academy of Sciences of the United States of America **100:**13887-13891.
113. **Leroy C, Cormier L, Kuras L.** 2006. Independent recruitment of mediator and SAGA by the activator Met4. Molecular and cellular biology **26:**3149-3163.
114. **Park JM, Werner J, Kim JM, Lis JT, Kim YJ.** 2001. Mediator, not holoenzyme, is directly recruited to the heat shock promoter by HSF upon heat shock. Molecular cell **8:**9-19.
115. **Cosma MP, Panizza S, 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.
116. **Esnault C, Ghavi-Helm Y, Brun S, Soutourina J, Van Berkum N, Boschiero C, Holstege F, Werner M.** 2008. Mediator-dependent recruitment of TFIIH modules in preinitiation complex. Molecular cell **31:**337-346.
117. **Sakurai H, Fukasawa T.** 2003. Artificial recruitment of certain Mediator components affects requirement of basal transcription factor IIE. Genes to cells : devoted to molecular & cellular mechanisms **8:**41-50.
118. **Sakurai H, Fukasawa T.** 1998. Functional correlation among Gal11, transcription factor (TF) IIE, and TFIIH in Saccharomyces cerevisiae. Gal11 and TFIIE cooperatively enhance TFIIH-mediated phosphorylation of RNA polymerase II carboxyl-terminal domain sequences. The Journal of biological chemistry **273:**9534-9538.
119. **Baek HJ, Kang YK, Roeder RG.** 2006. Human Mediator enhances basal transcription by facilitating recruitment of transcription factor IIB during preinitiation complex assembly. The Journal of biological chemistry **281:**15172-15181.
120. **Lee YC, Park JM, Min S, Han SJ, Kim YJ.** 1999. An activator binding module of yeast RNA polymerase II holoenzyme. Molecular and cellular biology **19:**2967-2976.
121. **Kato Y, Habas R, Katsuyama Y, Naar AM, He X.** 2002. A component of the ARC/Mediator complex required for TGF beta/Nodal signalling. Nature **418:**641-646.

122. **Yang F, Vought BW, Satterlee JS, Walker AK, Jim Sun ZY, Watts JL, DeBeaumont R, Saito RM, Hyberts SG, Yang S, Macol C, Iyer L, Tjian R, van den Heuvel S, Hart AC, Wagner G, Naar AM.** 2006. An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. Nature **442:**700-704.
123. **Ansari SA, He Q, Morse RH.** 2009. Mediator complex association with constitutively transcribed genes in yeast. Proceedings of the National Academy of Sciences of the United States of America **106:**16734-16739.
124. **Ansari SA, Ganapathi M, Benschop JJ, Holstege FC, Wade JT, Morse RH.** 2012. Distinct role of Mediator tail module in regulation of SAGA-dependent, TATA-containing genes in yeast. The EMBO journal **31:**44-57.
125. **Fondell JD, Ge H, Roeder RG.** 1996. Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proceedings of the National Academy of Sciences of the United States of America **93:**8329-8333.
126. **Rachez C, Suldan Z, Ward J, Chang CP, Burakov D, Erdjument-Bromage H, Tempst P, Freedman LP.** 1998. A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transactivation in a cell-free system. Genes & development **12:**1787-1800.
127. **Malik S, Guermah M, Yuan CX, Wu W, Yamamura S, Roeder RG.** 2004. Structural and functional organization of TRAP220, the TRAP/mediator subunit that is targeted by nuclear receptors. Molecular and cellular biology **24:**8244-8254.
128. **van de Peppel J, Kettelarij N, van Bakel H, Kockelkorn TT, van Leenen D, Holstege FC.** 2005. Mediator expression profiling epistasis reveals a signal transduction pathway with antagonistic submodules and highly specific downstream targets. Molecular cell **19:**511-522.
129. **Pavri R, Lewis B, Kim TK, Dilworth FJ, Erdjument-Bromage H, Tempst P, de Murcia G, Evans R, Chambon P, Reinberg D.** 2005. PARP-1 determines specificity in a retinoid signaling pathway via direct modulation of mediator. Molecular cell **18:**83-96.
130. **Larschan E, Winston F.** 2005. The Saccharomyces cerevisiae Srb8-Srb11 complex functions with the SAGA complex during Gal4-activated transcription. Molecular and cellular biology **25:**114-123.
131. **Belakavadi M, Fondell JD.** 2010. Cyclin-dependent kinase 8 positively cooperates with Mediator to promote thyroid hormone receptor-dependent transcriptional activation. Molecular and cellular biology **30:**2437-2448.

132. **Zhu X, Wiren M, Sinha I, Rasmussen NN, Linder T, Holmberg S, Ekwall K, Gustafsson CM.** 2006. Genome-wide occupancy profile of mediator and the Srb8-11 module reveals interactions with coding regions. Molecular cell **22:**169-178.
133. **Balamotis MA, Pennella MA, Stevens JL, Wasylyk B, Belmont AS, Berk AJ.** 2009. Complexity in transcription control at the activation domain-mediator interface. Science signaling **2:**ra20.
134. **Espinosa JM.** 2012. Get back TFIIF, don't let me Gdown1. Molecular cell **45:**3-5.
135. **Jishage M, Malik S, Wagner U, Uberheide B, Ishihama Y, Hu X, Chait BT, Gnatt A, Ren B, Roeder RG.** 2012. Transcriptional regulation by Pol II(G) involving mediator and competitive interactions of Gdown1 and TFIIF with Pol II. Molecular cell **45:**51-63.
136. **Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CA, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP, Florens L, Seidel CW, Lin C, Smith ER, Shilatifard A, Conaway RC, Conaway JW.** 2011. Human mediator subunit MED26 functions as a docking site for transcription elongation factors. Cell **146:**92-104.
137. **Diebold ML, Koch M, Loeliger E, Cura V, Winston F, Cavarelli J, Romier C.** 2010. The structure of an Iws1/Spt6 complex reveals an interaction domain conserved in TFIIS, Elongin A and Med26. The EMBO journal **29:**3979-3991.
138. **Sato S, Tomomori-Sato C, Parmely TJ, Florens L, Zybailov B, Swanson SK, Banks CA, Jin J, Cai Y, Washburn MP, Conaway JW, Conaway RC.** 2004. A set of consensus mammalian mediator subunits identified by multidimensional protein identification technology. Molecular cell **14:**685-691.
139. **Donner AJ, Ebmeier CC, Taatjes DJ, Espinosa JM.** 2010. CDK8 is a positive regulator of transcriptional elongation within the serum response network. Nature structural & molecular biology **17:**194-201.
140. **Galbraith MD, Allen MA, Bensard CL, Wang X, Schwinn MK, Qin B, Long HW, Daniels DL, Hahn WC, Dowell RD, Espinosa JM.** 2013. HIF1A employs CDK8-mediator to stimulate RNAPII elongation in response to hypoxia. Cell **153:**1327-1339.
141. **Nock A, Ascano JM, Barrero MJ, Malik S.** 2012. Mediator-regulated transcription through the +1 nucleosome. Molecular cell **48:**837-848.
142. **Mukundan B, Ansari A.** 2011. Novel role for mediator complex subunit Srb5/Med18 in termination of transcription. The Journal of biological chemistry **286:**37053-37057.

143. **Andrau JC, van de Pasch L, Lijnzaad P, Bijma T, Koerkamp MG, van de Peppel J, Werner M, Holstege FC.** 2006. Genome-wide location of the coactivator mediator: Binding without activation and transient Cdk8 interaction on DNA. Molecular cell **22:**179-192.
144. **Mukundan B, Ansari A.** 2013. Srb5/Med18-mediated termination of transcription is dependent on gene looping. The Journal of biological chemistry **288:**11384-11394.
145. **Carlsten JO, Zhu X, Gustafsson CM.** 2013. The multitalented Mediator complex. Trends in biochemical sciences **38:**531-537.
146. **Zhu X, Zhang Y, Bjornsdottir G, Liu Z, Quan A, Costanzo M, Davila Lopez M, Westholm JO, Ronne H, Boone C, Gustafsson CM, Myers LC.** 2011. Histone modifications influence mediator interactions with chromatin. Nucleic acids research **39:**8342-8354.
147. **Lorch Y, Beve J, Gustafsson CM, Myers LC, Kornberg RD.** 2000. Mediator-nucleosome interaction. Molecular cell **6:**197-201.
148. **Chen Z, Zhang C, Wu D, Chen H, Rorick A, Zhang X, Wang Q.** 2011. Phospho-MED1-enhanced UBE2C locus looping drives castration-resistant prostate cancer growth. The EMBO journal **30:**2405-2419.
149. **Phillips-Cremins JE, Sauria ME, Sanyal A, Gerasimova TI, Lajoie BR, Bell JS, Ong CT, Hookway TA, Guo C, Sun Y, Bland MJ, Wagstaff W, Dalton S, McDevitt TC, Sen R, Dekker J, Taylor J, Corces VG.** 2013. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. Cell **153:**1281-1295.
150. **Marsman J, Horsfield JA.** 2012. Long distance relationships: enhancer-promoter communication and dynamic gene transcription. Biochimica et biophysica acta **1819:**1217-1227.
151. **Cena A, Skoneczny M, Chelstowska A, Kowalec P, Natorff R, Kurlandzka A.** 2013. Cohesin Irr1/Scc3 is likely to influence transcription in Saccharomyces cerevisiae via interaction with Mediator complex. Acta biochimica Polonica **60:**233-238.
152. **Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS, Taatjes DJ, Dekker J, Young RA.** 2010. Mediator and cohesin connect gene expression and chromatin architecture. Nature **467:**430-435.
153. **Mehta GD, Rizvi SM, Ghosh SK.** 2012. Cohesin: a guardian of genome integrity. Biochimica et biophysica acta **1823:**1324-1342.
154. **Lai F, Orom UA, Cesaroni M, Beringer M, Taatjes DJ, Blobel GA, Shiekhattar R.** 2013. Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. Nature **494:**497-501.

155. **Meyer KD, Donner AJ, Knuesel MT, York AG, Espinosa JM, Taatjes DJ.** 2008. Cooperative activity of cdk8 and GCN5L within Mediator directs tandem phosphoacetylation of histone H3. The EMBO journal **27:**1447-1457.
156. **Murr R, Vaissiere T, Sawan C, Shukla V, Herceg Z.** 2007. Orchestration of chromatin-based processes: mind the TRRAP. Oncogene **26:**5358-5372.
157. **Ding N, Zhou H, Esteve PO, Chin HG, Kim S, Xu X, Joseph SM, Friez MJ, Schwartz CE, Pradhan S, Boyer TG.** 2008. Mediator links epigenetic silencing of neuronal gene expression with x-linked mental retardation. Molecular cell **31:**347-359.
158. **Lin JJ, Lehmann LW, Bonora G, Sridharan R, Vashisht AA, Tran N, Plath K, Wohlschlegel JA, Carey M.** 2011. Mediator coordinates PIC assembly with recruitment of CHD1. Genes & development **25:**2198-2209.
159. **Peng J, Zhou JQ.** 2012. The tail-module of yeast Mediator complex is required for telomere heterochromatin maintenance. Nucleic acids research **40:**581-593.
160. **Zhu X, Liu B, Carlsten JO, Beve J, Nystrom T, Myers LC, Gustafsson CM.** 2011. Mediator influences telomeric silencing and cellular life span. Molecular and cellular biology **31:**2413-2421.
161. **Liu Z, Myers LC.** 2012. Med5(Nut1) and Med17(Srb4) are direct targets of mediator histone H4 tail interactions. PloS one **7:**e38416.
162. **Askree SH, Yehuda T, Smolikov S, Gurevich R, Hawk J, Coker C, Krauskopf A, Kupiec M, McEachern MJ.** 2004. A genome-wide screen for Saccharomyces cerevisiae deletion mutants that affect telomere length. Proceedings of the National Academy of Sciences of the United States of America **101:**8658-8663.
163. **Oya E, Kato H, Chikashige Y, Tsutsumi C, Hiraoka Y, Murakami Y.** 2013. Mediator directs co-transcriptional heterochromatin assembly by RNA interference-dependent and -independent pathways. PLoS genetics **9:**e1003677.
164. **Carlsten JO, Szilagyi Z, Liu B, Lopez MD, Szaszi E, Djupedal I, Nystrom T, Ekwall K, Gustafsson CM, Zhu X.** 2012. Mediator promotes CENP-a incorporation at fission yeast centromeres. Molecular and cellular biology **32:**4035-4043.
165. **Thorsen M, Hansen H, Venturi M, Holmberg S, Thon G.** 2012. Mediator regulates non-coding RNA transcription at fission yeast centromeres. Epigenetics & chromatin **5:**19.
166. **Spaeth JM, Kim NH, Boyer TG.** 2011. Mediator and human disease. Seminars in cell & developmental biology **22:**776-787.
167. **Muncke N, Jung C, Rudiger H, Ulmer H, Roeth R, Hubert A, Goldmuntz E, Driscoll D, Goodship J, Schon K, Rappold G.**

2003. Missense mutations and gene interruption in PROSIT240, a novel TRAP240-like gene, in patients with congenital heart defect (transposition of the great arteries). Circulation **108:**2843-2850.

168. **Asadollahi R, Oneda B, Sheth F, Azzarello-Burri S, Baldinger R, Joset P, Latal B, Knirsch W, Desai S, Baumer A, Houge G, Andrieux J, Rauch A.** 2013. Dosage changes of MED13L further delineate its role in congenital heart defects and intellectual disability. European journal of human genetics : EJHG **21:**1100-1104.

169. **Chen CP, Chen YY, Chern SR, Wu PS, Su JW, Chen YT, Chen LF, Wang W.** 2013. Prenatal diagnosis and molecular cytogenetic characterization of de novo partial trisomy 12q (12q24.21-->qter) and partial monosomy 6q (6q27-->qter) associated with coarctation of the aorta, ventriculomegaly and thickened nuchal fold. Gene **516:**138-142.

170. **Krebs P, Fan W, Chen YH, Tobita K, Downes MR, Wood MR, Sun L, Li X, Xia Y, Ding N, Spaeth JM, Moresco EM, Boyer TG, Lo CW, Yen J, Evans RM, Beutler B.** 2011. Lethal mitochondrial cardiomyopathy in a hypomorphic Med30 mouse mutant is ameliorated by ketogenic diet. Proceedings of the National Academy of Sciences of the United States of America **108:**19678-19682.

171. **Landles C, Chalk S, Steel JH, Rosewell I, Spencer-Dene B, Lalani el N, Parker MG.** 2003. The thyroid hormone receptor-associated protein TRAP220 is required at distinct embryonic stages in placental, cardiac, and hepatic development. Molecular endocrinology **17:**2418-2435.

172. **Leal A, Huehne K, Bauer F, Sticht H, Berger P, Suter U, Morera B, Del Valle G, Lupski JR, Ekici A, Pasutto F, Endele S, Barrantes R, Berghoff C, Berghoff M, Neundorfer B, Heuss D, Dorn T, Young P, Santolin L, Uhlmann T, Meisterernst M, Sereda MW, Stassart RM, Meyer zu Horste G, Nave KA, Reis A, Rautenstrauss B.** 2009. Identification of the variant Ala335Val of MED25 as responsible for CMT2B2: molecular data, functional studies of the SH3 recognition motif and correlation between wild-type MED25 and PMP22 RNA levels in CMT1A animal models. Neurogenetics **10:**275-287.

173. **Rosman NP, Tarquinio DC, Datseris M, Hou W, Mannheim GB, Emigh CE, Rivkin MJ.** 2011. Postnatal-onset microcephaly: pathogenesis, patterns of growth, and prediction of outcome. Pediatrics **127:**665-671.

174. **Kaufmann R, Straussberg R, Mandel H, Fattal-Valevski A, Ben-Zeev B, Naamati A, Shaag A, Zenvirt S, Konen O, Mimouni-Bloch A, Dobyns WB, Edvardson S, Pines O, Elpeleg O.** 2010.

Infantile cerebral and cerebellar atrophy is associated with a mutation in the MED17 subunit of the transcription preinitiation mediator complex. American journal of human genetics **87:**667-670.

175. **Risheg H, Graham JM, Jr., Clark RD, Rogers RC, Opitz JM, Moeschler JB, Peiffer AP, May M, Joseph SM, Jones JR, Stevenson RE, Schwartz CE, Friez MJ.** 2007. A recurrent mutation in MED12 leading to R961W causes Opitz-Kaveggia syndrome. Nature genetics **39:**451-453.
176. **Rocha PP, Scholze M, Bleiss W, Schrewe H.** 2010. Med12 is essential for early mouse development and for canonical Wnt and Wnt/PCP signaling. Development **137:**2723-2731.
177. **Zhou H, Kim S, Ishii S, Boyer TG.** 2006. Mediator modulates Gli3-dependent Sonic hedgehog signaling. Molecular and cellular biology **26:**8667-8682.
178. **Janody F, Treisman JE.** 2011. Requirements for mediator complex subunits distinguish three classes of notch target genes at the Drosophila wing margin. Developmental dynamics : an official publication of the American Association of Anatomists **240:**2051-2059.
179. **Girard SL, Xiong L, Dion PA, Rouleau GA.** 2011. Where are the missing pieces of the schizophrenia genetics puzzle? Current opinion in genetics & development **21:**310-316.
180. **Philibert RA, Bohle P, Secrest D, Deaderick J, Sandhu H, Crowe R, Black DW.** 2007. The association of the HOPA(12bp) polymorphism with schizophrenia in the NIMH Genetics Initiative for Schizophrenia sample. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics **144B:**743-747.
181. **Schwartz CE, Tarpey PS, Lubs HA, Verloes A, May MM, Risheg H, Friez MJ, Futreal PA, Edkins S, Teague J, Briault S, Skinner C, Bauer-Carlin A, Simensen RJ, Joseph SM, Jones JR, Gecz J, Stratton MR, Raymond FL, Stevenson RE.** 2007. The original Lujan syndrome family has a novel missense mutation (p.N1007S) in the MED12 gene. Journal of medical genetics **44:**472-477.
182. **Wang X, Yang N, Uno E, Roeder RG, Guo S.** 2006. A subunit of the mediator complex regulates vertebrate neuronal development. Proceedings of the National Academy of Sciences of the United States of America **103:**17284-17289.
183. **Lee T, Seeman P.** 1980. Elevation of brain neuroleptic/dopamine receptors in schizophrenia. The American journal of psychiatry **137:**191-197.

184. **Schiano C, Casamassimi A, Rienzo M, de Nigris F, Sommese L, Napoli C.** 2014. Involvement of Mediator complex in malignancy. Biochimica et biophysica acta **1845:**66-83.
185. **Zhu Y, Qi C, Jain S, Le Beau MM, Espinosa R, 3rd, Atkins GB, Lazar MA, Yeldandi AV, Rao MS, Reddy JK.** 1999. Amplification and overexpression of peroxisome proliferator-activated receptor binding protein (PBP/PPARBP) gene in breast cancer. Proceedings of the National Academy of Sciences of the United States of America **96:**10848-10853.
186. **Kang YK, Guermah M, Yuan CX, Roeder RG.** 2002. The TRAP/Mediator coactivator complex interacts directly with estrogen receptors alpha and beta through the TRAP220 subunit and directly enhances estrogen receptor function in vitro. Proceedings of the National Academy of Sciences of the United States of America **99:**2642-2647.
187. **Nagalingam A, Tighiouart M, Ryden L, Joseph L, Landberg G, Saxena NK, Sharma D.** 2012. Med1 plays a critical role in the development of tamoxifen resistance. Carcinogenesis **33:**918-930.
188. **Rod NH, Hansen AM, Nielsen J, Schnohr P, Gronbaek M.** 2009. Low-risk factor profile, estrogen levels, and breast cancer risk among postmenopausal women. International journal of cancer. Journal international du cancer **124:**1935-1940.
189. **Cui J, Germer K, Wu T, Wang J, Luo J, Wang SC, Wang Q, Zhang X.** 2012. Cross-talk between HER2 and MED1 regulates tamoxifen resistance of human breast cancer cells. Cancer research **72:**5625-5634.
190. **Vijayvargia R, May MS, Fondell JD.** 2007. A coregulatory role for the mediator complex in prostate cancer cell proliferation and gene expression. Cancer research **67:**4034-4041.
191. **Kim HJ, Roh MS, Son CH, Kim AJ, Jee HJ, Song N, Kim M, Seo SY, Yoo YH, Yun J.** 2012. Loss of Med1/TRAP220 promotes the invasion and metastasis of human non-small-cell lung cancer cells by modulating the expression of metastasis-related genes. Cancer letters **321:**195-202.
192. **Ndong Jde L, Jean D, Rousselet N, Frade R.** 2009. Down-regulation of the expression of RB18A/MED1, a cofactor of transcription, triggers strong tumorigenic phenotype of human melanoma cells. International journal of cancer. Journal international du cancer **124:**2597-2606.
193. **Lu M, Zhang L, Maul RS, Sartippour MR, Norris A, Whitelegge J, Rao JY, Brooks MN.** 2005. The novel gene EG-1 stimulates cellular proliferation. Cancer research **65:**6159-6166.

194. **Yoon NK, Maresh EL, Elshimali Y, Li A, Horvath S, Seligson DB, Chia D, Goodglick L.** 2010. Elevated MED28 expression predicts poor outcome in women with breast cancer. BMC cancer **10:**335.
195. **Firestein R, Bass AJ, Kim SY, Dunn IF, Silver SJ, Guney I, Freed E, Ligon AH, Vena N, Ogino S, Chheda MG, Tamayo P, Finn S, Shrestha Y, Boehm JS, Jain S, Bojarski E, Mermel C, Barretina J, Chan JA, Baselga J, Tabernero J, Root DE, Fuchs CS, Loda M, Shivdasani RA, Meyerson M, Hahn WC.** 2008. CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. Nature **455:**547-551.
196. **Kapoor A, Goldberg MS, Cumberland LK, Ratnakumar K, Segura MF, Emanuel PO, Menendez S, Vardabasso C, Leroy G, Vidal CI, Polsky D, Osman I, Garcia BA, Hernando E, Bernstein E.** 2010. The histone variant macroH2A suppresses melanoma progression through regulation of CDK8. Nature **468:**1105-1109.
197. **Orioli A, Pascali C, Pagano A, Teichmann M, Dieci G.** 2012. RNA polymerase III transcription control elements: themes and variations. Gene **493:**185-194.
198. **Werner M, Thuriaux P, Soutourina J.** 2009. Structure-function analysis of RNA polymerases I and III. Current opinion in structural biology **19:**740-745.
199. **Kassavetis GA, Geiduschek EP.** 2006. Transcription factor TFIIIB and transcription by RNA polymerase III. Biochemical Society transactions **34:**1082-1087.
200. **Fernandez-Tornero C, Bottcher B, Riva M, Carles C, Steuerwald U, Ruigrok RW, Sentenac A, Muller CW, Schoehn G.** 2007. Insights into transcription initiation and termination from the electron microscopy structure of yeast RNA polymerase III. Molecular cell **25:**813-823.
201. **Brun I, Sentenac A, Werner M.** 1997. Dual role of the C34 subunit of RNA polymerase III in transcription initiation. The EMBO journal **16:**5730-5741.
202. **Ehara H, Sekine S, Yokoyama S.** 2011. Crystal structure of the C17/25 subcomplex from Schizosaccharomyces pombe RNA polymerase III. Protein science : a publication of the Protein Society **20:**1558-1565.
203. **Landrieux E, Alic N, Ducrot C, Acker J, Riva M, Carles C.** 2006. A subcomplex of RNA polymerase III subunits involved in transcription termination and reinitiation. The EMBO journal **25:**118-128.
204. **Carter R, Drouin G.** 2010. The increase in the number of subunits in eukaryotic RNA polymerase III relative to RNA polymerase II is

due to the permanent recruitment of general transcription factors. Molecular biology and evolution **27:**1035-1043.

205. **Noma K, Cam HP, Maraia RJ, Grewal SI.** 2006. A role for TFIIIC transcription factor complex in genome organization. Cell **125:**859-872.

206. **Teichmann M, Wang Z, Roeder RG.** 2000. A stable complex of a novel transcription factor IIB- related factor, human TFIIIB50, and associated proteins mediate selective transcription by RNA polymerase III of genes with upstream promoter elements. Proceedings of the National Academy of Sciences of the United States of America **97:**14200-14205.

207. **Schramm L, Hernandez N.** 2002. Recruitment of RNA polymerase III to its target promoters. Genes & development **16:**2593-2620.

208. **Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A.** 2007. The expanding RNA polymerase III transcriptome. Trends in genetics : TIG **23:**614-622.

209. **Roberts DN, Stewart AJ, Huff JT, Cairns BR.** 2003. The RNA polymerase III transcriptome revealed by genome-wide localization and activity-occupancy relationships. Proceedings of the National Academy of Sciences of the United States of America **100:**14695-14700.

210. **Guffanti E, Ferrari R, Preti M, Forloni M, Harismendy O, Lefebvre O, Dieci G.** 2006. A minimal promoter for TFIIIC-dependent in vitro transcription of snoRNA and tRNA genes by RNA polymerase III. The Journal of biological chemistry **281:**23945-23957.

211. **Bachellerie JP, Cavaille J, Huttenhofer A.** 2002. The expanding snoRNA world. Biochimie **84:**775-790.

212. **Kruszka K, Barneche F, Guyot R, Ailhas J, Meneau I, Schiffer S, Marchfelder A, Echeverria M.** 2003. Plant dicistronic tRNA-snoRNA genes: a new mode of expression of the small nucleolar RNAs processed by RNase Z. The EMBO journal **22:**621-632.

213. **Deng W, Zhu X, Skogerbo G, Zhao Y, Fu Z, Wang Y, He H, Cai L, Sun H, Liu C, Li B, Bai B, Wang J, Jia D, Sun S, He H, Cui Y, Wang Y, Bu D, Chen R.** 2006. Organization of the Caenorhabditis elegans small non-coding transcriptome: genomic features, biogenesis, and expression. Genome research **16:**20-29.

214. **Isogai Y, Takada S, Tjian R, Keles S.** 2007. Novel TRF1/BRF target genes revealed by genome-wide analysis of Drosophila Pol III transcription. The EMBO journal **26:**79-89.

215. **Braglia P, Percudani R, Dieci G.** 2005. Sequence context effects on oligo(dT) termination signal recognition by Saccharomyces

cerevisiae RNA polymerase III. The Journal of biological chemistry **280:**19551-19562.

216. **Nielsen S, Yuzenkova Y, Zenkin N.** 2013. Mechanism of eukaryotic RNA polymerase III transcription termination. Science **340:**1577-1580.
217. **Fernandez-Tornero C, Bottcher B, Rashid UJ, Steuerwald U, Florchinger B, Devos DP, Lindner D, Muller CW.** 2010. Conformational flexibility of RNA polymerase III during transcriptional elongation. The EMBO journal **29:**3762-3772.
218. **Rijal K, Maraia RJ.** 2013. RNA polymerase III mutants in TFIIFalpha-like C37 that cause terminator readthrough with no decrease in transcription output. Nucleic acids research **41:**139-155.
219. **Iben JR, Mazeika JK, Hasson S, Rijal K, Arimbasseri AG, Russo AN, Maraia RJ.** 2011. Point mutations in the Rpb9-homologous domain of Rpc11 that impair transcription termination by RNA polymerase III. Nucleic acids research **39:**6100-6113.
220. **Schmidt MJ, Norbury CJ.** 2010. Polyadenylation and beyond: emerging roles for noncanonical poly(A) polymerases. Wiley interdisciplinary reviews. RNA **1:**142-151.
221. **Tian B, Graber JH.** 2012. Signals for pre-mRNA cleavage and polyadenylation. Wiley interdisciplinary reviews. RNA **3:**385-396.
222. **Viphakone N, Voisinet-Hakil F, Minvielle-Sebastia L.** 2008. Molecular dissection of mRNA poly(A) tail length control in yeast. Nucleic acids research **36:**2418-2433.
223. **Lemay JF, Lemieux C, St-Andre O, Bachand F.** 2010. Crossing the borders: poly(A)-binding proteins working on both sides of the fence. RNA biology **7:**291-295.
224. **Hector RE, Nykamp KR, Dheur S, Anderson JT, Non PJ, Urbinati CR, Wilson SM, Minvielle-Sebastia L, Swanson MS.** 2002. Dual requirement for yeast hnRNP Nab2p in mRNA poly(A) tail length control and nuclear export. The EMBO journal **21:**1800-1810.
225. **Derry MC, Yanagiya A, Martineau Y, Sonenberg N.** 2006. Regulation of poly(A)-binding protein through PABP-interacting proteins. Cold Spring Harbor symposia on quantitative biology **71:**537-543.
226. **Weill L, Belloc E, Bava FA, Mendez R.** 2012. Translational control by changes in poly(A) tail length: recycling mRNAs. Nature structural & molecular biology **19:**577-585.
227. **Goldstrohm AC, Seay DJ, Hook BA, Wickens M.** 2007. PUF protein-mediated deadenylation is catalyzed by Ccr4p. The Journal of biological chemistry **282:**109-114.

228. **Barreau C, Paillard L, Osborne HB.** 2005. AU-rich elements and associated factors: are there unifying principles? Nucleic acids research **33:**7138-7150.
229. **Sartain CV, Cui J, Meisel RP, Wolfner MF.** 2011. The poly(A) polymerase GLD2 is required for spermatogenesis in Drosophila melanogaster. Development **138:**1619-1629.
230. **Rissland OS, Mikulasova A, Norbury CJ.** 2007. Efficient RNA polyuridylation by noncanonical poly(A) polymerases. Molecular and cellular biology **27:**3612-3624.
231. **Rissland OS, Norbury CJ.** 2009. Decapping is preceded by 3' uridylation in a novel pathway of bulk mRNA turnover. Nature structural & molecular biology **16:**616-623.
232. **LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D.** 2005. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell **121:**713-724.
233. **San Paolo S, Vanacova S, Schenk L, Scherrer T, Blank D, Keller W, Gerber AP.** 2009. Distinct roles of non-canonical poly(A) polymerases in RNA metabolism. PLoS genetics **5:**e1000555.
234. **Buhler M, Haas W, Gygi SP, Moazed D.** 2007. RNAi-dependent and -independent RNA turnover mechanisms contribute to heterochromatic gene silencing. Cell **129:**707-721.
235. **Keller C, Woolcock K, Hess D, Buhler M.** 2010. Proteomic and functional analysis of the noncanonical poly(A) polymerase Cid14. Rna **16:**1124-1129.
236. **Jia H, Wang X, Liu F, Guenther UP, Srinivasan S, Anderson JT, Jankowsky E.** 2011. The RNA helicase Mtr4p modulates polyadenylation in the TRAMP complex. Cell **145:**890-901.
237. **International Human Genome Sequencing C.** 2004. Finishing the euchromatic sequence of the human genome. Nature **431:**931-945.
238. **Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ.** 2008. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nature genetics **40:**1413-1415.
239. **Chen HC, Tseng CK, Tsai RT, Chung CS, Cheng SC.** 2013. Link of NTR-mediated spliceosome disassembly with DEAH-box ATPases Prp2, Prp16, and Prp22. Molecular and cellular biology **33:**514-525.
240. **Abelson J, Trotta CR, Li H.** 1998. tRNA splicing. The Journal of biological chemistry **273:**12685-12688.
241. **Trotta CR, Miao F, Arn EA, Stevens SW, Ho CK, Rauhut R, Abelson JN.** 1997. The yeast tRNA splicing endonuclease: a tetrameric enzyme with two active site subunits homologous to the archaeal tRNA endonucleases. Cell **89:**849-858.

242. **Phizicky EM, Hopper AK.** 2010. tRNA biology charges to the front. Genes & development **24:**1832-1860.
243. **Xiao S, Scott F, Fierke CA, Engelke DR.** 2002. Eukaryotic ribonuclease P: a plurality of ribonucleoprotein enzymes. Annual review of biochemistry **71:**165-189.
244. **Maraia RJ, Lamichhane TN.** 2011. 3' processing of eukaryotic precursor tRNAs. Wiley interdisciplinary reviews. RNA **2:**362-375.
245. **Copela LA, Fernandez CF, Sherrer RL, Wolin SL.** 2008. Competition between the Rex1 exonuclease and the La protein affects both Trf4p-mediated RNA quality control and pre-tRNA maturation. Rna **14:**1214-1227.
246. **Shan X, Russell TA, Paul SM, Kushner DB, Joyce PB.** 2008. Characterization of a temperature-sensitive mutation that impairs the function of yeast tRNA nucleotidyltransferase. Yeast **25:**219-233.
247. **Mori S, Kajita T, Endo T, Yoshihisa T.** 2011. The intron of tRNA-TrpCCA is dispensable for growth and translation of Saccharomyces cerevisiae. Rna **17:**1760-1769.
248. **Lund E, Dahlberg JE.** 1998. Proofreading and aminoacylation of tRNAs before export from the nucleus. Science **282:**2082-2085.
249. **Yoshihisa T, Yunoki-Esaki K, Ohshima C, Tanaka N, Endo T.** 2003. Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. Molecular biology of the cell **14:**3266-3279.
250. **El Yacoubi B, Bailly M, de Crecy-Lagard V.** 2012. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. Annual review of genetics **46:**69-95.
251. **Gerber AP, Keller W.** 1999. An adenosine deaminase that generates inosine at the wobble position of tRNAs. Science **286:**1146-1149.
252. **El Yacoubi B, Lyons B, Cruz Y, Reddy R, Nordin B, Agnelli F, Williamson JR, Schimmel P, Swairjo MA, de Crecy-Lagard V.** 2009. The universal YrdC/Sua5 family is required for the formation of threonylcarbamoyladenosine in tRNA. Nucleic acids research **37:**2894-2909.
253. **Sengupta R, Vainauskas S, Yarian C, Sochacka E, Malkiewicz A, Guenther RH, Koshlap KM, Agris PF.** 2000. Modified constructs of the tRNA TPsiC domain to probe substrate conformational requirements of m(1)A(58) and m(5)U(54) tRNA methyltransferases. Nucleic acids research **28:**1374-1380.
254. **Newby MI, Greenbaum NL.** 2001. A conserved pseudouridine modification in eukaryotic U2 snRNA induces a change in branch-site architecture. Rna **7:**833-845.

255. **Nameki N, Asahara H, Shimizu M, Okada N, Himeno H.** 1995. Identity elements of Saccharomyces cerevisiae tRNA(His). Nucleic acids research **23:**389-394.
256. **Muramatsu T, Nishikawa K, Nemoto F, Kuchino Y, Nishimura S, Miyazawa T, Yokoyama S.** 1988. Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification. Nature **336:**179-181.
257. **Glaunsinger BA, Lee YJ.** 2010. How tails define the ending: divergent roles for polyadenylation in RNA stability and gene expression. RNA biology **7:**13-17.
258. **Januszyk K, Lima CD.** 2014. The eukaryotic RNA exosome. Current opinion in structural biology **24C:**132-140.
259. **Morrissey JP, Deardorff JA, Hebron C, Sachs AB.** 1999. Decapping of stabilized, polyadenylated mRNA in yeast pab1 mutants. Yeast **15:**687-702.
260. **Hamill S, Wolin SL, Reinisch KM.** 2010. Structure and function of the polymerase core of TRAMP, a RNA surveillance complex. Proceedings of the National Academy of Sciences of the United States of America **107:**15045-15050.
261. **Ibrahim H, Wilusz J, Wilusz CJ.** 2008. RNA recognition by 3'-to-5' exonucleases: the substrate perspective. Biochimica et biophysica acta **1779:**256-265.
262. **Lee G, Bratkowski MA, Ding F, Ke A, Ha T.** 2012. Elastic coupling between RNA degradation and unwinding by an exoribonuclease. Science **336:**1726-1729.
263. **Liu Q, Greimann JC, Lima CD.** 2006. Reconstitution, activities, and structure of the eukaryotic RNA exosome. Cell **127:**1223-1237.
264. **Tomecki R, Kristiansen MS, Lykke-Andersen S, Chlebowski A, Larsen KM, Szczesny RJ, Drazkowska K, Pastula A, Andersen JS, Stepien PP, Dziembowski A, Jensen TH.** 2010. The human core exosome interacts with differentially localized processive RNases: hDIS3 and hDIS3L. The EMBO journal **29:**2342-2357.
265. **Wasmuth EV, Lima CD.** 2012. Exo- and endoribonucleolytic activities of yeast cytoplasmic and nuclear RNA exosomes are dependent on the noncatalytic core and central channel. Molecular cell **48:**133-144.
266. **Makino DL, Baumgartner M, Conti E.** 2013. Crystal structure of an RNA-bound 11-subunit eukaryotic exosome complex. Nature **495:**70-75.
267. **Schaeffer D, Tsanova B, Barbas A, Reis FP, Dastidar EG, Sanchez-Rotunno M, Arraiano CM, van Hoof A.** 2009. The exosome contains domains with specific endoribonuclease,

exoribonuclease and cytoplasmic mRNA decay activities. Nature structural & molecular biology **16:**56-62.

268. **Lorentzen E, Basquin J, Tomecki R, Dziembowski A, Conti E.** 2008. Structure of the active subunit of the yeast exosome core, Rrp44: diverse modes of substrate recruitment in the RNase II nuclease family. Molecular cell **29:**717-728.
269. **Tomecki R, Dziembowski A.** 2010. Novel endoribonucleases as central players in various pathways of eukaryotic RNA metabolism. Rna **16:**1692-1724.
270. **Hilleren P, McCarthy T, Rosbash M, Parker R, Jensen TH.** 2001. Quality control of mRNA 3'-end processing is linked to the nuclear exosome. Nature **413:**538-542.
271. **Schneider C, Kudla G, Wlotzka W, Tuck A, Tollervey D.** 2012. Transcriptome-wide analysis of exosome targets. Molecular cell **48:**422-433.
272. **Carneiro T, Carvalho C, Braga J, Rino J, Milligan L, Tollervey D, Carmo-Fonseca M.** 2007. Depletion of the yeast nuclear exosome subunit Rrp6 results in accumulation of polyadenylated RNAs in a discrete domain within the nucleolus. Molecular and cellular biology **27:**4157-4165.
273. **Hopper AK, Pai DA, Engelke DR.** 2010. Cellular dynamics of tRNAs and their genes. FEBS letters **584:**310-317.
274. **Callahan KP, Butler JS.** 2008. Evidence for core exosome independent function of the nuclear exoribonuclease Rrp6p. Nucleic acids research **36:**6645-6655.
275. **Halbach F, Reichelt P, Rode M, Conti E.** 2013. The yeast ski complex: crystal structure and RNA channeling to the exosome complex. Cell **154:**814-826.
276. **Butler JS, Mitchell P.** 2011. Rrp6, rrp47 and cofactors of the nuclear exosome. Advances in experimental medicine and biology **702:**91-104.
277. **Milligan L, Decourty L, Saveanu C, Rappsilber J, Ceulemans H, Jacquier A, Tollervey D.** 2008. A yeast exosome cofactor, Mpp6, functions in RNA surveillance and in the degradation of noncoding RNA transcripts. Molecular and cellular biology **28:**5446-5457.
278. **Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R, Pruijn GJ, Stoecklin G, Moroni C, Mann M, Karin M.** 2001. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. Cell **107:**451-464.
279. **Tran H, Schilling M, Wirbelauer C, Hess D, Nagamine Y.** 2004. Facilitation of mRNA deadenylation and decay by the exosome-bound, DExH protein RHAU. Molecular cell **13:**101-111.

280. **Grzechnik P, Kufel J.** 2008. Polyadenylation linked to transcription termination directs the processing of snoRNA precursors in yeast. Molecular cell **32:**247-258.
281. **Wlotzka W, Kudla G, Granneman S, Tollervey D.** 2011. The nuclear RNA polymerase II surveillance system targets polymerase III transcripts. The EMBO journal **30:**1790-1803.
282. **Orban TI, Izaurralde E.** 2005. Decay of mRNAs targeted by RISC requires XRN1, the Ski complex, and the exosome. Rna **11:**459-469.
283. **Fang Y, Bateman JF, Mercer JF, Lamande SR.** 2013. Nonsense-mediated mRNA decay of collagen -emerging complexity in RNA surveillance mechanisms. Journal of cell science **126:**2551-2560.
284. **Ge Z, Mehta P, Richards J, Karzai AW.** 2010. Non-stop mRNA decay initiates at the ribosome. Molecular microbiology **78:**1159-1170.
285. **Harigaya Y, Parker R.** 2010. No-go decay: a quality control mechanism for RNA in translation. Wiley interdisciplinary reviews. RNA **1:**132-141.
286. **Coller J, Parker R.** 2004. Eukaryotic mRNA decapping. Annual review of biochemistry **73:**861-890.
287. **LaGrandeur TE, Parker R.** 1998. Isolation and characterization of Dcp1p, the yeast mRNA decapping enzyme. The EMBO journal **17:**1487-1496.
288. **Jones CI, Zabolotskaya MV, Newbury SF.** 2012. The 5' --> 3' exoribonuclease XRN1/Pacman and its functions in cellular processes and development. Wiley interdisciplinary reviews. RNA **3:**455-468.
289. **Schwartz D, Decker CJ, Parker R.** 2003. The enhancer of decapping proteins, Edc1p and Edc2p, bind RNA and stimulate the activity of the decapping enzyme. Rna **9:**239-251.
290. **Castel SE, Martienssen RA.** 2013. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. Nature reviews. Genetics **14:**100-112.
291. **Lee SH, Chung BH, Park TG, Nam YS, Mok H.** 2012. Small-interfering RNA (siRNA)-based functional micro- and nanostructures for efficient and selective gene silencing. Accounts of chemical research **45:**1014-1025.
292. **Di Leva G, Garofalo M, Croce CM.** 2014. MicroRNAs in cancer. Annual review of pathology **9:**287-314.
293. **Luteijn MJ, Ketting RF.** 2013. PIWI-interacting RNAs: from generation to transgenerational epigenetics. Nature reviews. Genetics **14:**523-534.
294. **Saini HK, Griffiths-Jones S, Enright AJ.** 2007. Genomic analysis of human microRNA transcripts. Proceedings of the National

Academy of Sciences of the United States of America **104:**17719-17724.

295. **Mattioli C, Pianigiani G, Pagani F.** 2013. A competitive regulatory mechanism discriminates between juxtaposed splice sites and pri-miRNA structures. Nucleic acids research **41:**8680-8691.
296. **Shabalina SA, Koonin EV.** 2008. Origins and evolution of eukaryotic RNA interference. Trends in ecology & evolution **23:**578-587.
297. **Tan GS, Chiu CH, Garchow BG, Metzler D, Diamond SL, Kiriakidou M.** 2012. Small molecule inhibition of RISC loading. ACS chemical biology **7:**403-410.
298. **Kawamata T, Tomari Y.** 2010. Making RISC. Trends in biochemical sciences **35:**368-376.
299. **Khvorova A, Reynolds A, Jayasena SD.** 2003. Functional siRNAs and miRNAs exhibit strand bias. Cell **115:**209-216.
300. **Wilson RC, Doudna JA.** 2013. Molecular mechanisms of RNA interference. Annual review of biophysics **42:**217-239.
301. **Hauptmann J, Dueck A, Harlander S, Pfaff J, Merkl R, Meister G.** 2013. Turning catalytically inactive human Argonaute proteins into active slicer enzymes. Nature structural & molecular biology **20:**814-817.
302. **Iwasaki S, Tomari Y.** 2009. Argonaute-mediated translational repression (and activation). Fly **3:**204-206.
303. **Eulalio A, Huntzinger E, Izaurralde E.** 2008. GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. Nature structural & molecular biology **15:**346-353.
304. **Lingel A, Simon B, Izaurralde E, Sattler M.** 2004. Nucleic acid 3'-end recognition by the Argonaute2 PAZ domain. Nature structural & molecular biology **11:**576-577.
305. **Buker SM, Motamedi MR.** 2011. Purification of native Argonaute complexes from the fission yeast Schizosaccharomyces pombe. Methods in molecular biology **725:**1-13.
306. **MacRae IJ, Doudna JA.** 2007. Ribonuclease revisited: structural insights into ribonuclease III family enzymes. Current opinion in structural biology **17:**138-145.
307. **MacRae IJ, Zhou K, Doudna JA.** 2007. Structural determinants of RNA recognition and cleavage by Dicer. Nature structural & molecular biology **14:**934-940.
308. **Gan J, Tropea JE, Austin BP, Court DL, Waugh DS, Ji X.** 2006. Structural insight into the mechanism of double-stranded RNA processing by ribonuclease III. Cell **124:**355-366.

309. **Cenik ES, Fukunaga R, Lu G, Dutcher R, Wang Y, Tanaka Hall TM, Zamore PD.** 2011. Phosphate and R2D2 restrict the substrate specificity of Dicer-2, an ATP-driven ribonuclease. Molecular cell **42:**172-184.
310. **Tsutsumi A, Kawamata T, Izumi N, Seitz H, Tomari Y.** 2011. Recognition of the pre-miRNA structure by Drosophila Dicer-1. Nature structural & molecular biology **18:**1153-1158.
311. **Maida Y, Masutomi K.** 2011. RNA-dependent RNA polymerases in RNA silencing. Biological chemistry **392:**299-304.
312. **Wang XB, Wu Q, Ito T, Cillo F, Li WX, Chen X, Yu JL, Ding SW.** 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America **107:**484-489.
313. **Lehmann E, Brueckner F, Cramer P.** 2007. Molecular basis of RNA-dependent RNA polymerase II activity. Nature **450:**445-449.
314. **Motamedi MR, Verdel A, Colmenares SU, Gerber SA, Gygi SP, Moazed D.** 2004. Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell **119:**789-802.
315. **Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ.** 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature **389:**251-260.
316. **Davey CA, Sargent DF, Luger K, Maeder AW, Richmond TJ.** 2002. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. Journal of molecular biology **319:**1097-1113.
317. **Kowalski A, Palyga J.** 2012. Linker histone subtypes and their allelic variants. Cell biology international **36:**981-996.
318. **Mueller-Planitz F, Klinker H, Becker PB.** 2013. Nucleosome sliding mechanisms: new twists in a looped history. Nature structural & molecular biology **20:**1026-1032.
319. **Karmodiya K, Krebs AR, Oulad-Abdelghani M, Kimura H, Tora L.** 2012. H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. BMC genomics **13:**424.
320. **French BT, Straight AF.** 2013. Swapping CENP-A at the centromere. Nature cell biology **15:**1028-1030.
321. **Sharma U, Stefanova D, Holmes SG.** 2013. Histone variant H2A.Z functions in sister chromatid cohesion in Saccharomyces cerevisiae. Molecular and cellular biology **33:**3473-3481.

322. **Hansen KR, Hazan I, Shanker S, Watt S, Verhein-Hansen J, Bahler J, Martienssen RA, Partridge JF, Cohen A, Thon G.** 2011. H3K9me-independent gene silencing in fission yeast heterochromatin by Clr5 and histone deacetylases. PLoS genetics **7:**e1001268.
323. **Thompson SL, Bakhoum SF, Compton DA.** 2010. Mechanisms of chromosomal instability. Current biology : CB **20:**R285-295.
324. **Tanaka K.** 2013. Regulatory mechanisms of kinetochore-microtubule interaction in mitosis. Cellular and molecular life sciences : CMLS **70:**559-579.
325. **Black BE, Bassett EA.** 2008. The histone variant CENP-A and centromere specification. Current opinion in cell biology **20:**91-100.
326. **Heun P, Erhardt S, Blower MD, Weiss S, Skora AD, Karpen GH.** 2006. Mislocalization of the Drosophila centromere-specific histone CID promotes formation of functional ectopic kinetochores. Developmental cell **10:**303-315.
327. **Sullivan BA, Karpen GH.** 2004. Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. Nature structural & molecular biology **11:**1076-1083.
328. **Bergmann JH, Rodriguez MG, Martins NM, Kimura H, Kelly DA, Masumoto H, Larionov V, Jansen LE, Earnshaw WC.** 2011. Epigenetic engineering shows H3K4me2 is required for HJURP targeting and CENP-A assembly on a synthetic human kinetochore. The EMBO journal **30:**328-340.
329. **Nechemia-Arbely Y, Fachinetti D, Cleveland DW.** 2012. Replicating centromeric chromatin: spatial and temporal control of CENP-A assembly. Experimental cell research **318:**1353-1360.
330. **Cheeseman IM, Desai A.** 2008. Molecular architecture of the kinetochore-microtubule interface. Nature reviews. Molecular cell biology **9:**33-46.
331. **Joglekar AP, Bouck D, Finley K, Liu X, Wan Y, Berman J, He X, Salmon ED, Bloom KS.** 2008. Molecular architecture of the kinetochore-microtubule attachment site is conserved between point and regional centromeres. The Journal of cell biology **181:**587-594.
332. **Alper BJ, Lowe BR, Partridge JF.** 2012. Centromeric heterochromatin assembly in fission yeast--balancing transcription, RNA interference and chromatin modification. Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology **20:**521-534.
333. **Pidoux AL, Allshire RC.** 2005. The role of heterochromatin in centromere function. Philosophical transactions of the Royal Society of London. Series B, Biological sciences **360:**569-579.

334. **Chan FL, Wong LH.** 2012. Transcription in the maintenance of centromere chromatin identity. Nucleic acids research **40:**11178-11188.
335. **Thon G.** 2008. Histone modifications: cycling with chromosomal replication. Current biology : CB **18:**R380-382.
336. **Lejeune E, Bayne EH, Allshire RC.** 2010. On the connection between RNAi and heterochromatin at centromeres. Cold Spring Harbor symposia on quantitative biology **75:**275-283.
337. **Zhang K, Mosch K, Fischle W, Grewal SI.** 2008. Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. Nature structural & molecular biology **15:**381-388.
338. **Buscaino A, Lejeune E, Audergon P, Hamilton G, Pidoux A, Allshire RC.** 2013. Distinct roles for Sir2 and RNAi in centromeric heterochromatin nucleation, spreading and maintenance. The EMBO journal **32:**1250-1264.
339. **Kratz A, Arner E, Saito R, Kubosaki A, Kawai J, Suzuki H, Carninci P, Arakawa T, Tomita M, Hayashizaki Y, Daub CO.** 2010. Core promoter structure and genomic context reflect histone 3 lysine 9 acetylation patterns. BMC genomics **11:**257.
340. **Scott KC, White CV, Willard HF.** 2007. An RNA polymerase III-dependent heterochromatin barrier at fission yeast centromere 1. PloS one **2:**e1099.
341. **Palm W, de Lange T.** 2008. How shelterin protects mammalian telomeres. Annual review of genetics **42:**301-334.
342. **Chan SR, Blackburn EH.** 2004. Telomeres and telomerase. Philosophical transactions of the Royal Society of London. Series B, Biological sciences **359:**109-121.
343. **Tomas-Loba A, Flores I, Fernandez-Marcos PJ, Cayuela ML, Maraver A, Tejera A, Borras C, Matheu A, Klatt P, Flores JM, Vina J, Serrano M, Blasco MA.** 2008. Telomerase reverse transcriptase delays aging in cancer-resistant mice. Cell **135:**609-622.
344. **Frydrychova RC, Mason JM, Archer TK.** 2008. HP1 is distributed within distinct chromatin domains at Drosophila telomeres. Genetics **180:**121-131.
345. **Blasco MA.** 2007. The epigenetic regulation of mammalian telomeres. Nature reviews. Genetics **8:**299-309.
346. **Zhu X, Gustafsson CM.** 2009. Distinct differences in chromatin structure at subtelomeric X and Y' elements in budding yeast. PloS one **4:**e6363.
347. **de Lange T.** 2005. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes & development **19:**2100-2110.

348. **Stansel RM, de Lange T, Griffith JD.** 2001. T-loop assembly in vitro involves binding of TRF2 near the 3' telomeric overhang. The EMBO journal **20:**5532-5540.
349. **Bianchi A, Smith S, Chong L, Elias P, de Lange T.** 1997. TRF1 is a dimer and bends telomeric DNA. The EMBO journal **16:**1785-1794.
350. **Chikashige Y, Hiraoka Y.** 2001. Telomere binding of the Rap1 protein is required for meiosis in fission yeast. Current biology : CB **11:**1618-1623.
351. **Loayza D, De Lange T.** 2003. POT1 as a terminal transducer of TRF1 telomere length control. Nature **423:**1013-1018.
352. **Luo K, Vega-Palas MA, Grunstein M.** 2002. Rap1-Sir4 binding independent of other Sir, yKu, or histone interactions initiates the assembly of telomeric heterochromatin in yeast. Genes & development **16:**1528-1539.
353. **Choy JS, Acuna R, Au WC, Basrai MA.** 2011. A role for histone H4K16 hypoacetylation in Saccharomyces cerevisiae kinetochore function. Genetics **189:**11-21.
354. **Hecht A, Laroche T, Strahl-Bolsinger S, Gasser SM, Grunstein M.** 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell **80:**583-592.
355. **Imai S, Armstrong CM, Kaeberlein M, Guarente L.** 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403:**795-800.
356. **Suka N, Luo K, Grunstein M.** 2002. Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. Nature genetics **32:**378-383.
357. **Khorosjutina O, Wanrooij PH, Walfridsson J, Szilagyi Z, Zhu X, Baraznenok V, Ekwall K, Gustafsson CM.** 2010. A chromatin-remodeling protein is a component of fission yeast mediator. The Journal of biological chemistry **285:**29729-29737.
358. **Mozdy AD, Podell ER, Cech TR.** 2008. Multiple yeast genes, including Paf1 complex genes, affect telomere length via telomerase RNA abundance. Molecular and cellular biology **28:**4152-4161.
359. **Suzuki Y, Nishizawa M.** 1994. The yeast GAL11 protein is involved in regulation of the structure and the position effect of telomeres. Molecular and cellular biology **14:**3791-3799.
360. **Allshire RC, Karpen GH.** 2008. Epigenetic regulation of centromeric chromatin: old dogs, new tricks? Nature reviews. Genetics **9:**923-937.

361. **Walfridsson J, Bjerling P, Thalen M, Yoo EJ, Park SD, Ekwall K.** 2005. The CHD remodeling factor Hrp1 stimulates CENP-A loading to centromeres. Nucleic acids research **33:**2868-2879.
362. **Enomoto S, Berman J.** 1998. Chromatin assembly factor I contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. Genes & development **12:**219-232.
363. **Choi ES, Stralfors A, Castillo AG, Durand-Dubief M, Ekwall K, Allshire RC.** 2011. Identification of noncoding transcripts from within CENP-A chromatin at fission yeast centromeres. The Journal of biological chemistry **286:**23600-23607.
364. **Reyes-Turcu FE, Zhang K, Zofall M, Chen E, Grewal SI.** 2011. Defects in RNA quality control factors reveal RNAi-independent nucleation of heterochromatin. Nature structural & molecular biology **18:**1132-1138.
365. **McGovern SL, Qi Y, Pusztai L, Symmans WF, Buchholz TA.** 2012. Centromere protein-A, an essential centromere protein, is a prognostic marker for relapse in estrogen receptor-positive breast cancer. Breast cancer research : BCR **14:**R72.