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KADRI PEIL

RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*

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RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*

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Press

Department of Cell Biology, Institute of Molecular and Cell Biology,
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LIST OF ORIGINAL PUBLICATIONS

Current dissertation is based on the following original publications which will be referred to in the text by their Roman numerals:

I Värv, S; Kristjuhan, K; **Peil, K**; Lõoke, M; Mahlakõiv, T; Paapsi, K; Kristjuhan, A. (2010) Acetylation of H3 K56 Is Required for RNA Polymerase II Transcript Elongation through Heterochromatin in Yeast. *Mol Cell Biol* (6): 1467–77.

II Lõoke, M; Reimand, J; Sedman, T; Sedman, J; Järvinen, L; Värv, S; **Peil, K**; Kristjuhan, K; Vilo, J; Kristjuhan, A. (2010) Relicensing of Transcriptionally Inactivated Replication Origins in Budding Yeast. *J Bio Chem*, 285 (51): 40004–40011.

III **Peil, K**; Värv, S; Lõoke, M; Kristjuhan, K; Kristjuhan, A. (2011) Uniform Distribution of Elongating RNA Polymerase II Complexes in Transcribed Gene Locus. *J Bio Chem*, 286 (27): 23817–23822.

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My contribution to the articles is as follows:

Ref. I I participated in performing the experiments and analyzing the data.
Ref. II I participated in performing the experiments and analyzing the data.
Ref. III I participated in experimental design, performed the experiments, analyzed the data and wrote the manuscript.

LIST OF ABBREVIATIONS

ac	acetylation
ARS	autonomously replicating sequence
CF	cleavage factor
Chap	histone chaperons
ChIP	chromatin immunoprecipitation
CPF	cleavage and polyadenylation factor
CTD	carboxy terminal domain of RNAPII Rpb1 subunit
CUT	cryptic unstable transcript
DRB	5,6-dichloro-1-β-D-ribofuranoside
DSIF	DRB-sensitivity-inducing factor
gal	galactose
GFP	green fluorescent protein
glc	glucose
GRO-seq	Global Run-on Sequencing
GTF	general transcription factor
HAT	histone acetyltransferase
HDAC	histone deacetylase
MCM	minichromosome maintenance
Met	methylation
MNase	Micrococcal nuclease
mRNA	messenger RNA
NELF	negative elongation factor
NNS	Nrd1-Nab3-Sen1-dependent
nt	nucleotide
ORC	origin recognition complex
ORF	open reading frame
PIC	pre-initiation complex
pre-RC	pre-replicative complex
P-TEFb	Positive Transcription Elongation Factor b
qPCR	quantitative polymerase chain reaction
raf	raffinose
rDNA	ribosomal DNA
RNAPII	RNA polymerase II
rRNA	ribosomal RNA
RT-PCR	reverse transcriptase polymerase chain reaction
SAGE	Serial Analysis of Gene expression
Ser_2-P	phosphorylation of Ser_2
SIR	silent information regulator
snoRNA	small nucleolar RNA
snRNA	small nuclear RNA
TAF	TBP-associated factor
TBP	TATA-binding protein
TFIIS	transcription elongation factor S
tRNA	transfer RNA

INTRODUCTION

Transcription of eukaryotic protein-coding genes to produce mRNA is carried out by RNA polymerase II (RNAPII). The process of mRNA synthesis is divided into three steps: initiation, elongation and termination. For a long time, it was believed that transcription by RNAPII was mainly controlled during the initiation phase. However, it has recently become clear that transcription elongation is also a highly regulated process. Furthermore, latest data indicate that elongation is not a smooth continuous process as previously thought. Transcription takes place in the context of chromatin, and therefore the elongating RNAPII often encounters various obstacles – from DNA damage to protein complexes involved in different DNA-related processes.

A higher-order chromatin structure known as hetero- or silenced chromatin has been considered to hinder transcription by obstructing transcription machinery's access to chromatin. Surprisingly, some studies have shown that heterochromatin in *Saccharomyces cerevisiae* allows binding of complexes required for transcription initiation. This raises the question of how repressive heterochromatin influences already elongating RNAPII. In addition to heterochromatin, transcribing RNAPII encounters other protein complexes on the DNA, such as pre-replicative complexes loaded onto replication origins. While it has been shown that most of the replication origins in *S. cerevisiae* are located in the intergenic regions, transcription of non-coding regions is widespread in yeast. Therefore, it is possible that a large fraction of replication origins is transcribed, leading to collisions between RNAPII and pre-replicative complexes.

The level of transcription varies significantly among different genes, and the amount of RNAPII complexes recruited to a transcribed locus is generally proportional to the rate of gene transcription. However, the average distribution of RNAPII on actively transcribed loci has been shown to be uneven on occasion, with elevated RNAPII signal obtained from the beginning or from the end of a gene. As the observation of RNAPII distribution on transcribed loci is based on an average signal measured from the whole cell population, it does not address the question of spacing of RNAPII complexes in a single cell.

The current thesis focuses on mechanisms of transcription in budding yeast *S. cerevisiae*, and explores the effects of various DNA-based obstacles on RNAPII elongation rate. Distribution of individual RNAPII complexes on transcribed DNA in a single cell is also examined.

I. REVIEW OF LITERATURE

1. Eukaryotic RNA polymerase II

The number of RNA polymerases differs between eukaryotes, bacteria and archaea. While the latter two have only one polymerase, most eukaryotes use three different nuclear RNA polymerases (Pol I, Pol II, Pol III) for synthesis of different types of RNA. RNA Pol I produces ribosomal RNA (rRNA), while RNA Pol III synthesizes small non-coding RNAs, such as transfer RNA (tRNA). The central player in eukaryotic transcription of all protein-coding genes into messenger RNA (mRNA) is RNA polymerase II (RNAPII). The RNAPII holoenzyme is a large (514 kDa) twelve-subunit complex (Figure 1A) (Cramer et al., 2008). The twelve subunits can be divided into two groups based on whether they are shared with other nuclear polymerases or are unique to RNAPII. The first group comprises five subunits (Rpb5, Rpb6, Rpb8, Rpb10 and Rpb12) shared between all three nuclear polymerases. The second group consists of the core domain subunits (Rpb1, Rpb2, Rpb3, Rpb9 and Rpb11) and the Rpb4/Rpb7 subcomplex. Subunits from the second group are unique to RNAPII but they also have homologues in bacterial and archaeal polymerase and in other eukaryotic polymerases (Ebright, 2000; Hahn, 2004). RNAPII structure can also be divided into a 10-subunit "core" enzyme and the Rpb4/Rpb7 subcomplex as the Rpb4 and Rpb7 subunits form a heterodimer that easily dissociates from the decameric "core" (Edwards et al., 1991). While RNAPII lacking Rpb4/Rpb7 subcomplex is able to elongate with similar activity to the 12-subunit holoenzyme, it fails to initiate RNA transcription (Christie et al., 1994; Edwards et al., 1991). Of the twelve RNAPII subunits only two, Rpb4 and Rpb9, are not essential for cell viability under optimal growth conditions. However, both are important for tolerance to different stress factors such as extreme temperatures (Woychik et al., 1991; Woychik and Young, 1989).

An important step in understanding eukaryotic RNAPII structure was made in 2000 when crystallographic structure of *Saccharomyces cerevisiae* RNAPII lacking the Rpb4/Rpb7 subcomplex was determined at 3.0 Å resolution (Cramer et al., 2000). Based on this analysis, RNAPII structure can be divided into four distinct modules: "core", "clamp", "shelf" and "jaw-lobe" (Figure 1B). Half of the RNAPII mass is located in the "core" module, which comprises Rpb3, Rpb10, Rpb11 and Rpb12 subunits together with regions from Rpb1 and Rpb2 that form the active centre. Three other modules are located next to the positively charged DNA-binding cleft along which DNA enters into the active centre. The "jaw-lobe" consists of the upper "jaw" formed by regions from Rpb1 and Rpb9, as well as the "lobe" element of Rpb2. The "shelf" module contains Rpb5, Rpb6 and regions from Rpb1. The "clamp" was first identified as a mobile element and consists of regions from Rpb1 and Rpb2 (Cramer et al., 2001). During transcription complex formation, the "clamp" moves over the cleft, trapping the template and transcript (Gnatt et al., 2001). In 2003, an X-ray structure of the entire 12-subunit RNAPII holoenzyme was determined at 4.1 Å

resolution (Bushnell and Kornberg, 2003). This structure of the complete holoenzyme RNAPII shows that the Rpb4/Rpb7 subcomplex binds to the pocket at the base of the "clamp" close to the carboxy terminal part of Rpb1, and that Rpb7 locks the "clamp" in the closed conformation (Armache et al., 2003; Bushnell and Kornberg, 2003). This suggests that double-stranded DNA never enters the active site cleft, but that the single-stranded template strand is inserted deep into the cleft to reach the active site (Armache et al., 2003).

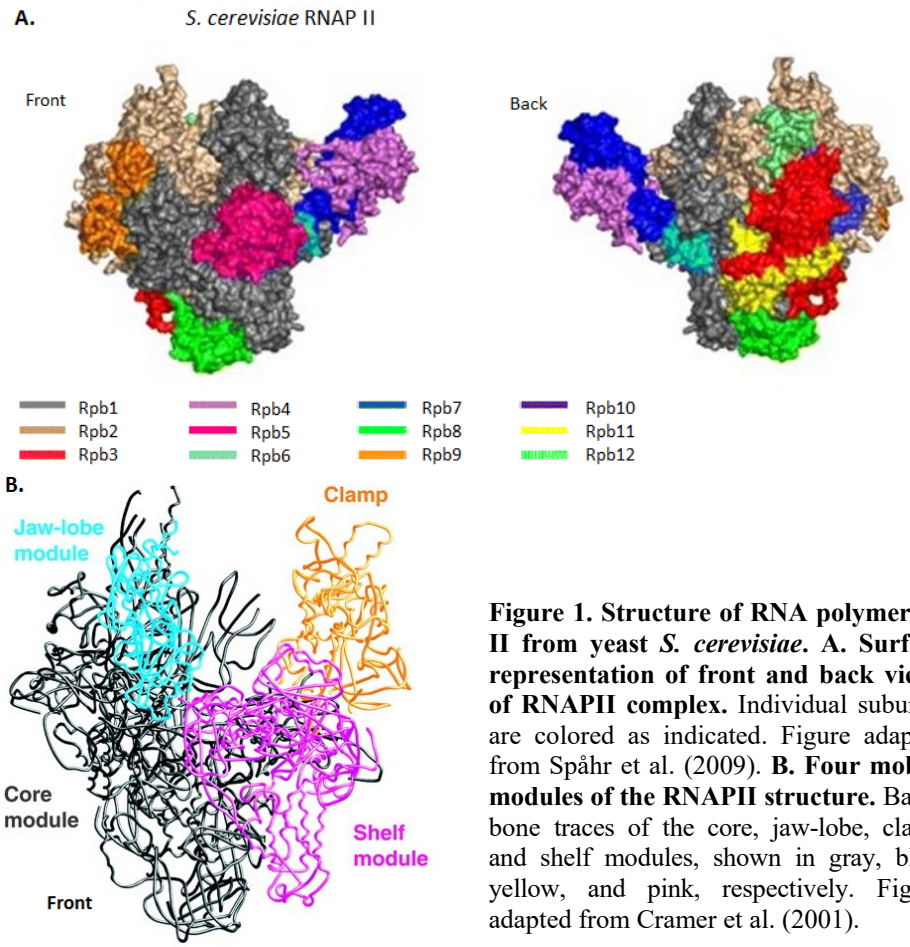

Figure 1. Structure of RNA polymerase II from yeast *S. cerevisiae*. A. Surface representation of front and back views of RNAPII complex. Individual subunits are colored as indicated. Figure adapted from Spåhr et al. (2009). **B. Four mobile modules of the RNAPII structure.** Backbone traces of the core, jaw-lobe, clamp and shelf modules, shown in gray, blue, yellow, and pink, respectively. Figure adapted from Cramer et al. (2001).

Under the active site lies an important mobile element of the Rpb1 subunit, called the trigger loop, that is involved in RNAPII fidelity (Brueckner and Cramer, 2008; Kaplan et al., 2008a). The trigger loop interacts with the incoming nucleotide, and the two fail to align properly in case of nucleotide mismatch in the active site (Kaplan et al., 2008a; Wang et al., 2006). This, in turn, leads to a substantial reduction in the rate of phosphodiester bond formation (Kaplan et al., 2008a; Wang et al., 2006). When the structure of elongating

RNAPII was analyzed, an 8 bp RNA:DNA hybrid was observed in the active center region of RNAPII (Westover et al., 2004). The second loop of Rpb1, the "lid", acts as a barrier that separates the DNA and RNA strands and guides the RNA along the exit path (Westover et al., 2004). The emerging mRNA exiting the polymerase interacts with the Rpb7 subunit (Ujvari and Luse, 2006).

A very important characteristic of RNAPII is that in addition to moving forward on the DNA template, it can also move backward. This is achieved by using the Brownian two-pawl ratchet mechanism, where the incoming template is thought to act as a stationary pawl, allowing only forward movement of RNAPII (Nudler, 2009). An important structural element of RNAPII (the bridge helix) acts as a reciprocating pawl. It not only pushes RNAPII forward on the nucleic acid scaffold, but also permits backward movement so that the newly formed mRNA 3'-terminus comes out of the alignment with the active site (Nudler, 2009). As a result of brownian motion, the 3'-end of RNA can enter the active site again. Alternatively, the polymerase is also capable of endonucleolytic cleavage of the transcript producing a new 3'-hydroxyl group in the catalytic site and thereby enabling RNAPII to continue with elongation (Rudd et al., 1994). This endonucleolytic cleavage is greatly enhanced by the elongation factor TFIIS (Izban and Luse, 1992; Reines, 1992).

1.1 Function of the carboxy terminal domain (CTD) of Rpb1, the largest RNAPII subunit

The largest subunit of RNAPII (Rpb1) contains several repeats of an unusual heptapeptide sequence Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7 at its carboxy terminus (Allison et al., 1985; Corden et al., 1985). The copy number of this consensus repeat varies greatly, ranging from 26 copies in *S. cerevisiae* to 52 repeats in mammals (Allison et al., 1985; Corden et al., 1985). In *S. cerevisiae*, almost all the repeats follow the consensus sequence, while in mammals there are significant deviations from it, especially in the distal part of the CTD (Eick and Geyer, 2013). These repeats are unique and essential for RNAPII activity – at least 8 repeats are required for viability in yeast, and cells containing RNAPII with a truncated CTD (9-11 repeats) possess reduced growth rates and reduced transcription level (Nonet et al., 1987; West and Corden, 1995). Five amino acids (Tyr_1, Ser_2, Thr_4, Ser_5 and Ser_7) from the CTD repeat can be reversibly phosphorylated, while Pro_3 and Pro_6 can exist in either *cis* or *trans* isomeric state (Cadena and Dahmus, 1987; Yaffe et al., 1997; Zhang and Corden, 1991). The dynamic phosphorylation pattern of the CTD generates negative charges and is known to be directly involved in binding and release of different RNAPII-associated factors (Egloff et al., 2012). The *cis-trans* proline isomerization alters the spatial conformation of the CTD structure. For example, the polyadenylation/termination factor Pcf11 binds to the CTD only when Ser_2 is phosphorylated and both prolines are in *trans* configuration (Noble et al., 2005). Conversely, the phosphatase Ssu72 interacts with the CTD when Ser_5 is

phosphorylated and the Pro_6 is in the *cis* configuration (Werner-Allen et al., 2011). In addition to phosphorylation, Thr_4, Ser_2 and Ser_5 are subject to O-glycosylation in higher eukaryotes, a modification that considerably increases the size and mass of CTD but whose functional significance is not clear (Kelly et al., 1993; Ranuncolo et al., 2012). The CTD is not visible in the crystal structure of RNAPII due to its mobile nature. However, it is likely to be compact in its unphosphorylated state and to become more extended upon phosphorylation because of charge repulsion (Cramer et al., 2001; Meinhart et al., 2005). In its extended conformation, the CTD could theoretically reach any location on the surface of RNAPII (Cramer et al., 2001).

Although all repeats of the CTD can be dynamically modified, the best-studied modification is phosphorylation of Ser_2 and Ser_5 (Ser_2-P and Ser_5-P). Komarnitsky and colleagues demonstrated as early as 2000 that levels of RNAPII CTD Ser_5-P remain high at the beginning of a transcribed gene and decrease further downstream (Komarnitsky et al., 2000). In contrast, Ser_2-P accumulates in later stages of transcription as, when RNAPII progresses through a gene (Komarnitsky et al., 2000). The same was shown to be true for majority of protein-coding genes in a genome-wide analysis in yeast, and later in mammalian cells (Mayer et al., 2010; Odawara et al., 2011). This dynamic pattern of phosphorylation is achieved due to a complicated interplay between CTD kinases and phosphatases. During the early phase of transcription, Ser_5 is mostly phosphorylated by the cyclin dependent kinase Kin28, which is a component of the general transcription factor TFIIH in *S. cerevisiae* (Feaver et al., 1991; Feaver et al., 1994; Valay et al., 1995). Another cyclin dependent kinase, Srb10, a component of the Mediator complex, is also hypothesized to be involved in Ser_5 phosphorylation, but its precise role remains unclear (Liao et al., 1995). Two phosphatases – Ssu72 and Rtr1 – have been identified as responsible for the removal of phospho-group from Ser_5-P in yeast (Krishnamurthy et al., 2004; Mosley et al., 2009).

Ser_2 can be phosphorylated by two kinases, Bur1 and Ctk1, both of which are essential for normal cell growth in yeast (Chu et al., 2006; Lee and Greenleaf, 1991; Yao et al., 2000). The current model suggests that Bur1 phosphorylates Ser_2, thereby stimulating subsequent phosphorylation of this residue by Ctk1 (Qiu et al., 2009). The homolog of Bur1 in mammalian cells is Cdk9, a kinase that together with Cyclin T forms a complex called positive transcription elongation factor b (P-TEFb) (Marshall et al., 1996). At the end of a transcription cycle, Ser_2-P is removed by the Fcp1 phosphatase, enabling RNAPII to enter the next round of transcription (Cho et al., 2001). This dynamic pattern of phosphorylation and dephosphorylation allows for coupling of transcription with other nuclear processes like chromatin modification and RNA processing by recruiting different factors during appropriate stages of transcription (Buratowski, 2009). One example of chromatin-modifying enzymes recruited to the transcription machinery by CTD phosphorylation is the H3K4 methyltransferase Set1. Set1 was found in a complex with the Ser_5-phosphorylated form of RNAPII, which is characteristic of early transcribed regions of a gene

(Ng et al., 2003). In addition to CTD Ser_5-P, the Polymerase-Associated Factor 1 complex (Paf1), an important cofactor for elongating RNAPII, is required for Set1 recruitment *in vivo* (Krogan et al., 2003a; Ng et al., 2003). Set2, another methyltransferase responsible for H3K36 methylation, is recruited to transcribed regions, again in association with the Paf1 complex and Ser_2-phosphorylated CTD (Krogan et al., 2003b).

2. Transcription cycle of eukaryotic RNA polymerase II

The transcription cycle of RNAPII can be divided into three main phases: 1) initiation, during which RNAPII is recruited to a gene's promoter region and mRNA synthesis begins; 2) elongation, when RNAPII extends the mRNA transcript and, 3) termination, during which both RNAPII and the synthesized mRNA transcript disengage from the DNA template.

2.1. Transcription initiation

In bacteria, the only necessary factor for RNAP to bind to the promoters is the σ-subunit (Sugiura et al., 1970). In eukaryotes, this RNAP subunit's function is conducted by a much larger set of polypeptides. For example, there are six general transcription factors (GTFs) composed of more than 30 polypeptides in the RNAPII transcription machinery (Hahn, 2004). Transcription of protein-coding genes by RNAPII starts with the binding of gene-specific regulatory factors near the site of transcription initiation (Fuda et al., 2009). This results in positioning of RNAPII, six general transcription factors (TFIIA, -B, -D, -E, -F and -H) and the Mediator complex onto the core promoter region, where they form the pre-initiation complex (PIC) (Woychik and Hampsey, 2002). The best known DNA element to guide the transcription machinery to the promoter is the so-called TATA box, a DNA region containing the TATA(A/T)A(A/T)(A/G) sequence (Basehoar et al., 2004). Although only 20% of yeast genes contain a TATA box in their promoter, it has been shown that GTFs are located genome-wide on most promoters in yeast. This in turn suggests that the general initiation complex architecture is similar at both TATA-containing and TATA-less promoters (Basehoar et al., 2004; Rhee and Pugh, 2012). An important feature of promoter regions is that in many eukaryotes non-coding transcripts are produced divergently from the protein-coding genes (Wei et al., 2011). For example, most of the widely transcribed cryptic unstable transcripts (CUTs) in yeast are products of divergent transcription from promoters, indicating that promoter regions are intrinsically bidirectional (Neil et al., 2009; Xu et al., 2009).

The first to bind to the core promoter is the TFIID complex composed of the TATA-binding protein (TBP) and TBP-associated factors (TAFs) (Nikolov and Burley, 1997). The binding of TBP distorts the TATA sequence and facilitates

the assembly of other TFs (Nikolov and Burley, 1997). TFIIA stabilizes the TBP-DNA interaction and strongly promotes binding of TFIID to DNA (Kokubo et al., 1998; Weideman et al., 1997). The next factor to bind is TFIIB, which is important for transcription start site selection (Lagrange et al., 1998). TFIIB binds to RNAPII, which is then recruited into the PIC (Pardee et al., 1998; Pinto et al., 1992). RNAPII joins the PIC in complex with TFIIF and the Mediator complex (Buratowski and Zhou, 1993; Soutourina et al., 2011). The last general transcription factors to enter the PIC are TFIIH and its stimulatory factor TFIIE (Maxon et al., 1994). The helicase activity of TFIIH starts to unwind DNA, PIC forms an open complex, and RNAPII initiates transcription (Goodrich and Tjian, 1994; Holstege et al., 1996).

Recently, the structure of the 32-protein, 1.5 MDa pre-initiation complex comprising the complete set of GTFs and assembled with RNAPII and promoter DNA was solved (Murakami et al., 2013). Based on this structure, it was suggested that the promoter DNA interacts only with GTFs and not with RNAPII itself (Murakami et al., 2013). Furthermore, the structure revealed that the GTFs position DNA above the RNAPII cleft, and that the polymerase interacts with DNA only after the latter is melted and can bend into the RNAPII cleft. After newly synthesized mRNA reaches a length of approximately 30 nucleotides, RNAPII releases its contacts with the core promoter and enters the stage of transcription elongation (Heintzman and Ren, 2007). RNAPII is mostly unphosphorylated during initial promoter binding, which contributes to CTD interactions with factors stabilizing the PIC, such as the Mediator complex (Myers et al., 1998). Recently, the architecture of the RNAPII-Mediator core initiation complex was solved, suggesting that Mediator forms a cradle-like structure that may position the CTD and the TFIIH kinase inside itself, thereby stimulating RNAPII phosphorylation (Plaschka et al., 2015). After this step, the CTD is phosphorylated at Ser_5, possibly destabilizing the interactions between RNAPII and transcription factors on the promoter and leading to the release of RNAPII from the promoter (Jiang et al., 1996; Søgaard and Svejstrup, 2007). While RNAPII carries on with elongation, Mediator and many of the GTFs are thought to remain behind, bound to the promoter to form a scaffold complex that facilitates subsequent rounds of transcription (Yudkovsky et al., 2000).

2.2. Transcription elongation

In higher eukaryotes, the density of RNAPII at promoter proximal regions is extremely high, but only a small fraction of these polymerases are either in the pre-initiation or arrested complexes (Core et al., 2012). Instead, the majority of the promoter proximal polymerases were shown to be transcriptionally engaged (Core et al., 2012). This suggests that RNAPII needs some activating signal to continue elongation from this pause, and this is considered to be an important regulatory step. This pausing phenomenon has been well described in transcription of the *Drosophila* heat-shock gene. Under normal uninduced condi-

tions, RNAPII synthesizes 25-50 nt of mRNA before the pause (Rasmussen and Lis, 1993; Rougvie and Lis, 1988). Since formation of the mRNA 5'-end cap takes place when the nascent transcript has reached the length of 25-30 nucleotides, these two processes appear to overlap. Interestingly, it was shown that this is indeed the case for the gene encoding the hsp70 heat shock protein. However, in the case of hsp27, most mRNAs are capped before RNAPII pauses (Rasmussen and Lis, 1993). It was also shown that in uninduced cells these paused RNAPII complexes have phosphorylation on Ser_5 but not on Ser_2 of the CTD (Boehm et al., 2003). Presently, RNAPII pausing during early elongation is considered to be a common phenomenon, as revealed by several genome-wide analyses (Core et al., 2008; Min et al., 2011; Muse et al., 2007).

The pausing of RNAPII in higher eukaryotes depends on two factors – DSIF and NELF, which bind to RNAPII and inhibit its function (Wada et al., 1998; Yamaguchi et al., 1999a; Yamaguchi et al., 1999b). As NELF is not present in yeast and DSIF acts only as a positive elongation factor in the absence of NELF, promoter-proximal pausing is restricted to higher eukaryotes (Hartzog et al., 1998; Rodriguez-Gil et al., 2010). It was shown that immunodepleting either DSIF or NELF in *Drosophila* reduced the level of paused polymerase (Wu et al., 2003). Both factors also co-localized together with hypophosphorylated RNAPII at the hsp70 genes, small heat shock genes, as well as at many other chromatin locations (Wu et al., 2003). When RNAPII elongation was induced with heat shock, DSIF remained associated with RNAPII, while NELF dissociated from the elongation complex (Wu et al., 2003). To overcome the negative effect of DSIF and NELF and to enable RNAPII to proceed to productive elongation, Positive Transcription Elongation Factor b (P-TEFb) is required (Marshall and Price, 1995). P-TEFb is a cyclin-dependent kinase that phosphorylates NELF and DSIF in addition to Ser_2 of RNAPII CTD (Peterlin and Price, 2006). This finding has led to the creation of the current model, in which phosphorylation of all three components mediates the release of paused RNAPII, phosphorylated NELF dissociates from the complex and phosphorylated DSIF becomes a positive elongation factor (Jonkers and Lis, 2015).

Just recently, a very intriguing paper was published, showing pause-like distributions of elongating RNAPII immediately downstream from the transcription start sites in fission yeast *Schizosaccharomyces pombe* (Booth et al., 2016). In this paper 28% of active genes were identified as paused and it was determined that deletion of Spt4 (subunit of DSIF) reduces promoter-proximal pausing of RNAPII (Booth et al., 2016). Deletion of Spt4 also inceased RNAPII density in the gene body, so it was proposed that Spt4-Spt5 complex (DSIF) prevents the premature release of RNAPII from promoter-proximal sites during early transcription elongation (Booth et al., 2016).

When nascent mRNA has reached the length of 25-30 nucleotides, its 5'-terminus gets modified by RNA guanylyltransferase and RNA (guanine-7)-methyltransferase to form the 7-methylguanosine cap structure necessary for mRNA stability (Hagler and Shuman, 1992). In order to recognize mRNAs transcribed by RNAPII, the capping enzyme guanylyltransferase does not need

specific RNA sequences (Cho et al., 1997; McCracken et al., 1997). Instead, it caps mRNAs co-transcriptionally and is targeted to the RNAPII initiation complex by phosphorylation of Ser_5 in the CTD (Cho et al., 1997; McCracken et al., 1997). It has been proposed that the Rpb7 subunit, which lies just above the Rpb1 CTD domain, acts as a scaffold that helps the 5'-end-capping machinery interact with the nascent mRNA as it exits elongating RNAPII (Chen et al., 2009).

For a long time, the initiation phase was considered to be the critical part of transcription. That changed when the importance of regulation during early steps of elongation was discovered, as described above. But even after RNAPII is released from its promoter-proximal pause, the subsequent productive elongation is not smooth and steady. Rather, it is a process of variable speed, offering several mechanisms for regulation.

2.3. Transcription termination

Transcription termination is the last step in the transcription cycle. There are two pathways for termination: 1) the poly(A)-dependent pathway, which is responsible for 3'-end processing of most protein-coding mRNAs and 2) Nrd1-Nab3-Sen1-dependent (NNS) pathway functioning in 3'-end formation of non-coding RNA and short RNAs such as cryptic unstable transcripts (CUTs) (Kuehner et al., 2011). Several 3'-end processing factors interact with the CTD of RNAPII, and it has been shown that its Ser_2 phosphorylation is important for recruiting these factors to the 3'-ends of coding regions (Ahn et al., 2004; Skaar and Greenleaf, 2002). In the case of poly(A)-dependent termination, interactions with the pre-mRNA are also important, as an intact polyadenylation signal is required for proper termination (Kim et al., 2004a). In *S. cerevisiae*, transcription termination of protein-coding genes depends mainly on the CPF-CF termination factor complex (Porrua and Libri, 2015). During transcription termination, the components of CPF-CF are recruited to the 3'-ends of genes. Nascent RNA is subsequently cleaved by the CPF endonuclease at the poly(A) site, which is followed by the addition of poly(A) tails by the CPF-associated poly(A) polymerase Pap1 (Chanfreau et al., 1996; Patel and Butler, 1992). Two alternative models for RNAPII termination following transcript cleavage have been proposed. According to the allosteric model, the loss of elongation factors and/or conformational changes in the RNAPII complex destabilize the elongation complex and lead to release of RNAPII (Ahn et al., 2004; Kim et al., 2004a). The torpedo model states that after endonucleolytic cleavage of the poly(A) site, the Rat1 exonuclease is targeted to the newly generated 5'-end of the transcript where it degrades the nascent mRNA up to the transcribing RNAPII, leading to dissociation of the elongation complex (Kim et al., 2004b).
The second pathway dependent on the previously mentioned NNS complex is needed for transcription termination of small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs) and CUTs in *S. cerevisiae* (Arigo et al., 2006; Steinmetz et al., 2001; Thiebaut et al., 2006). The Nrd1 component of NNS

interacts with the Ser_5-phosphorylated RNAPII CTD (Vasiljeva et al., 2008). This allows the NNS complex to be recruited to elongating RNAPII already during an early phase of elongation, and enables its Nrd1 and Nab3 components to recognize specific RNA sequence motifs as soon as they emerge from the transcribing RNAPII complex (Carroll et al., 2007; Porrua et al., 2012). This, in turn, leads to the recruitment of the Sen1 helicase that translocates on the nascent RNA to get to the elongating RNAPII and trigger the termination (Porrua and Libri, 2013). In the next step, the RNA-bound Nrd1-Nab3 heterodimer recruits the TRAMP complex, which promotes polyadenylation of the transcript and its processing by the exosome (Tudek et al., 2014; Vasiljeva and Buratowski, 2006). This processing leads to generation of mature snRNAs and snoRNAs, or to complete degradation of CUTs (Allmang et al., 1999; Arigo et al., 2006; Gudipati et al., 2012; Thiebaut et al., 2006).

After termination, RNAPII is ready to initiate a new round of transcription. The transcription initiation factor TFIIB interacts with factors from the 3'-end processing complex, bringing promoter and terminator DNA close together. This results in so-called gene looping, which enables RNAPII recycling and rapid reinitiation (Singh and Hampsey, 2007).

3. Transcription in the context of chromatin

3.1. Structure of chromatin

In all eukaryotic cells, chromosomal DNA is assembled into chromatin. This packaging starts with the basic unit called nucleosome, which consists of 147 bp of DNA wrapped around an octamer of histones. Each octamer contains four types of so-called core histones (H2A, H2B, H3, H4), assembled into two copies of H2A-H2B and H3-H4 dimers (Luger et al., 1997). The central part of the nucleosome is an H3/H4 heterotetramer, which is flanked on either side by an H2A/H2B dimer. The majority of each histone protein forms a compact structure called the histone-fold (Luger et al., 1997). Additionally, each core histone contains an unstructured and highly mobile N-terminal "tail". The "tail" is subjected to a number of post-translational modifications, among which acetylation, methylation, phosphorylation, ubiquitylation and sumoylation are the most common ones (Kouzarides, 2007). These modifications play important roles in chromatin dynamics by influencing the stability of nucleosomes as well as participating in recruitment of different proteins to chromatin (Fuchs et al., 2009). The most prevalent of these modifications is acetylation, which is carried out by nine different histone acetyltransferases (HATs) in budding yeast (Fuchs et al., 2009). Histone acetylation is commonly associated with transcription activation, and in the chromatin context it has been shown to weaken the interaction of histones with DNA (Wang and Hayes, 2008). There are also a number of different histone deacetylases (HDACs) in yeast that restore a positive charge of lysine residues in histones, leading to re-establishment of a more compact

chromatin structure (Keogh et al., 2005). The histone "tails" are also hypothesized to be involved in generating higher order chromatin structures (Luger et al., 1997). Chromatin is divided into active, decondensed regions known as euchromatin and inactive, silenced regions known as heterochromatin (Rusche et al., 2003). Heterochromatin was initially described cytologically as highly condensed blocks of chromatin structures that remain inert throughout the cell cycle (Schultz, 1936). It consists mostly of highly regular nucleosome arrays with modified histones that are thought to facilitate formation of higher order structures (Grewal and Moazed, 2003; Sun et al., 2001).

3.1.1 Formation of heterochromatin in *S. cerevisiae*

In budding yeast, three main regions subject to silencing by heterochromatin formation are telomeres, the ribosomal DNA (rDNA) locus and the silent mating type loci (Rusche et al., 2003). Formation of heterochromatin in these regions requires the silent information regulator (SIR) complex. SIR interacts with silencers, which determine the regions to be heterochromatinized (Rusche et al., 2003). For example, when different reporter genes were inserted adjacent to telomeric repeats, expression of these genes was reversibly repressed (Gottschling et al., 1990).

The *cis*-acting elements responsible for this type of position–effect regulation are very well studied for the *HM* loci. In addition to the *MAT* locus, which determines whether haploid cells are of α or a mating type, *S. cerevisiae* has an unexpressed copy of the *MATα* genes at the *HML* locus, and another unexpressed copy of the *MATa* genes at the *HMR* locus. These *HM* loci are flanked by silencers *E* and *I* (Loo and Rine, 1994). The elements that are necessary for the functioning of the silencer are well described for the *HMR-E*. It was established that the binding sites for three essential factors – origin recognition complex (ORC), Rap1 and Abf1 – are the only elements required for silencing function. A synthetic sequence containing these binding sites was able to repress transcription of the *HMRa1* gene (McNally and Rine, 1991). When these three essential factors are bound to the silencer, the Sir1 protein is recruited to the locus via an interaction with the Orc1 protein (Triolo and Sternglanz, 1996). Sir1 is important for establishing silencing, but it does not spread throughout silenced chromatin and is dispensable for maintenance of silencing (Rusche et al., 2002). The next step is recruitment of the Sir4 protein, which interacts with the Sir1 and Rap1 proteins (Moretti and Shore, 2001; Triolo and Sternglanz, 1996). This, in turn, leads to the recruitment of Sir2 and Sir3, both of which bind to Sir4 (Hecht et al., 1996; Moazed et al., 1997). After that, the deacetylase activity of Sir2 modifies the "tails" of histones H3 and H4 on the neighbouring nucleosome. This creates new high-affinity binding sites for Sir3 and Sir4, as these proteins bind to histone "tails" more tightly when they are hypoacetylated (Hecht et al., 1995). When new Sir3 and Sir4 molecules bind to the deacetylated nucleosome, additional Sir2 deacetylase is recruited and the process repeats itself (Figure 2A) (Rusche et al., 2002).

The sequential deacetylation model also proposes a mechanism for interrupting the spreading of Sir proteins and therefore the silenced chromatin formation. It is predicted that disruption of the nucleosome array – for example, by a DNA-binding protein – would make it impossible for the Sir2 deacetylase to create new binding sites for the Sir3 and Sir4 proteins. Additionally, a localized histone acetylase that acetylates histones more effectively than Sir2 can deacetylate them would prevent spreading of heterochromatin (Figure 2B) (Rusche et al., 2002).

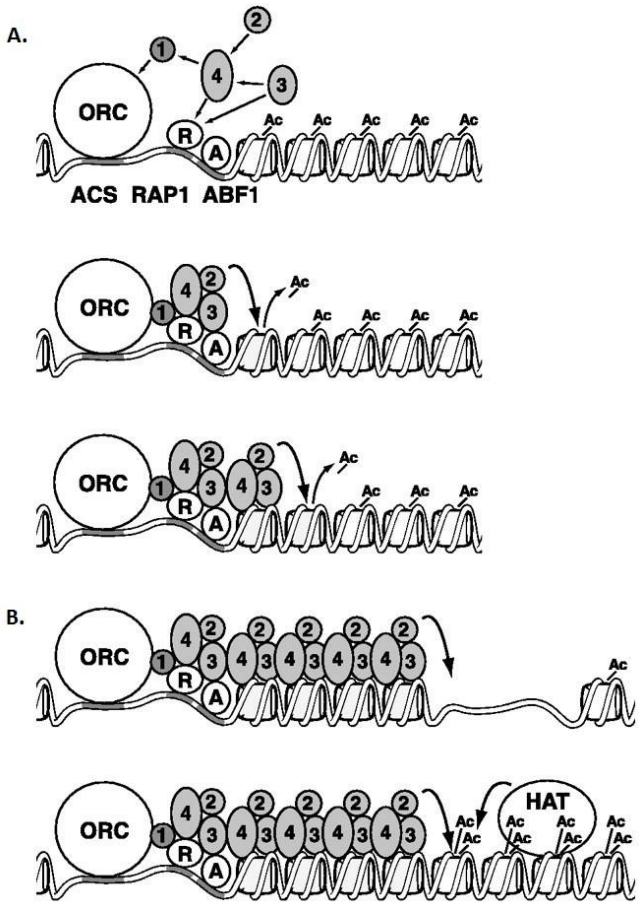

Figure 2. Models for action of Sir proteins. A. Formation of silenced chromatin. Sir proteins (1-4) associate with ORC, Rap1 (R) and Abf1 (A) as shown with straight arrows. Sir2 then deacetylates the "tails" of H3 and H4 on neighbouring nucleosomes (line 2), creating binding sites for Sir3 and Sir4 (line 3). Sir2 deacetylates the next nucleosome and the process repeats itself**. B. Boundaries of silenced chromatin.** Spread of Sir proteins could be counteracted either by a gap in the nucleosome array (line1) or a highly acetylated nucleosome (line 2) (ac – acetylation; HAT – histone acetyltransferase). Figure adapted from Rusche et al. (2002).

3.2. RNAPII elongation through chromatin

Even in open euchromatin, transcription has to contend with histones. The hypothesis that histones may inhibit DNA-based processes, including transcription, was proposed as early as 1951 by Ellen and Edgar Stedman (Stedman, 1951). *In vitro* experiments showed that even a single nucleosome is an obstacle for RNAPII to initiate transcription, but that an elongating RNAPII is capable of displacing nucleosomes (Lorch et al., 1987). However, it is clear that nucleosomes present a formidable barrier to RNAPII as elongation rates on nucleosomal templates are severely inhibited compared to those on naked DNA (Izban and Luse, 1991). On a naked DNA template, only 3% of RNAPII molecules paused before the intrinsic pause site compared to 73% of RNAPII molecules on a chromatin template (Izban and Luse, 1991). To tackle this issue, eukaryotic cells have developed a mechanism of disrupting chromatin structure in front of an elongating RNAPII. Experiments performed in *S. cerevisiae* have shown that histone density on different galactose-inducible genes is inversely correlated with transcriptional activity, suggesting that histones are evicted from DNA by elongating RNAPII machinery (Kristjuhan and Svejstrup, 2004; Schwabish and Struhl, 2004). It was also shown that the major factor for defining the region of nucleosome removal in transcribed genes is the elongating RNAPII itself (Värv et al., 2007).

The structure of nucleosomes requires that during chromatin disassembly the outer H2A/H2B dimers are removed from the nucleosome first, followed by removal of H3/H4 (Bohm et al., 2011). When incorporation of new epitope-tagged histones into the yeast genome was analyzed, it was observed that while exchange of H2B occurred rapidly upon transcription elongation, H3 exchange was less dynamic and only took place within highly transcriptionally active genes (Jamai et al., 2007). Another study analyzing nucleosome dynamics throughout the yeast genome showed that exchange of H3 within coding regions is a common feature of highly transcribed genes (Dion et al., 2007). It was also observed that rates of H3 replacement within coding regions correlated with RNAPII density, thereby supporting the idea that the elongating polymerase stimulates nucleosome eviction (Dion et al., 2007; Ivanovska et al., 2011; Jamai et al., 2007). Similar effect was seen in human cells, where histone exchange was monitored with green fluorescent protein (GFP)-tagged histones (Kimura and Cook, 2001). Replication-independent exchange of H2B-GFP took place rapidly and throughout the genome, whereas exchange of H3-GFP and H4-GFP occurred mostly in euchromatic regions, indicating its dependence on transcription (Kimura and Cook, 2001).

An alternative mechanism that relies on acetylation allows RNAPII elongation through chromatin without a substantial displacement of nucleosomes (Kristjuhan and Svejstrup, 2004). Most likely, only eviction of either one or both H2A/H2B dimers occurs during this process (Kireeva et al., 2002). It was demonstrated that when RNAPII reaches an area of strong DNA-histone interactions, DNA can unwind from the nucleosome and form a small intra-

nucleosomal loop that contains the transcribing RNAPII. Formation of this loop allows for rapid restoration of DNA-histone interactions behind RNAPII, so that at least some H3 and H4 molecules are associated with DNA at any point in time (Kulaeva et al., 2009).

Although several studies have shown that low-level transcription does not lead to eviction of nucleosomes while histones within highly transcribed genes are replaced, a genome-wide investigation in yeast demonstrated that for some genes the rate of histone exchange is higher or lower than would be predicted from their transcription rate (Gat-Viks and Vingron, 2009). Such unexpected histone dynamics occurred along gene's entire coding regions and appeared to be a gene-specific property rather than a regional effect. Moreover, it was observed that trimethylation of H3K79 is depleted from coding regions where histone exchange rate is higher than expected and enriched in coding regions where histones are replaced less frequently than predicted (Gat-Viks and Vingron, 2009). Therefore, it is possible that histone exchange within coding regions is a possibility to add or remove certain histone modifications and thereby provides another way of transcription elongation regulation.

3.2.1 Factors influencing RNAPII elongation through chromatin

There are several factors affecting RNAPII passage through chromatin. First, RNAPII has an intrinsic ability to backtrack and possibly arrest when encountering obstacles. Likely due to this, transcription elongation factor S (TFIIS), which stimulates cleavage of mRNA stalled at arrest sites, is required for efficient elongation through chromatin (Guermah et al., 2006). Secondly, some DNA sequences may be more difficult to transcribe because of their topology. For example, G-rich DNA can result in formation of R-loops (an RNA-DNA hybrid structure) that may induce RNAPII pausing (Huertas and Aguilera, 2003). Another important factor for RNAPII elongation are covalent histone modifications, mainly located in the unstructured mobile N-terminal "tails" (Allfrey and Mirsky, 1964). Histones can be modified by numerous enzymes, and these modifications can either tighten or loosen DNA binding to nucleosomes, thereby restricting or promoting elongation efficiency (Das and Tyler, 2013). These modifications can also act as binding sites for different non-histone proteins affecting numerous cellular processes (Das and Tyler, 2013). One common modification of histones is acetylation, predominantly found in promoter regions of transcribed genes (Pokholok et al., 2005). However, several histone acetyltransferases (HATs) and histone deacetylases (HDACs) are also associated with coding regions of transcribed genes (Carrozza et al., 2005; Govind et al., 2007; Vogelauer et al., 2000; Wang et al., 2002). The importance of acetylation during transcription elongation was demonstrated by studies in yeast, where deletion of HATs GCN5 and ELP3 resulted in lower histone acetylation levels in coding regions of many genes. This, in turn, led to dramatically reduced transcription levels (Kristjuhan et al., 2002). It was also

observed that the major H4 lysine acetyltransferase complex NuA4 is recruited co-transcriptionally to coding regions, leading to increased H4 acetylation, enhanced recruitment of chromatin remodellers and increased histone eviction (Ginsburg et al., 2009). Recruitment of NuA4 to coding regions also stimulates the rate of RNAPII transcription elongation (Ginsburg et al., 2009). Taken together, these data indicate that histone acetylation within coding regions is dynamic and important for elongating RNAPII.

The importance of histone methylation in transcription elongation has been best characterized for histone H3 lysine 36 (H3K36) (Krogan et al., 2003b). The elongating RNAPII with its CTD phosphorylated on Ser_2 is recognized by the histone methyltransferase Set2 (Li et al., 2003; Schaft et al., 2003; Xiao et al., 2003). Thanks to its interaction with the elongating RNAPII, Set2 methylates H3K36 in the coding regions of transcriptionally active genes (Krogan et al., 2003b; Li et al., 2003; Schaft et al., 2003). This methylation mark is in turn recognized by the HDAC Rpd3, which deacetylates histones and promotes chromatin refolding (Carrozza et al., 2005; Keogh et al., 2005). When cells fail to methylate H3K36, coding regions remain hyperacetylated and available for RNAPII to initiate transcription from cryptic initiation sites (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005). Therefore, the purpose of this modification appears to be to restore the chromatin structure after RNAPII passage.

In a recent article on *S.cerevisiae*, a model was proposed whereby methylation of H3K4 and H3K36 regulates H3 and H4 acetylation and deacetylation during transcription elongation (Ginsburg et al., 2014). According to this model, the NuA4 acetyltransferase recognizes nucleosomes containing monomethylated H3K4 and H3K36, leading to H4 acetylation. Nucleosomes with acetylated H4 “tails” are bound by the SAGA complex, which in turn acetylates the H3 “tail”. Nucleosomes with dimethylated H3K4 and H3K36 can still be acetylated by NuA4, but they are also recognized by HDACs Rpd3 and Set3/Hos2, leading to competition between acetylation and deacetylation (Ginsburg et al., 2014). Taken together, these data show that maintaining the proper balance of acetylation and deacetylation within coding regions during transcription elongation is critical, and histone methylation plays an important role in accomplishing this (Figure 3).

The next set of important factors that help RNAPII elongation through chromatin could be divided into two categories: 1) ATP-dependent chromatin remodellers, and 2) histone chaperones. For example, in *S. cerevisiae*, the ATP-dependent chromatin remodeller Chd1 interacts with the Paf, Spt4/5 (DSIF) and FACT elongation factor complexes and co-localizes with Paf and Spt4/5 within the coding regions of several highly expressed genes (Simic et al., 2003). This localization is transcription-dependent, strongly suggesting an important role for Chd1 in chromatin remodelling during transcription elongation (Simic et al., 2003). Another ATP-dependent remodeller, the RSC complex, has been shown to facilitate transcription elongation (Ginsburg et al., 2009). When RSC occupancy within the coding region of the *GAL1* gene was reduced, a drop in

elongation rate and histone eviction was also observed (Ginsburg et al., 2009). Later, it was demonstrated that RSC is recruited to the coding regions of actively transcribed genes genome-wide (Spain et al., 2014). In the same study, the catalytic subunit of the RSC complex, Sth1, was depleted, resulting in a drastic reduction of RNAPII occupancy in the coding regions of weakly transcribed genes (Spain et al., 2014).

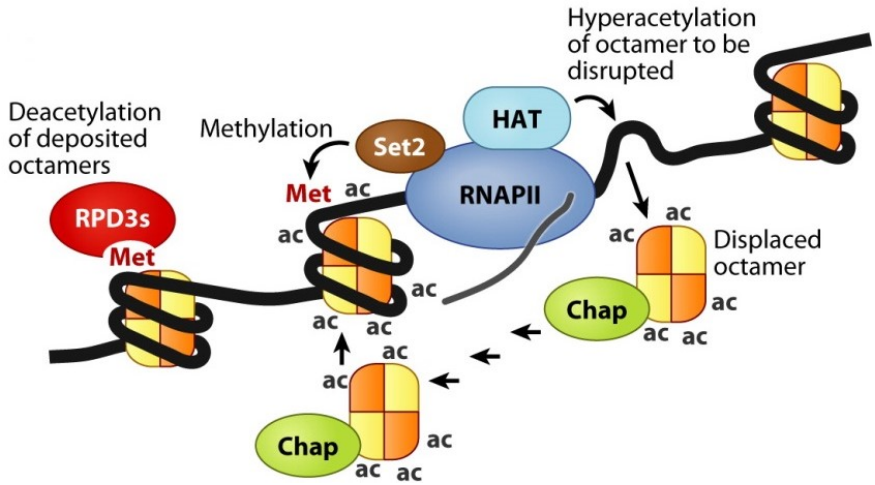

Figure 3. Transcription through nucleosomes. Histone acetyltransferases (HATs) acetylate histones as RNAPII transcribes through chromatin. This stimulates nucleosome dissociation and mobility of histone proteins. Upon reloading onto DNA (or in cases where RNAPII traverses a nucleosome without completely displacing it), the hyperacetylated nucleosome becomes methylated by Set2. This, in turn, leads to recruitment of the histone deacetylase complex Rpd3S and methylated nucleosomes become deacetylated, so that chromatin structure is restored (ac – acetylation; Met – methylation; Chap – histone chaperons). Figure adapted from Selth et al. (2010).

Histone chaperones facilitate histone deposition, exchange or eviction from chromosomal DNA (Figure 3) (Park and Luger, 2008). One of these chaperones, Spt6, is important for maintaining proper chromatin structure in yeast. It plays a role in chromatin structure remodelling during transcription elongation and prevents transcription from cryptic promoters within coding regions (Bortvin and Winston, 1996; Ivanovska et al., 2011; Kaplan et al., 2003). For example, Youdell and colleagues showed in 2008 that H3K36 methylation by Set2 requires Spt6 (Youdell et al., 2008). Such a requirement shows a tight connection of different factors facilitating RNAPII elongation through chromatin.

Another histone chaperon complex, FACT, is required for transcription elongation through nucleosomes (Orphanides et al., 1998). FACT has been shown to destabilize the nucleosomal structure by disassembling H2A-H2B dimers and thereby facilitating RNAPII passage through chromatin (Belotser-

kovskaya et al., 2003). After the passage of RNAPII, the disassembled H2A-H2B dimer can be redeposited onto DNA by FACT (Xin et al., 2009).

Considering the numerous processes that assist RNAPII transcription through chromatin, one can conclude that cells have evolved several different and sometimes overlapping or even redundant mechanisms to ensure proper RNAPII elongation and maintenance of chromatin structure at the same time.

3.3. The rate of RNAPII elongation

Studies published over the past decade used a variety of methods and model organisms or cell lines to reveal that elongation rate of RNAPII can differ more than four-fold between genes, ranging from ~1.0 to 4.3 kilobases per minute (Ardehali and Lis, 2009). It is argued that some of these differences are due to different model organisms used. For example, the average length of an mRNA coding gene in yeast is 1.45 kb, whereas human genes are much longer, averaging around 27 kb (Dujon, 1996; Venter et al., 2001). This difference has led to the suggestion that mammals might have developed mechanisms that allow their RNAPII to elongate at higher rates (Ardehali and Lis, 2009). Yet, the elongation rate in mammals has been estimated to range from 1.3 to 4.3 kb/min, varying by a factor of 3 between different studies (Darzacq et al., 2007; Femino et al., 1998; Tennyson et al., 1995). In 2009, Singh and Padgett developed a method for measuring the transcription elongation rate at several relatively long (~100-580 kb) human genes in their endogenous environments (Singh and Padgett, 2009). The authors used the reversible RNAPII elongation inhibitor 5,6-dichloro-1-β-D-ribofuranoside (DRB) and measured the emergence of nascent pre-mRNA with RT-PCR after removal of DRB. The results showed that the RNAPII elongation rate over long distances was about 3.8 kb/min, with rates being similar for most of the ten genes analyzed (Singh and Padgett, 2009). The rate of 3.8kb/min corresponds to roughly 80% of the maximal elongation rate in mammals, calculated as the rate of pure "rapid elongation" without pausing (Darzacq et al., 2007). The authors suggested that the previous lower elongation rate estimations could have come from analyzing inducible genes that were transcriptionally silent before their activation (Femino et al., 1998; Tennyson et al., 1995).

More recently, Global Run-on Sequencing (GRO-seq) was used to measure RNAPII elongation rates at 140 native human genes in the MCF-7 cell line after induction of these genes with the physiological, non-toxic inducer 17β-estradiol (E2) (Danko et al., 2013). It was observed that RNAPII elongation rate, induced by a common stimulus, was very different for different genes, varying from 0.37 to 3.57 kb/min, with a median rate of 2.1 kb/min. In the same study, 26 genes from a different human cell line (AC16) were stimulated with TNFα, a cytokine that rapidly produces cellular response. In this case, the observed median RNAPII elongation rate was 2.8 kb/min (Danko et al., 2013). Moreover, for two genes that were activated by both treatments, the rate of RNAPII

elongation was 25-40% faster in the case of TNFα induction, suggesting that elongation rate of RNAPII can vary in response to different signals and that the DNA sequence itself is not the primary determinant for measured elongation rate differences (Danko et al., 2013). When the elongation rate along the genes was analyzed in more detail, it emerged that RNAPII is slower near promoters and accelerates as it moves along the gene (Danko et al., 2013; Fuchs et al., 2014; Jonkers et al., 2014). When RNAPII elongation at more than 1000 genes was analyzed in mouse embryonic stem cells, it was confirmed that there are gene-to-gene variations in elongation rates, ranging from 0.5 to 4 kb/min (Jonkers et al., 2014). It was also observed that exons have a negative effect on the elongation rate, slowing RNAPII down (Jonkers et al., 2014). Such an effect could be important for splice site recognition and co-transcriptional splicing or, alternatively, the splicing process itself could be responsible for slower RNAPII elongation rate (Jonkers et al., 2014). When genome-wide analysis of RNAPII elongation rates was performed in human cells, transcription elongation rates were found to vary between 2 and 6 kb/min, with the average of 3.5 kb/min (Fuchs et al., 2014). Different studies have revealed that dimethylation of H3K79 has a positive effect on RNAPII elongation rate, as it is enriched within the genes with the highest elongation rate. However, it remains to be determined whether this histone modification accelerates elongation or is itself the consequence of faster elongation (Fuchs et al., 2014; Jonkers et al., 2014; Veloso et al., 2014). Taken together, these data demonstrate that transcription is not only controlled during the initiation phase, but that elongation is also a variable, regulated and rate-limiting step for controlling gene expression levels.

3.4. Distribution of RNAPII on protein-coding genes

RNAPII density has been investigated across many genes in different organisms (Guenther et al., 2007; Muse et al., 2007; Steinmetz et al., 2006; Venters and Pugh, 2009; Zeitlinger et al., 2007). When RNAPII occupancy was measured on 3852 open reading frames (ORFs) in *S. cerevisiae*, it correlated well with previously estimated transcription activity for most of the genes (Steinmetz et al., 2006). However, exceptions were observed as well. For example, a subset of highly transcribed ribosomal protein genes exhibited only moderate (1.5-2.5x) RNAPII enrichment (Steinmetz et al., 2006). RNAPII was depleted at most telomeres and in regions where repressed loci are known to be present. One such example is the FLO11 gene – its transcription is repressed if cells grow in rich medium, and it is strongly depleted of RNAPII (Steinmetz et al., 2006). Surprisingly, RNAPII was observed at the silent mating type loci, *HML* and *HMR*, and on some genes that neither produce stable transcripts nor are actively silenced or repressed (Steinmetz et al., 2006).

When RNAPII distribution in *S. cerevisiae* was analyzed in finer detail, its enrichment was detected at promoter regions of more than 2/3 of the investigated genes (2077 genes out of 3041), suggesting that RNAPII spends on

average more time at promoters than at any other locations in transcribed regions (Venters and Pugh, 2009). For the remaining 964 genes, a different distribution pattern was observed, with RNAPII located rather evenly across the gene bodies or enriched at their 3'-ends (Venters and Pugh, 2009). It was also observed that most genes have relatively little RNAPII bound to them, as more than half of the genome showed less than 5% of the maximum RNAPII density, indicating that most genes are not transcribed frequently (Venters and Pugh, 2009). Using the genomic run-on technique, distribution of transcriptionally active RNAPII was measured on 261 genes in yeast (Rodriguez-Gil et al., 2010). This analysis showed similar patterns of RNAPII distribution, with the polymerase either enriched at the edges of transcription units or distributed evenly along gene bodies (Rodriguez-Gil et al., 2010). Therefore, RNAPII distribution appears to be an intrinsic characteristic of each transcription unit, independent of gene length or expression level (Rodriguez-Gil et al., 2010).

Interestingly, when the distribution of RNAPII determined via genomic run-on experiments was compared to chromatin immunoprecipitation (ChIP) data, it was discovered that the density of RNAPII observed in ChIP assays was more uniform. This suggests that some RNAPII complexes on transcribed regions are arrested and transcriptionally inactive, and therefore do not produce a run-on signal despite detection via ChIP (Rodriguez-Gil et al., 2010). Enrichment of RNAPII at the 5'-ends of genes is also common in *Drosophila* and in mammals, suggesting that transcription may be largely regulated after RNAPII has been recruited (Guenther et al., 2007; Muse et al., 2007; Zeitlinger et al., 2007). Approximately 30% of all genes display promoter-proximal pausing of RNAPII in higher eukaryotes (human, mouse, *Drosophila*), leading to detection of higher levels of RNAPII near promoter regions compared to gene bodies (Core et al., 2008; Larschan et al., 2011; Min et al., 2011). Substantial accumulation of RNAPII signal has also been detected at the ends of transcription units, just downstream of the polyA site (Core et al., 2008). This accumulation is probably the result of RNAPII slowing down at the end of the gene to facilitate the coupling of transcript cleavage with termination. Likewise, slowdown and therefore higher levels of RNAPII can also be caused by the exons, which probably facilitates splicing (Jonkers et al., 2014; Kwak et al., 2013).

OBJECTIVES OF THE PRESENT STUDY

During transcription elongation, RNAPII has to contend with different obstacles, from highly condensed heterochromatin structures to replication complexes. To determine what happens when the elongating RNAPII encounters different impediments, the following aims were set:

1. To analyze whether RNAPII transcript elongation is affected when encountering heterochromatic structures.
2. To characterize the factors necessary for transcript elongation through heterochromatin.
3. To determine what happens to pre-replicative complexes that are formed in transcriptionally active regions.

In addition, we wanted to explore distribution of RNAPII to answer following question:

4. Can sequential chromatin immunoprecipitation assay (re-ChIP) be used to determine whether the distribution of elongating RNAPII complexes at a highly transcribed model gene on single cell level differs from that previously established at the whole population level?

II. RESULTS AND DISCUSSION

1. Description of the model system

The *S. cerevisiae* strain W303 was used as a model organism for all experiments performed and presented in this thesis. As the genome of budding yeast is very compact – the average length of yeast genes is only 1.45 kb – it is complicated to distinguish between the transcription-coupled events taking place during transcription at promoters from the changes originating within coding regions. To overcome this problem, we chose a long (9433 bp) non-essential gene *VPS13* at its natural locus as our model. We inserted a galactose-inducible promoter (*GAL10* promoter) in front of *VPS13*, so that transcription of this synthetic *GAL-VPS13* fusion gene could be activated or repressed by changing the carbon source in the growth medium. When glucose (glc) is used as the carbon source, transcription from the model gene is repressed, whereas in the presence of galactose (gal) transcription is activated (Kristjuhan and Svejstrup, 2004). In order to study the mechanism of transcription elongation, yeast strains containing insertions of different foreign DNA sequences at different locations in the coding region of *GAL-VPS13* were constructed (Figure 4).

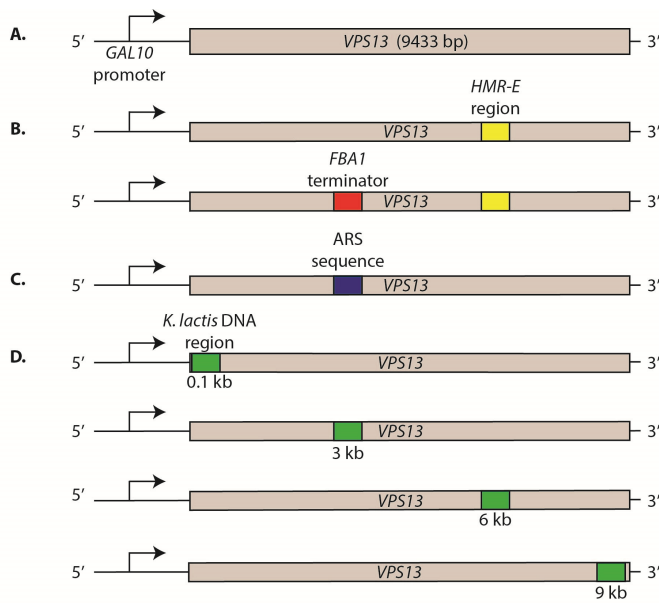

Figure 4. Modificatons of *S. cerevisiae VPS13* gene. A. Schematic representation of the galactose-inducible *VPS13* model gene, with the *GAL10* promoter inserted in front of the *VPS13* ORF. **B-D.** *GAL-VPS13* model gene with different foreign DNA inserts in the coding region. *HMR-E* region (yellow rectangle) 6 kb from the promoter (B); *FBA1* terminator region (red rectangle) 3 kb from the promoter (B); ARS sequence (blue rectangle) 3 kb from the promoter (C); *Kluyveromyces lactis VPS13* DNA insertion (green rectangle) 0.1 kb, 3 kb, 6 kb and 9 kb from the promoter (D).

2. Elongating RNAPII can contend with heterochromatic structures at a highly transcribed locus (Ref. I)

It is generally considered that heterochromatin represses transcription by limiting access of sequence-specific factors required for recruitment of transcription machinery (Kornberg and Lorch, 1991). However, binding of transcription activators and formation of PIC at promoter regions of repressed genes has been observed in *S. cerevisiae* (Sekinger and Gross, 1999, 2001). Subsequently, it was demonstrated that repression of transcription by heterochromatin formation targets the transition between RNAPII initiation and elongation (Gao and Gross, 2008). This leads to the question whether RNAPII that has cleared the promoter and is already elongating can contend with heterochromatic structures.

In order to analyze the influence of heterochromatin structures on transcription elongation, we constructed yeast strains where the *HMR-E* silencer was inserted into the *GAL-VPS13* model gene at a distance of 6 kb downstream of the *VPS13* start codon (Figure 4B; Ref. I, Figure 1A). We used chromatin immunoprecipitation (ChIP) and quantitative PCR (qPCR) methods to determine the distribution of Sir3 protein representing heterochromatin formation at different locations within the model gene. When cells were grown in glucose, which represses transcription of *GAL-VPS13*, SIR complexes were efficiently recruited to the *HMR-E* sequence (*GAL-VPS13-HMR-E*) (Ref. I, Figure 1B). These complexes spread bidirectionally from the silencer sequence, preferentially towards the 5'-end of the gene, although the promoter and the very beginning of the gene remained free of heterochromatin (Ref. I, Figure 1B). No accumulation of SIR complexes on the *GAL-VPS13-HMR-E* gene was detected in a *sir4Δ* strain, which is deficient in heterochromatin and SIR complex formation (Ref. I, Figure 1B).

When transcription of the *GAL-VPS13-HMR-E* gene was induced, SIR complexes were removed from the entire locus, indicating that heterochromatic structures can be removed as a consequence of transcription (Ref. I, Figure 1B). To determine how SIR complexes influence the level of transcription induction and elongation, we compared the density of RNAPII and nucleosomes on *GAL-VPS13-HMR-E* and *GAL-VPS13* model genes in respective yeast strains. Surprisingly, after induction of transcription by galactose, the levels of RNAPII and nucleosomes upstream of the *HMR-E* silencer sequence on the model gene were similar in both strains (Ref. I, Figure 2A-B). However, we could not detect RNAPII recruitment or loss of nucleosomes downstream of the *HMR-E* element in *GAL-VPS13-HMR-E* (Ref. I, Fig. 2A-B). We were also unable to detect *VPS13* transcripts beyond the *HMR-E* sequence with reverse transcriptase PCR (RT-PCR). This further indicated that although RNAPII can elongate through heterochromatin formed upstream of the *HMR-E* silencer, it is unable to traverse this region itself (Ref. I, Figure 5).

To investigate whether transcription termination was caused by the heterochromatin structure or another factor, we performed similar experiments in the

previously described s*ir4Δ* strain. Again, RNAPII was incapable of transcribing through the *HMR-E* sequence, clearly demonstrating that this silencer element on its own represents a strong obstacle for transcription, as no SIR complexes and therefore no heterochromatin were present on the model gene in this strain (Ref. I, Figure 2C).

Although the loss of SIR complexes from the *GAL-VPS13-HMR-E* model gene was concurrent with the appearance of elongating RNAPII in the coding region, we wanted to confirm that the disassembly of heterochromatic structure was a direct result of RNAPII elongation and was not merely caused by promoter activation. For this we constructed a yeast strain where the *FBA1* terminator sequence was inserted into *GAL-VPS13-HMR-E* at 3 kb downstream from the promoter, creating a model gene where RNAPII elongation is terminated before it reaches the heterochromatic region in the locus (Ref. I, Figure 4A). As expected, upon transcription activation, RNAPII was detected upstream of the terminator sequence (Ref. I, Figure 4B). Importantly, SIR complexes were not removed downstream of the terminator sequence, confirming that elongation through heterochromatic region is indeed required for displacement of SIR complexes.

These results confirmed that RNAPII is capable of transcribing through heterochromatic region and that SIR complexes were removed from the model gene in a transcription-dependent manner. Therefore, we next asked whether encountering these structures changes the kinetics of transcription. To analyze the efficiency of RNAPII elongation, we monitored the accumulation of *VPS13* mRNA after induction of *GAL-VPS13-HMR-E* and *GAL-VPS13* model genes. RNA samples collected at different time points after galactose induction were analyzed by RT-PCR. Surprisingly, mRNA accumulation was nearly identical in *GAL-VPS13* and *GAL-VPS13-HMR-E* strains (Ref. I, Figure 5), indicating that presence of heterochromatin does not change the kinetics of gene induction and RNAPII elongation through the region.

Histone H3 lysine 56 acetylation is required for RNAPII elongation through heterochromatin (Ref. I)

Our next question was whether any chromatin modifying factors are needed for efficient elongation of RNAPII through heterochromatin in the *GAL-VPS13-HMR-E* locus. In order to address this, we constructed twelve yeast strains with deletions of genes necessary for different transcription-coupled chromatin modifications and analyzed accumulation of *VPS13* mRNA by RT-PCR upon induction of *GAL-VPS13-HMR-E*. Deletion of *BRE1* (necessary for transcription-coupled H2B K123 monoubiquitylation and subsequent methylation of H3K4 and K79 (Wood et al., 2003); H3 methyltransferase genes *SET1*, *SET2* and *DOT1*; chromatin remodelling factor gene *RSC1*; and histone deacetylase gene *RPD3* had no adverse effect on *GAL-VPS13-HMR-E* transcription induction (Ref. I, Figure 6A). We detected slightly slower appearance of *GAL-VPS13-HMR-E* mRNA in *sas2Δ* and *gcn5Δ* strains (Ref I, Figure 6A), in concordance with previous results showing that both of these HATs are

important for control of heterochromatin spreading (Kimura et al., 2002; Kristjuhan et al., 2003; Suka et al., 2002).

Strikingly, severe impairment of *GAL-VPS13-HMR-E* induction was detected in *rtt109Δ*, *asf1Δ and htz1Δ* strains (Ref. I, Figure 6A). Rtt109 and Asf1 are both required for H3K56 acetylation – Rtt109 has acetyltransferase activity, while the histone chaperone Asf1 is responsible for Rtt109 binding to H3 (Driscoll et al., 2007; Han et al., 2007; Recht et al., 2006; Schneider et al., 2006; Tsubota et al., 2007). Htz1 is a histone variant of H2A, mainly incorporated into nucleosomes in subtelomeric regions to restrict spreading of the SIR complex, as well as in promoter regions of genes transcribed by RNAPII to facilitate rapid opening of chromatin at these loci (Li et al., 2005; Meneghini et al., 2003; Zhang et al., 2005).

In order to confirm that the defects in *GAL-VPS13-HMR-E* transcript formation were specific to our model gene containing heterochromatic structures, we determined the induction efficiency of the endogenous *GAL10* gene in the same samples (Ref. I, Figure 6B). Induction of *GAL10* was severely delayed in the *htz1Δ* strain, indicating that deletion of *HTZ1* leads to general impairment of transcription of genes with the *GAL10* promoter. Since a possibility existed that in *rtt109Δ*, *asf1Δ and htz1Δ* strains, transcription of long genes was affected by general defects in transcription elongation, we introduced the same deletions into the strain containing the *GAL-VPS13* model gene without the *HMR-E* sequence. Again, induction of the *GAL-VPS13* model gene was significantly delayed in the *htz1Δ* strain (Ref. I, Figure 6C). This confirmed that the loss of *GAL-VPS13-HMR-E* induction previously observed in the *htz1Δ* background was not caused specifically by the heterochromatic structures in the locus, but rather by an overall defect in induction of *GAL10* promoter-driven genes. This result is in accordance with previous studies that demonstrated slow induction of inducible genes in the *htz1Δ* strain (Adam et al., 2001; Santisteban et al., 2000).

On the contrary, deletion of *RTT109* and *ASF1* caused slow induction of transcription primarily in the locus covered with heterochromatic structures (Ref. I, Figure 6). Interestingly, all three proteins: Rtt109, Asf1 and Htz1 were required only for initial rapid induction of the heterochromatin-covered gene, as *GAL-VPS13-HMR-E* mRNA was detected in all strains after overnight growth in galactose-containing medium (Ref. I, Figure 6A). Thus, it is possible that during long-term growth under inducing conditions, heterochromatic structures are removed during DNA replication, allowing RNAPII to access the locus and inhibit re-formation of SIR complexes.

As Rtt109 and Asf1 proteins are both required for H3K56 acetylation, we decided to investigate the importance of this particular acetylation for RNAPII elongation through heterochromatic region in more detail. We constructed a yeast strain containing the *GAL-VPS13-HMR-E* model gene and carrying the H3K56R mutation, in which the H3 lysine at position 56 has been replaced by arginine to mimic the deacetylated state. As seen in the *rtt109Δ* and *asf1Δ* strains, mRNA production from the heterochromatic locus was severely

impaired in the H3K56R strain (Ref. I, Figure 6D), whereas induction of the endogenous *GAL10* or the heterochromatin-free *GAL-VPS13* gene was essentially unaffected (Ref. I, Figure 6C-D). To further analyze the effect constitutive H3K56 acetylation would have on transcription of *GAL-VPS13-HMR-E*, we generated two additional strains: one with deletion of the two major H3K56 deacetylases, *HST3* and *HST4*, and another strain with the H3K56Q mutation, where the lysine has been replaced with glutamine to permanently mimic the acetylated state of the histone. We saw no impairment of transcription of the heterochromatic locus in these strains and, most importantly, when we combined the H3K56Q mutation with deletion of *RTT109*, the inhibitory effect of *rtt109Δ* was reversed (Ref. I, Figure 6E). This confirmed that acetylation of H3K56, and not the Rtt109 protein itself, is responsible for efficient transcription elongation through the heterochromatic region.

As acetylation of H3K56 is cell cycle-regulated and occurs predominantly in the S-phase (Masumoto et al., 2005), we wanted to test whether progression through the S-phase is needed for effective transcription of the *GAL-VPS13-HMR-E* locus. In order to address this question, we arrested cells in the G1-phase using the yeast α-factor pheromone and monitored the induction of *GAL-VPS13* and *GAL-VPS13-HMR-E* mRNA in respective strains (Ref. I, Figure 7A-B). The two strains had very similar induction patterns in arrested cells, and the overall transcription kinetics of our model gene was essentially the same as in asynchronous cells (Ref. I, Figure 5). Severe impairment of *GAL-VPS13-HMR-E* transcript synthesis was observed only in the H3K56R background (Ref. I, Figure 7C). Taken together, these results demonstrate that acetylation of H3K56 is essential for transcribing a heterochromatic locus, while progression through the S-phase is not necessary.

To confirm that acetylation of H3K56 actually occurs in the *GAL-VPS13-HMR-E* locus during transcription, we used ChIP to directly compare the presence of acetylated H3K56 before, during and after the induction of transcription from *GAL-VPS13* and *GAL-VPS13-HMR-E*. We detected elevated acetylation levels of H3K56 in both strains at a region 5.5kb downstream of the promoter 120 minutes after galactose induction and shortly after repression of transcription (Ref. I, Figure 8). However, no significant change in H3K56 acetylation levels was detected within the 6.4 kb non-transcribed region after transcription induction in the *GAL-VPS13-HMR-E* strain (Ref. I, Figure 8), confirming that H3K56 acetylation at this locus was transcription-dependent.

Considering our overall results, we propose that the role of H3K56 acetylation may be either promotion of nucleosome removal during transcription elongation or inhibition of re-association of the SIR complex with the transcribed locus. As K56 is located in the part of the globular domain of histone H3 that contacts DNA and is close to the DNA entry/exit site (Hyland et al., 2005; Ozdemir et al., 2005), modification of this residue is good candidate for regulating histone-DNA or nucleosome-nucleosome interactions. Nucleosomes with H3K56 acetylation are bound more loosely to DNA and therefore can be removed more easily. During elongation, RNAPII displaces nucleo-

somes, and new histones with acetylated H3K56 are then re-loaded onto chromatin in the wake of its passage (Kaplan et al., 2008b; Rufiange et al., 2007; Schneider et al., 2006). This may create a more favourable chromatin environment for the next elongating RNAPII molecule.

In addition, H3K56 acetylation may directly interfere with the ability of the SIR complex to re-associate with the transcribed locus, leading to opening of heterochromatic structures. It has been shown that acetylation of H3K56 disrupts telomeric silencing and that in silenced loci K56 is maintained mostly in a hypoacetylated state (Miller et al., 2008; Xu et al., 2007; Yang et al., 2008). This possibility is also supported by *in vitro* experiments by Oppikofer and colleagues (Oppikofer et al., 2011). They demonstrated that acetylation of H3K56 reduced the affinity of the SIR complex for chromatin. Moreover, acetylated H3K56 also increased the accessibility of linker DNA to Micrococcal nuclease (MNase), suggesting that this acetylation leads to spontaneous unwrapping of DNA from the histone octamer (Oppikofer et al., 2011). Therefore, both DNA mobility and SIR complex re-association mechanisms may account for the anti-silencing effect of acetylated H3K56.

Additionally, it is worth noting that the borders of heterochromatic regions of DNA are not strictly defined, but are rather the product of the constantly changing equilibrium between SIR complex binding and dissociation. Therefore, it is possible that SIR recruitment is inhibited by the replacement of nucleosomes during transcription elongation with new nucleosomes containing acetylated H3K56. This, in turn, leads to shifting of the border between eu- and heterochromatin in favour of euchromatin. When acetylation of H3K56 is abolished (either by deleting *ASF1* or *RTT109* genes or by mutating H3K56 to arginine), reloaded nucleosomes do not restrict spreading of the SIR complex, and opening of a heterochromatic locus becomes considerably slower that in wild type cells.

3. Transcriptionally inactivated replication origins are repetitively licensed after transcription (Ref. II)

Cells start preparing for genome replication before the actual start of the S-phase. Already in the early G1-phase, ORC recruits the minichromosome maintenance (MCM) helicase to replication origins. These pre-replicative complexes (pre-RCs) remain inactive until cells enter the S-phase. It was demonstrated that replication origins in *S. cerevisiae* are mostly located in intergenic regions, so that transcription should not interfere with their functioning (Wyrick et al., 2001). However, the yeast genome is highly compact and bidirectional transcription initiation is common, giving rise to transcription of CUTs from intergenic regions (David et al., 2006; Davis and Ares, 2006; Neil et al., 2009; Wyers et al., 2005; Xu et al., 2009). Thus, it is highly likely that elongating RNAPII will encounter pre-replicative complexes at origins. This raises

questions about the consequences of such collisions and about the frequency with which replication origins are transcribed in budding yeast.

Our first goal was to determine the proportion of replication origins affected by transcription in *S. cerevisiae*. To answer this question, our colleagues from the Institute of Computer Science analyzed the tiling array data of the total transcriptome published by Neil *et al*. (2009). The analysis compared transcription of 336 replication origins defined as "confirmed autonomously replicating sequences (ARS)" in *S. cerevisiae* OriDB (Nieduszynski et al., 2007) to the average level of non-coding DNA transcription genome-wide. Results showed that over 10% of replication origins (34 of 336) had higher levels of transcription than an average non-coding region (Ref. II, Figure 1A). However, this analysis probably underestimated the number of transcribed replication origins due to the fact that in yeast cells transcription of non-coding DNA is widespread, which increases the background signal significantly.

To study in more detail whether transcription of non-coding DNA overlaps with replication origins, the genome-wide locations of CUTs as defined by the Serial Analysis of Gene expression (SAGE) by Neil et al. (2009) were analyzed. Replication origins were considered as untranscribed when no CUTs overlapped their location; as moderately transcribed when overlapped by 1-10 CUTs; and as extensively transcribed with 11-43 overlapping CUTs. This analysis revealed that more than one-third of all replication origin sequences are transcribed as CUTs (Ref. II, Figure 1B). Importantly, this analysis allowed us to identify 128 transcribed replication origins, compared to only 34 such origins revealed by the tiling array analysis of the total transcriptome (Ref. II, Figure 1C). Our results indicate that transcription of replication origins is common in *S. cerevisiae*, and that replication origins are mainly transcribed as CUTs. Although most of the replication origins are located in non-coding regions with low transcriptional activity, some efficient and active replication origins like ARS305 and ARS519 are highly transcribed as CUTs. This raises the question of how these two processes, transcription and replication origin functioning, influence one another.

Formation of the Pre-RC in a transcriptionally active locus (Ref. II)

To study what happens when an elongating RNAPII encounters a replication origin loaded with the pre-RC, we inserted different replication origins, referred to as ARS (ARS605, ARS607, ARS609 and ARS409) into our galactose-inducible *GAL-VPS13* model gene at a location 3 kb downstream of the *VPS13* start codon (Figure 4C; Ref. II, Figure 2A). We used ChIP to detect the transcribing RNAPII and monitor the binding of pre-RC components at *GAL-VPS13-ARS* loci. Insertion of the different ARS sequences into our model gene did not interfere with RNAPII transcription elongation, as evidenced by the fact that after transcription induction with galactose, we could detect RNAPII both upstream (at 2.6 kb) and downstream (at 3.5 kb) of the ARS sequence (Ref. II, Figure 2B). Although the signal of RNAPII was ~30% weaker at 3.5 kb

compared to 2.6 kb, this reduction was similar to that seen in the *GAL-VPS13* model gene without an ARS sequence (Ref. II, Figure 2B).

We confirmed formation of the pre-RC in *GAL-VPS13-ARS* by monitoring the binding of Orc2 and Mcm4 proteins to the origin region. When transcription was repressed, we detected a 6-12 fold enrichment of Orc2 binding to different *GAL-VPS13-ARS* loci, while no Orc2 signal was detected in the *GAL-VPS13* locus without an ARS sequence (Ref. II, Figure 2C). When transcription was induced with galactose, recruitment of Orc2 to *GAL-VPS13-ARS* loci was significantly reduced compared to non-induced conditions (Ref. II, Figure 2C). Orc2 occupancy was also measured at corresponding natural ARS loci and showed very similar levels to those found in transcriptionally repressed *GAL-VPS13-ARS* loci (Ref. II, Figure 2C). Importantly, no significant changes in Orc2 binding were detected in different growth media at natural ARS loci, confirming that loss of Orc2 from the *GAL-VPS13-ARS* model gene came as a result of transcription in the locus (Ref. II, Figure 2C).

We performed similar experiments with the MCM helicase component Mcm4 in two selected strains (*GAL*-VPS13 model gene with either ARS609 or ARS409 inserted). Again, we detected that binding of Mcm4 to *GAL-VPS13-ARS* loci was reduced upon transcriptional induction (Ref. II, Figure 2D). These results indicate that the replication origins within the transcriptionally inactive *GAL-VPS13-ARS* model gene were efficiently licensed. As before, no reduction of Mcm4 occupancy was observed at corresponding natural ARS sequences when cells were grown in galactose-containing medium and GAL transcription was induced (Ref. II, Figure 2D).

To investigate the RNAPII transcription-dependent removal of the pre-RC in more detail, we induced transcription of the *GAL-VPS13-ARS607* locus with galactose-containing medium for 120 minutes and then repressed it with glucose-containing medium for 30 minutes. Within 120 minutes of transcription, virtually all MCM and ORC complexes were removed from the ARS607 insert (Ref. II, Figure 3A-B). Strikingly, after 10 minutes of transcription repression, almost 50% of both complexes were reloaded onto the locus. (Ref. II, Figure 3A-B). For reference, we analyzed the Mcm4 and Orc2 signal at the natural chromosomal location of ARS607 and observed no significant changes in different media during the time course of the experiment (Ref. II, Figure 3A-B). We also monitored the amount of RNAPII at the 3.5 kb location from the start site of *GAL-VPS13-ARS607* and, as expected, found it to correlate negatively with the amounts of pre-RC components (Ref. II, Figure 3C).

The speed of ORC and MCM complex reloading suggested that cells do not need to go through the S-phase for relicensing of origins, and that origins within the *GAL-VPS13-ARS* loci are reloaded with pre-RCs immediately after transcription of the model gene is terminated. To verify this, we arrested cells in the G1-phase with α-factor and observed the removal and reloading of Mcm4 while maintaining cells in the G1-phase throughout the experiment (Ref. II, Figure 4A). In all four strains with different ARS sequences in the model gene,

we detected MCM removal from *GAL-VPS13-ARS* in response to transcription induction (Ref. II, Figure 4B). After 60 minutes of transcription repression of the model gene, the MCM complex was efficiently reloaded onto *GAL-VPS13-ARS*, even though cells were still arrested in the G1-phase (Ref. II, Figure 4B). No MCM removal or reloading was detected during the course of the experiment at natural ARS loci, as these origins in their natural chromosomal positions are not affected by galactose-inducible transcription (Ref. II, Figure 4C).

As we confirmed that reloading of transcriptionally removed pre-RCs onto replication origins is possible in the G1-phase, we wanted to know whether these relicensed origins were competent for replication initiation in the following S-phase. To monitor replication origin firing, the Cdc45 protein has been used as a reliable marker, as it associates with origins at the time of replication initiation (Aparicio et al., 2004; Zou and Stillman, 2000). We performed ChIP assays to detect Cdc45 throughout the course of the S-phase at the ARS609 origin inserted into *GAL-VSP13* (Ref. II, Figure 5A). After arresting cells with α-factor, pre-RC proteins were displaced by transcription, and then allowed to re-associate with DNA during 60 minutes in transcriptionally repressive conditions. Cells were then released into the S-phase, and binding of Cdc45 to the *GAL-VPS13-ARS609* locus was monitored by ChIP (Ref. II, Figure 5A). In parallel, we analyzed cells that had been kept continuously in transcriptionally repressive conditions, as well as cells where transcription of *GAL-VPS13-ARS609* was actively ongoing (Ref. II Fig. 5A). For reference, we also determined Cdc45 recruitment to the natural ARS609 locus (Ref. II Fig. 5A). We detected that when transcription was repressed, Cdc45 bound to the *GAL-VPS13-ARS609* locus (both *Raf-Gal-Glc* and *Glc* sample) and to the native ARS609 locus ~60 minutes after release from the G1-arrest, indicating that replication was initiated from these origins in the S-phase of the same cell cycle. As expected, when transcription was continuously activated, no binding of Cdc45 to the *GAL-VPS13-ARS609* replication origin was observed (Ref. II, Figure 5A). These results not only confirmed that a relicensed ARS609 origin in the *GAL-VPS13* locus was efficiently activated, but that there was no change in the timing or efficiency of replication origin activation compared to native ARS609 or to a *GAL-VPS13-ARS609* origin that had been continuously kept in transcriptionally repressive conditions.

Finally, we analyzed DNA replication intermediate structures of *GAL-VPS13-ARS609* loci from cells grown either in transcriptionally repressed or active conditions, or in conditions allowing relicensing of the origin. Detection of the replication bubble arc by two-dimensional DNA electrophoresis analysis confirmed that a relicensed *GAL-VPS13-ARS609* origin successfully fired 60 minutes after release into the S-phase (Ref. II, Figure 5C). Origin firing was also detected in cells grown in transcriptionally repressed conditions, but not in cells where transcription of the *GAL-VPS13-ARS609* locus was ongoing (Ref. II, Figure 5D-E).

Taken together, our results indicated that even though majority of *S. cerevisiae* replication origins are located in intergenic regions, at least one-third of them are transcribed, leading to displacement of pre-RC components (Ref. II, Figure 1; 2). However, our analysis also demonstrated that some of the replication origins are located in transcriptionally active regions, raising the possibility that replication machinery may benefit from transcription-coupled modifications and remodelling of chromatin. After the passage of RNAPII, transcribed regions may become more easily accessible to pre-RC components and facilitate initiation of replication. We also confirmed that when elongating RNAPII encounters a pre-RC, the latter is removed from the origin, yet elongation by RNAPII is unaffected (Ref. II, Figure 2). As individual RNAPII complexes often need to transcribe very long genes without dissociation, it is important that they remain active and stable on DNA even upon collision with other complexes. Our data support this concept, as the relative amount of RNAPII on our model gene was not affected by encountering and inducing removal of pre-RC components (Ref. II, Figure 2). Importantly, after transcription stops, transcriptionally inactivated replication origins are quickly reloaded with pre-RC components. This happens during the same G1-phase and allows for DNA replication to be initiated from these relicensed origins in the following S-phase. Such relicensing provides cells with a rescue mechanism for replication origins loaded with pre-RCs in case of sporadic transcription and helps maintain a sufficient number of functional replication origins. The existence of this mechanism further supports the idea that even though elongating RNAPII causes removal of pre-RC components, changes in chromatin structure induced by transcription may help replication machinery. Such a relicensing mechanism may also explain how replication origins can be located in transcriptionally active regions in other eukaryotes. For example, it was shown that in mouse embryonic stem cells, 85% of the replication origins are located within transcriptional units (Sequeira-Mendes et al., 2009).

4. Elongating RNAPII is uniformly distributed on a highly transcribed locus (Ref III)

Although the amount RNAPII recruited to a gene usually corresponds to the levels of produced transcript, several genome-wide studies of RNAPII occupancy have reported uneven distribution of the enzyme on different genes. For example, within some genes, elevated RNAPII signal has been detected in 5'-regions, while in others the polymerase appears to be concentrated either at the end of the coding region or at both 5'- and 3'-ends (Glover-Cutter et al., 2008; Rodriguez-Gil et al., 2010; Steinmetz et al., 2006). Additionally, when transcription from the galactose-inducible *GAL-VPS13* model gene was activated, considerably higher amount of RNAPII was detected in the 5'-region compared to the 3'-end of the gene in *S. cerevisiae* (Kristjuhan and Svejstrup, 2004). Such uneven distribution is expected immediately following induction of

transcription. However, we observed similar uneven distribution of RNAPII on *GAL-VPS13* even in cells that had been growing overnight in galactose-containing medium. In these cells, distribution of RNAPII should be stably established across the body of the *GAL-VPS13* gene.

One explanation for the gradual decline of the RNAPII density along the *GAL-VPS13* model gene could be acceleration of RNAPII during transcription, which would increase the distance between elongating polymerases. Alternatively, only a fraction of cells may produce a full-length transcript, while in others transcription terminates at random points in the gene. In this case, detection of RNAPII with ChIP in a population of cells would create deceptive enrichment of RNAPII at the beginning of the gene, as most cells would have RNAPII molecules at the beginning of the gene, but only some would have it at the end of the gene.

To analyze the distribution of elongating RNAPII complexes on the *GAL-VPS13* model gene in more detail, we performed ChIP analysis of cells where GAL-regulated transcription had been induced for 30 minutes by growth in galactose-containing medium. We observed that the amount of RNAPII was inversely correlated to the distance from the promoter. It decreased gradually, so that at the end of the coding region the density of RNAPII was approximately four times lower than next to the promoter region (Ref. III, Figure 1). To investigate the distribution of RNAPII at different regions of our model gene without variations in analyzed DNA sequence, we inserted a 700 bp DNA fragment (originating from the *Kluyveromyces lactis VPS13* gene) into the coding region of *GAL-VPS13* at different locations (at 0.1 kb, 3 kb, 6 kb or 9 kb downstream of the *VPS13* start codon) (Figure 4D; Ref. III, Figure 2A). These four yeast strains allowed us to analyze the amount of RNAPII on the exact same DNA sequence at various distances from the promoter, eliminating any possible influence from the DNA template. Using this approach, we observed that after overnight induction of GAL-regulated transcription, the relative amount of elongating RNAPII on the DNA insert remained dependent on its distance from the promoter, being approximately three times higher at the beginning of the gene than at its 3'-end (Ref. III, Figure 2B).

To exclude the possibility that differences in RNAPII levels might originate from different induction of galactose-regulated genes, we compared the amount of RNAPII recruited to the coding region of the *GAL10* gene and to the 2.6 kb region of the *GAL-VPS13* model gene in all four yeast strains (Ref. III, Figure 2C). The results showed very similar induction dynamics of *GAL10* and *GAL-VPS13* in different strains. This confirmed that the differences in RNAPII levels along the model gene reflected a genuine decrease of RNAPII density towards the end of the gene and were not caused by our experimental conditions.

Determination of uniform RNAPII distribution in the actively transcribed *GAL-VPS13* locus by re-ChIP assay (Ref. III)

Previous studies had demonstrated considerable variability in gene expression levels in individual yeast cells by measuring real-time GFP-bound mRNA

synthesis and using *in situ* hybridization assays (Larson et al., 2011; Zenklusen et al., 2008). These methods are suitable for estimating the amount of actively transcribing polymerases at a specific locus within a single cell. However, the distribution of individual RNAPII complexes along a transcribed gene remained unclear. By contrast, the conventional ChIP assay allows analysis of RNAPII distribution along a specific transcribed gene, but it reflects the average density of polymerase complexes on this gene in a population of cells. Therefore, this method is unable to determine whether an elevated RNAPII signal at the beginning of a gene reflects a genuinely higher density of RNAPII complexes in the 5'-region of the gene in all cells. Alternatively, it is possible that a stronger polymerase signal in the 5'-end is detected because transcription of the model gene is induced in most cells, but only a small number of them completely transcribe to the end of the gene.

To distinguish multiple elongating RNAPII complexes in a single *GAL-VPS13* locus, we constructed a yeast strain where the Rpb3 subunit of RNAPII was tagged with the E2 epitope in its genomic locus, while two additional copies of the *RPB3* gene with either E4 or myc-tag were inserted into the *HIS3* and *LEU2* loci, respectively. Because the RNAPII complex contains only one subunit of Rpb3, every RNAPII molecule in this strain could contain only E2, E4 or myc-tag, but not multiple tags at the same time. Equal incorporation of differently tagged Rpb3 subunits into functional RNAPII complexes was confirmed by ChIP, with all three variants of the complex efficiently recruited to the *GAL-VPS13* locus after galactose induction (Ref. III, Figure 3).

Next, we used this strain for sequential ChIP analysis (re-ChIP) to detect multiple RNAPII complexes on the same DNA fragment and to analyze the distribution of transcribing polymerases on our *GAL-VPS13* model gene. We induced transcription of the *GAL-VPS13* gene and used an antibody against the E2-tag for the first round of ChIP to precipitate RNAPII complexes containing Rpb3 with this tag (approximately one third of all polymerases in the cell extract). For the second round of ChIP, we used antibodies against either the E4 or myc-tag to recover DNA fragments bound by multiple polymerases. If only a single RNAPII complex was bound to a DNA fragment at one time, the second round of immunoprecipitation would yield no signal. However, successful recovery of RNAPII-DNA complexes in the second immunoprecipitation indicated that at least two differently tagged RNAPII molecules could occupy the same DNA fragment simultaneously. Importantly, this confirms that the whole RNAPII-DNA complex originated from the same cell, giving us a unique opportunity to detect multiple transcribing polymerases on a single chromatin fragment.

We compared the recovery of RNAPII from the second round of re-ChIP with different antibodies throughout the entire *GAL-VPS13* locus. The amount of RNAPII precipitated with the 4H8 antibody that recognizes the Rpb1 subunit and therefore all RNAPII complexes, regardless of their epitope tag, was defined as maximal possible recovery of RNAPII complexes in the second round of re-ChIP. Re-precipitation with antibodies recognizing either the E4 or

myc-tag recovered 30-40 % of RNAPII complexes within the *GAL-VPS13* locus compared to precipitation with the 4H8 antibody (Ref. III, Figure 4A). Intriguingly, no major differences in the efficiency of RNAPII recovery in different regions of the *GAL-VPS13* gene were detected; the amount of recovered RNAPII was rather uniform, regardless of the distance from the promoter (Ref. III, Figure 4A). This result indicates that uneven distribution of RNAPII on our model gene that was detected using conventional ChIP reflects the heterogeneity of the cell population, rather than different spacing of RNAPII molecules on this gene in individual cells.

When we applied re-ChIP assay to the less intensively transcribed *FBA1*, *TAN1* and *ARN1* genes, the recovery of multiple polymerases from the highly transcribed *FBA1* locus was slightly lower than from *GAL-VPS13* (Ref. III, Figure 4A). However, we could not recover any RNAPII-DNA complexes from the relatively weakly expressed *ARN1* and *TAN1* loci in the re-ChIP assay (data not shown), indicating that either the re-ChIP assay is not sufficiently sensitive or that only a single RNAPII transcribes these loci at any moment in time. Several other genome-wide studies support the latter possibility, as it has been shown that transcription of most genes is a rare event, taking place only a few times during one cell cycle (Holstege et al., 1998; Nagalakshmi et al., 2008; Pelechano et al., 2010). Additionally, the *in situ* hybridization analysis of transcription dynamics in living yeast cells has revealed that majority of constitutively expressed genes are transcribed by a single RNAPII complex (Zenklusen et al., 2008).

Finally, we wanted to determine the number of transcribing RNAPII molecules in a single DNA-protein complex. We used an equation to calculate how the probability of precipitating a second epitope tag increases as more polymerases are bound to the same DNA fragment. Experimentally acquired values from re-ChIP assay (Ref. III, Figure 4A) were fitted to the probability curve of multiple RNAPII complex detection (Ref. III, Figure 4B). For simplicity, it was assumed that all RNAPII complexes present at the locus were cross-linked during cell extract preparation. While this assumption probably overestimates the real cross-linking efficiency, it allows us to define the minimal number of RNAPII complexes bound to the same DNA fragment. Our results show that on average at least 2-2.5 RNAPII complexes occupy the same DNA fragment within the *GAL-VPS13* model gene (Ref. III, Figure 4B). The number of recovered RNAPII complexes in the *FBA1* locus, one of the most highly transcribed constitutively expressed genes in yeast, was slightly lower, averaging around 1.8 polymerases on a single DNA fragment (Ref. III, Figure 4B). We confirmed that the maximal length of DNA fragments in our re-ChIP assay was ~1000 bp (Ref. III, Figure 4C), although the majority of them were roughly 500 bp as assessed by DNA electrophoresis. This also indicates that the DNA fragments in our lysates were short enough to distinguish between RNAPII-DNA complexes bound to different regions of the *GAL-VPS13* model gene. As the real cross-linking efficiency of RNAPII is probably considerably lower than the theoretical maximal efficiency used in our calculations, it is

important to emphasize that these estimations should be considered as the minimal number of RNAPII complexes on a single fragment of DNA.

Our data show that spacing between different elongating RNAPII complexes remains stable along the entire highly transcribed model gene (Ref. III, Figure 4). In cases of random abortion of transcription and steady speed of elongation by transcribing RNAPII, we should see lower density of polymerases in the distal part of the gene. Therefore, it appears that two different ways to obtain a uniform distribution of RNAPII on a gene can be used. First, some elongating polymerases could abort in a co-ordinated way. For example, DNA damage might result in co-ordinated abortion of all elongating RNAPII complexes, as polymerases have to be removed and degraded for efficient DNA repair in the coding regions of genes (Somesh et al., 2005; Woudstra et al., 2002). Second, it is possible that the leading RNAPII complex elongates more slowly than the ones following it. This way, if any RNAPII complex prematurely aborts transcription, the faster polymerases can catch up with the leading polymerase, preserving even distribution of elongating RNAPII complexes. There are several reasons for the leading RNAPII complex to be slower than the following ones. For example, opening the chromatin structure and temporary removal of nucleosomes in coding regions of highly transcribed genes (Kristjuhan and Svejstrup, 2004; Lee et al., 2004; Schwabish and Struhl, 2004; Värv et al., 2007) could slow down the leading polymerase, while the following polymerases can transcribe more easily through the open chromatin in its wake. Even if nucleosomes are re-assembled behind the leading RNAPII complex, it is still possible that since newly synthesized nucleosomes are acetylated on H3K56, they are not as tightly bound to DNA and are therefore more easily removed.

Alternatively, the leading RNAPII complex could abort transcription more easily than the ones following it. It has been shown that many regulated genes are expressed as transcriptional bursts, where after rapid initiation of multiple mRNAs, the promoter remains inactive for relatively long periods (Chubb et al., 2006; Pare et al., 2009; Zenklusen et al., 2008). In this case, a group of polymerases transcribe through the entire locus together, and if the first one aborts, the next one can take over as the leading complex. This mechanism preserves uniform distribution of RNAPII complexes on genes, as we have shown in our experiments. Such a mechanism could be the most efficient strategy for inducible gene expression in response to external signals because cooperation of multiple RNAPII complexes may lower the nucleosomal barrier to transcription and thus ensure synthesis of full-length transcripts. This idea is supported by *in vitro* experiments done by Kulaeva and colleagues, showing that transcription efficiency through the nucleosomal barrier was greatly increased when the second RNA polymerase complex followed closely behind the leading one (Kulaeva et al., 2010). It was proposed that most likely the second RNA polymerase complex prevents backtracking of the first one.

Additionally, our data show that the average ChIP signal detected in a cell population should be interpreted with care, as such results could conceal the actual situation in individual cells.

CONCLUSIONS

Transcription elongation is a highly complex process, influenced by a variety of different factors. This study focuses mainly on the mechanisms of RNAPII-dependent transcription elongation in *S. cerevisiae*, examining the fate of elongating RNAPII complexes as they encounter different obstacles such as heterochromatin or pre-replication complexes in their path. As it was already known that transcription repression by heterochromatin targets the transition between RNAPII initiation and elongation, we wanted to find out whether already elongating RNAPII complexes could transcribe through these structures. Since we saw that RNAPII can contend with heterochromatin without a detrimental effect on its elongation efficiency, our next goal was to determine how transcription elongation is influenced by a replication origin loaded with pre-RC complexes in the early G1-phase. To study this, we inserted a replication origin into the highly transcribed *GAL-VPS13* model gene and studied its effect on transcription. Our results indicated that replication origins inserted into the model gene did not hinder transcription elongation by RNAPII. However, we repeatedly noticed that the amount of RNAPII complexes gradually decreased along the model gene. As this observation was based on the average signal from the whole cell population, we employed sequential chromatin immunoprecipitation assay (re-ChIP) to detect multiple RNAPII complexes on the same DNA fragment. Intriguingly, our results revealed that in cases where transcription proceeds all the way to the 3'-end of the coding region, elongating RNAPII complexes are distributed uniformly throughout the entire length of the *GAL-VPS13* model gene.

The following specific conclusions can be drawn from the current thesis:

1. Elongating RNAPII can transcribe through heterochromatin, leading to displacement of silencing complexes from the transcribed locus.
2. Acetylation of H3K56 is essential for RNAPII elongation through heterochromatin in budding yeast.
3. Many replication origins are transcribed in the budding yeast genome, mostly as cryptic unstable transcripts (CUTs).
4. Pre-RC complexes formed on replication origins in a highly transcribed locus are removed by elongating RNAPII, and this encounter does not interfere with transcription elongation.
5. Transcription-coupled inactivation of replication origins is reversible via reassembly of pre-RC components onto origins when transcription stops. These relicensed replication origins are fully functional in the following S-phase.
6. Elongating RNAPII complexes are uniformly distributed throughout a highly transcribed locus.

SUMMARY IN ESTONIAN

RNA polümeraas II-sõltuva transkriptsiooni elongatsioon pagaripärmis *Saccharomyces cerevisiae*

Kõikides eukarüootsetes rakkudes viib valke kodeerivate geenide transkriptsiooni läbi RNA polümeraas II (RNAPII). Protsessi, mille käigus sünteesitakse mRNA, nimetatakse transkriptsioonitsükliks ning see tsükkel jaotatakse kolmeks etapiks: transkriptsiooni initsiatsioon, elongatsioon ja terminatsioon. Eukarüootsetes organismides on rakutuumades paiknev genoom pakitud kromatiiniks, mis jaguneb sõltuvalt pakituse tasemest eu- või heterokromatiiniks. Sõltumata oma pakituse tasemest takistab kromatiin kõiki DNA-ga toimuvaid protsesse. Lisaks ülaltoodule jääb transkriptsiooni läbi viiva RNAPII kompleksi teele ka muid takistusi, nagu näiteks DNA kahjustused või DNA-ga seotud protsessides osalevad makromolekulaarsed kompleksid, mis võivad samuti transkriptsiooni elongatsiooni häirida.

Pikka aega arvati, et RNAPII poolt läbi viidavat transkriptsiooni kontrollitakse rakkudes peamiselt vaid initsiatsiooni tasemel, kuid nüüdseks on teada, et ka transkriptsiooni elongatsioon on väga keeruline ja tugeva kontrolli all olev protsess. Käesolevas töös uurisin RNAPII elongatsiooni mehhanisme, kasutades mudelorganismina pagaripärmi *Saccharomyces cerevisiae* ja mudelgeenina ühte pikka geeni *GAL-VPS13*, mille ekspressiooni saab süsinikuallikaga söötmes reguleerida. Töö esmaseks eesmärgiks oli tekitada kõrgel tasemel transkribeeritava pika mudelgeeni sisse nn. vaigistavate valgukomplekside abil heterokromatiinne ala ning uurida, kuidas selline struktuur mõjutab transkriptsiooni. Kuna elongatsioonietapis olev RNAPII võib transkriptsiooni käigus puutuda kokku erinevate DNA-ga seotud makromolekulaarsete kompleksidega, küsisime, mis juhtub siis, kui kõrgel tasemel transkribeeritavasse mudelgeeni viia sisse replikatsiooni alguspiirkond (*origin*), millel moodustuvad pre-replikatiivsed kompleksid. Eelnevates, terveid rakupopulatsioone hõlmavates katsetes oli näidatud, et RNAPII kompleksid jaotusid geenidel ebaühtlaselt, millest tulenevalt oli käesoleva töö järgmiseks eesmärgiks uurida RNAPII komplekside jaotumist ühel kõrgelt transkribeeritaval mudelgeenil ühes rakus.

Kokkuvõtvalt saab teha antud uurimustöö tulemuste põhjal sellised järeldused:

1. Elongatsiooni etapis olev RNAPII suudab transkribeerida läbi heterokromatiini, mis viib vaigistavate valgukomplekside eemaldamisele.
2. RNAPII elongatsiooniks läbi heterokromatiini on pagaripärmis vajalik histooni H3 56. positsioonis (H3K56) oleva lüsiinijäägi atsetüleerimine.
3. Pagaripärmi genoomis paikneb enamus replikatsiooni alguspunkte küll valke kodeerivate geenide vahelises alas, kuid kuna paljud pärmi promootorid suudavad initsieerida transkriptsiooni kahesuunaliselt, siis transkribeeritakse neist replikatsiooni alguspunktidest vähemalt kolmandikku.

4. Elongatsioonietapis olev RNAPII suudab kõrgel tasemel transkribeeritavalt geenilt eemaldada sellel moodustunud pre-replikatiivsed kompleksid, ilma et selline kokkupuude häiriks juba toimuvat transkriptsiooni protsessi.
5. Pärast transkriptsiooni lõppemist taastatakse pre-replikatiivsed kompleksid RNAPII transkriptsiooni poolt inaktiveeritud replikatsiooni alguspiirkondadel ning sellised uuesti moodustunud pre-replikatsioonikompleksid on järgnevas S-faasis funktsionaalsed.
6. Kõrgel tasemel transkribeeritaval geenil paiknevad RNAPII kompleksid ühtlaselt kogu geeni ulatuses.

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PUBLICATIONS

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Scientific work

My main scientific interest has been to study the mechansims of eukaryotic transcription elongation in *Saccharomyces cerevisiae*.

List of publications

Peil, K.; Värv, S.; Lõoke, M.; Kristjuhan, K.; Kristjuhan, A. (2011). Uniform distribution of elongating RNA polymerase II complexes in transcribed gene locus. Journal of Biological Chemistry, 286(27), 23817–23822.

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PATENT APPLICATION 60/652390 Inventors: SILLA, Toomas; HÄÄL, Ingrid; GEIMANEN, Jelizaveta; **JANIKSON, Kadri**, ABROI, Aare; USTAV, Ene; USTAV, Mart and; MANDEL, Tiiu Title: Vectors, cell lines and their use in obtaining extended episomal maintenance replication of hybrid plasmids and expression of gene products. 2005

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Teadustöö

Minu uurimustöö põhisuunaks on eukarüootse transkriptsiooni elongatsiooni mehhanismide uurimine pagaripärmis *Saccharomyces cerevisiae*.

Teaduspublikatsioonid

Peil, K.; Värv, S.; Lõoke, M.; Kristjuhan, K.; Kristjuhan, A. (2011). Uniform distribution of elongating RNA polymerase II complexes in transcribed gene locus. Journal of Biological Chemistry, 286(27), 23817–23822.

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