DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
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SIGNE VÄRV

Studies on the mechanisms of RNA polymerase II-dependent transcription elongation

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LIST OF ORIGINAL PUBLICATIONS

The current thesis is based on the following original publications, referred to in the text by their Roman numerals.

I **Värv, S**., Kristjuhan, K., Kristjuhan, A. (2007) RNA polymerase II determines the area of nucleosome loss in transcribed gene loci. *Biochem Biophys Res Commun* 358(2):666–71.

II **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.

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):23817–22.

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My personal contribution to the articles referred to in this thesis is as follows:

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

LIST OF ABBREVIATIONS

ac	acetylated
ADP	adenosine diphosphate
CBP	CREB binding protein (CREB cAMP response element binding protein)
CHD	chromodomain, helicase, DNA binding
ChIP	chromatin immunoprectipitation
CoA	coenzyme A
CTD	C-terminal domain
C-terminal	carboxy-terminal
ELL	eleven-nineteen lysine-rich leukaemia (elongation factor)
FACT	facilitates chromatin transcription
GAL	galactose
GNAT	Gcn5-related N-acetyltransferases
GTF	general transcription factor
HAT	histone acetyltransferase
HDAC	histone deacetylase
HML	hidden MAT left
HMR	hidden MAT right
INO80	inositol requiring 80
ISWI	imitation switch
MYST	acetyltransferase family (MOZ, YBF2/SAS3, SAS2, Tip60)
MAT	mating type locus in budding yeast
NFR	nucleosome free region
NPS	nucleosome positioning sequence
N-terminal	amino-terminal
NTP	nucleoside triphosphate
ORC	origin recognition complex
PAU	seripauperin
PIC	pre-initiation complex
P-TEFb	positive transcription elongation factor b
PTM	post-translational modification
RNAPII	RNA polymerase II
RSC	remodels the structure of chromatin
TBP	TATA-binding protein
TF	transcription factor
TSS	transcription start site
SAGA	Spt-Ada-Gcn5 acetyltransferase
SIR	silencing information regulator
SWI/SNF	switching defective/sucrose nonfermenting
YSPTSPS	tyrosine-serine-proline-threonine-serine-proline-serine

PROLOGUE

It was the sequential appearance of proteins in the replication complex, explained by my biology teacher, that appealed to me and triggered my interest for molecular biology. I was fascinated by this seemingly ordered and logical process – one event being the prerequisite for the next. First year at the university supported this impression. The same coordinated system worked in repair processes, in transcription, in translation. Things seemed logical and nicely regulated. My following studies made some corrections to this concept of clarity and easiness.

INTRODUCTION

During RNA polymerase II (RNAPII)-dependent transcription one strand of DNA is used to synthesise complementary mRNA. RNA synthesis is divided into three main phases – initiation, elongation and termination. Correct regulation of all these stages of transcription and gene expression in general is crucial for the viability of an organism. Therefore the process of RNAPII-dependent transcription has been the subject for intensive studies for decades. So far, over 10 000 scientific articles have been written about the basic mechanisms of transcription, most of them concern transcription initiation. Just around 1700 deal with transcription termination and about 1400 with elongation (PubMed, May 2011).

In all eukaryotic cells DNA is assembled into chromatin by forming complexes with histone proteins. The structural elements consisting of DNA and histones are called nucleosomes. Like all processes involving DNA as a substrate, the transcriptional machinery encounters higher structures of chromatin, which hinder the access to DNA primary sequence. Therefore it is inevitable to wonder how RNAPII overcomes this barrier. Previous studies have determined additional factors facilitating RNAPII elongation through chromatin by histone post-translational modifications and nucleosome remodelling. In the case of a high transcription level, nucleosomes are fully evicted form the transcribed gene during elongation and reassembled after transcription repression. In the current thesis we have experimentally determined the area of nucleosome loss and whether pre-existing or newly synthesised histones are assembled in new nucleosomes upon transcription inhibition. Further, more complex and closed structures of chromatin exist, called hetero- or silenced chromatin. The classical view has considered heterochromatic structures impenetrable obstacle for the RNAPII. The second purpose of this study was to investigate this presumption as previous studies have shown the existence of transcription initiation complexes on promoters within silenced chromatin.

The transcription levels and levels of elongating RNAPII on different genes vary significantly as the requirements for the gene products are not identical. Several genome-wide studies, which have addressed the question of the distribution of the components of transcriptional machinery have provided us with valuable information. But as these studies draw conclusions on the average signal from the whole cell population they fail to describe processes occurring in a single cell. Thus, our third goal was to determine the distribution of RNAPII complexes on a single chromatin fragment.

The literature overview in this study describes the structure of chromatin, its remodelling and modifying and gives a short description of RNAPII-dependent transcription with the emphasis on the budding yeast (*Saccharomyces cerevisiae*) which was used as a model organism in our experiments. The results obtained from our studies add new information to the basic knowledge of gene transcription and enhance the understanding about the mechanisms of RNAPII-dependent elongation providing the scientific community with surprising data on less studied topics.

I. OVERVIEW OF LITERATURE

1. Structure of chromatin

Around 1880s German biologist Walther Flemming gave the name "chromatin" to the substance that strongly absorbed basophilic dyes and was found in the nuclei of cells (Flemming, 1882; Olins and Olins, 2003). The name "chromatin" has stuck to the complex of DNA and its associated proteins since then. At the first glance the main purpose of packing DNA into chromatin seems to be the need to fit this enormous molecule inside the nucleus in an ordered manner. However, the dynamics of chromatin structure plays a significant role in regulating different cellular processes that require access to DNA.

1.1. Structure of the nucleosome

The first level of higher order packaging of chromosomal DNA is the nucleosome – a complex of DNA and histone molecules (Figure 1). Histones are positively charged nuclear proteins consisting of functionally different "histone body" and "histone tail". DNA is wound around the histone octamer for 1.65 superhelical left-hand turns, leading to about sixfold reduction of the original DNA length. For different eukaryotes the number of DNA base pairs in a nucleosomal particle may vary from 157 to 240 bp. When digested with nucleases the "core particle" of the nucleosome consists of 145–147 bp of DNA and the histone octamer. Rest of the DNA forms a linker between nucleosomes. The final diameter of the core particle is about 11 nm and the height is approximately 5.5 nm (Luger et al., 1997; Olins and Olins, 2003; Richmond et al., 1984).

The histone octamer consists of four histone dimers defined by H2A-H2B and H3-H4 histone pairs. The two H3-H4 pairs interact with each other via H3 proteins. H2A and H2B pairs both interact with one of the H4 histones in the tetramer via H2B molecules (Figure 1). In solutions with physiologically relevant ionic strength, H3-H4 tetramer and the H2A-H2B dimer are present as stable aggregates (Luger et al., 1997).

All four histone proteins contain a common structural element called histone-fold domain that facilitates the interaction between DNA and histone proteins. It consists of three alpha helices connected by two loops. In an artificial nucleosome formed from recombinant histone proteins and human α-satellite DNA, 121 bp of DNA is bound directly to the histone-fold-domains. Each dimer from the histone pairs is associated with 27–28 bp of DNA, leaving 4 bp first-hand unbound linkers between the interactions (Luger et al., 1997). A study revealing 1.9 Å resolution crystal structure of the nucleosome shows that binding with histone proteins induces changes in DNA conformation when compared to free oligonucleotides and non-histone protein-DNA complexes. These differences might play a major role in allowing the precise recognition of nucleosomal DNA by nuclear factors (Richmond and Davey, 2003).

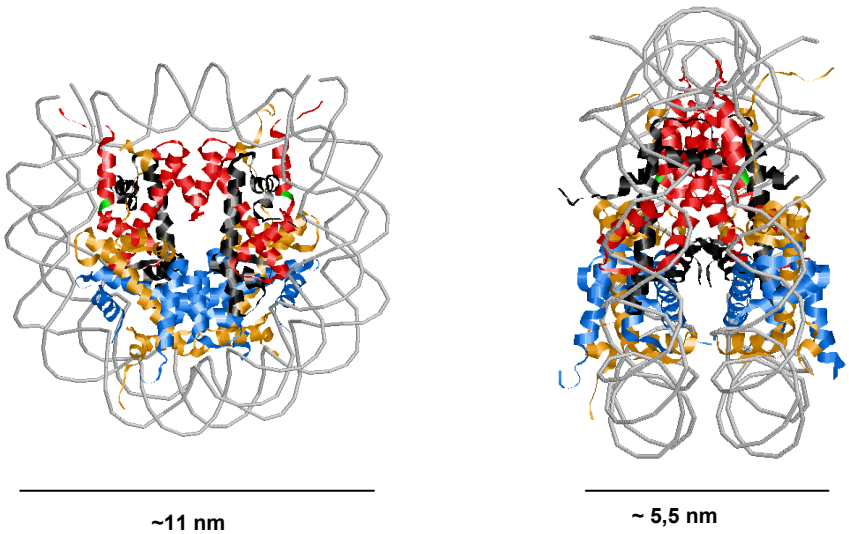

Figure 1. Structure of the nucleosome core particle. Top and side view of 146 bp DNA (light grey ribbon) in the complex with the eight histone proteins (red – H3; orange – H2A; black – H4; blue – H2B). Green spots on H3 proteins indicate the location of the K56 residue. Source: Protein Data Bank ID: 1AOI (Luger et al., 1997).

On the electron density map it is seen that the N-terminal tails of histones are out of the DNA-octamer fold for over about one third of their total length. The tails of H2B and H3 penetrate through the DNA super helix structure between two minor grooves situated side by side on the histone octamer. Nevertheless, due to weak electron density for the further tail sequences, the conformation of the extra-nucleosomal part is not known. It is very likely that no specific conformation exists. As these regions are highly basic and contain sites for covalent post-translational modifications (PTM), they are probably involved in the formation of higher order chromatin structure through interactions with neighbouring nucleosomes (Luger et al., 1997).

1.1.1. Histone post-translational modifications

As one way of contributing to the dynamics of chromatin, histones carry covalent modifications. These modifications are mainly found on histone tails, but some of them have been located also to histone bodies (Figure 2). Discovered in 1960s by Vincent Allfrey (Allfrey et al., 1964) but at that time considered somewhat artifactual, the histone modifications made their grand reappearance to the front pages in the end of the 1990s (Latham and Dent, 2007). In the year 2000, the "histone code" hypothesis was proposed, arguing that specific combinatorial sets of histone modifications can guide the

recruitment of particular transactivating factors to carry out specific functions (Barth and Imhof, 2010; Jenuwein and Allis, 2001; Strahl and Allis, 2000).

So far, well-known modifications of histones include co-translational acetylation of the N-terminus, post-translational lysine acetylation, lysine and arginine methylation, serine phosphorylation, lysine ubiquitylation and sumoylation. Less studied are glutamic acid ADP ribosylation, arginine deimination (citrullination), proline isomerization, lysine biotinylation and addition of the sugar residue *β-N-acetylglycosamine* (O-GlcNAc) (Iizuka and Smith, 2003; Kouzarides, 2007; Sakabe et al., 2010).

There are two main mechanisms through which histone modifications work. Firstly, some modifications change the overall charge of histones (in the case of acetylation, less positive charge is created due to the loss of ε-NH^{2+} groups on lysines). This can alter the strength of histone-DNA interaction. Secondly, histone modifications help to recruit nonhistone proteins to specific regions of DNA, therefore facilitating different cellular processes. First evidence for such regulation was found in case of bromodomains and chromodomains recognizing acetylated or methylated lysines, respectively (Dhalluin et al., 1999; Ferreira et al., 2007; Jacobs and Khorasanizadeh, 2002).

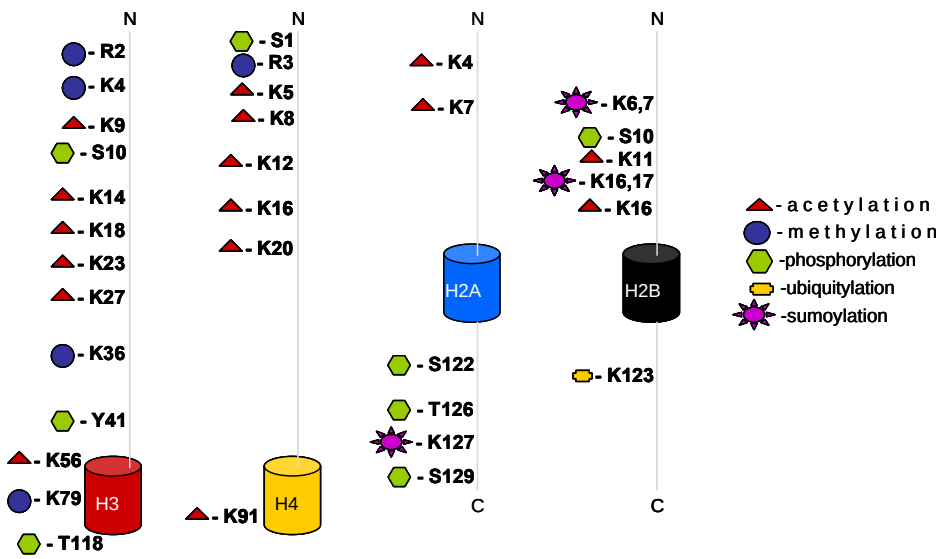

Figure 2. Common histone modifications in budding yeast. Numbers on the figure indicate modified amino acid residues on the corresponding histone proteins. Different modifications have been indicated by the colour code and different shapes (red triangle – acetylation; blue sphere – methylation; green hexagon – phosphorylation; yellow cross – ubiquitylation; magenta sun – sumoylation). N and C indicate amino- and carboxy-termini of histone proteins, respectively.

Histone modifications and their patterns are an intriguing topic as the vast number of possible combinations complicates the understanding of these patterns significantly. There are approximately 60 amino acid residues that are modified on histones. But each histone in the nucleosome might have its own modification pattern changing in time as modifications (acetylation, methylation, phosphorylation, deimination) can appear and disappear according to signals arriving from the environment. In addition, one modification may appear in different forms, for example, mono-, di- and trimethylation is common for lysines, mono- or dimethylation for arginines. It all indicates that nucleosomes have some kind of modifications all the time, but all these modifications are in permanent change. What more, there are some very specific histone modifying enzymes (mainly methyltransferases and kinases), shown to covalently attach chemical groups only to specific histone residues. But at the same time there are histone modifiers, whose substrate discrimination is not so restricted, modifying also other proteins in cells.

1.1.1.1. Histone acetylation

Two types of histone acetyltransferases (HATs; type B and type A) carry out the transfer of acetyl group from acetyl-CoA to the ε-amino group of lysine side chains on histones. Type B HATs are located to the cytoplasm and are responsible for acetylating newly synthesised free histone proteins. Right after synthesis histone H4 is acetylated at positions K5 and K12, histone H3 preferentially at position K9. When incorporated to nucleosomes, histones are quickly deacetylated by histone deacetylases (HDACs). Further modifications take place while histones are already components of nucleosomes (Bannister and Kouzarides, 2011; Jackson et al., 1976; Kuo et al., 1996; Parthun, 2007).

Type A HATs can be divided into at least three groups (GNAT, MYST, CBP/p300) according to their protein structure and amino acid sequence homology. All the enzymes in this family modify multiple sites within the N-terminal tails of histones but also additional sites within the histone core (Figure 2). Highly conserved acetylation sites in histone H4, in addition to previously mentioned ones, are K8, K16 and K20. N-terminal acetylation sites for H3 encompass also residues K14, K18, K23 and K27 (Bannister and Kouzarides, 2011). Whilst most modifications take place on histone tails, which are open for possible docking of proteins and complexes with specific enzymatic activities, acetylation mark of H3K56 is situated in the core domain of the histone. K56 residue is facing toward the major groove of the DNA in the nucleosome, being in a very good position to disrupt DNA-histone contact when acetylated by HAT Rtt109 (Han et al., 2007; Xu et al., 2005). Acetylation sites on H2A and H2B are less conserved and also less studied. Nevertheless, in yeast, acetylation of K11 and K16 on H2B and K4, K7 on H2A has been described (Ahn et al., 2006; Fuchs et al., 2009; Suka et al., 2001).

Type A HATs can be frequently found in large multi-subunit complexes, including the transcriptional machinery. Being involved in bigger complexes modulates HATs' activity and specificity. For example, purified Gcn5 acety-

lates free histones but not histones within a nucleosome. When part of the SAGA complex, it can efficiently acetylate also histones in nucleosomes (Grant et al., 1997).

Some general conclusions on the distribution of histone acetylation can be drawn in *S. cerevisiae*. On actively transcribed genes more acetylation signal is detected in the promoter and also 5' part of the gene, encompassing different acetylation sites like H3K9, H3K14, H3K18, H4K5, H4K12 and H2AK7. Nevertheless, on two nucleosomes surrounding the transcription start site (TSS), H2BK16, H4K8 and H4K16 acetylation level is low (Liu et al., 2005; Pokholok et al., 2005). When analysing acetylation patterns on single genes, a bit more controversial results may be obtained. For example, in case of H3K9 acetylation, data on "average gene" demonstrates that this modification peaks at the predicted TSSs of active genes and correlates with transcription rates genome-wide. Whereas data specifically on *GAL1–10* genes show that these genes have higher activation levels if at least 3 acetylation sites on H3 – K9, K14, K18 or K23 – are substituted with arginine or glycine (Mann and Grunstein, 1992; Pokholok et al., 2005). Hence indicating that histone acetylation is not always prerequisite for efficient gene expression.

The activity of nine HATs found in budding yeast is reversed by a number of HDACs whose activity restores the positive charge of lysines. There are four classes of HDACs of which three are represented in budding yeast. Classes I and II comprise of enzymes related to deacetylases Rpd3 and Hda1, class III (sirtuins) enzymes are homologous to yeast Sir2 protein and of all the HDACs are the ones who need NAD^+ as cofactor for their activity. In mammals also class IV of HDACs exists but has only one lonely member – HDAC11. Compared to HATs, HDACs have low substrate specificity by themselves and they are able to deacetylate different histone residues in a single nucleosome (Fuchs et al., 2009; Kurdistani and Grunstein, 2003; Rundlett et al., 1996; Yang and Seto, 2007). It is very likely that continuous balance between the activity of HATs and HDACs is one of the determinants for gene transcription level.

1.1.1.2. Histone methylation

Histone methylation occurs on lysine and arginine side chains and is carried out by methyltransferases. Methylation can be a multilevel process as mono-, di- and trimethylation is possible. Trimethylation occurs only on lysine residues whereas arginine can be dimethylated either symmetrically or asymmetrically (Bedford and Richard, 2005). Methyltransferases are much more substrate specific than HATs and so far 3 methylation sites for lysines on histone H3 in budding yeast are known – H3K4, H3K36 and H3K79, methylated by Set1, Set2 and Dot1, respectively (Briggs et al., 2001; Feng et al., 2002; Strahl et al., 2002) (Ng et al., 2002). Arginine methylations appear on H3R2 and H4R3 and are catalysed by arginine methylases. Methylation does not change the charge of histone tails and therefore the predominant role of these modifications is most likely the recruitment of additional factors needed for the execution of cellular processes on DNA. Proteins with chromodomains and PHD domains can

recognise methyllysines, whereas proteins with Tudor domain recognise both, methyllysines and –arginines (Cote and Richard, 2005; Fuchs et al., 2009; Jacobs and Khorasanizadeh, 2002; Taverna et al., 2006).

All lysine methylations in budding yeast have been associated with active transcription. On the coding region trimethylation of H3K36 and H3K79 is associated with elongating RNAPII although H3K79 methylation is also found on the promoter regions. Methylation of K36 plays a somewhat controversial role as this mark is a signal for recruiting HDAC Rpd3 to repress transcription. Trimethylated H3K4 is more specific to the 5' end of genes and to regulatory regions (Carrozza et al., 2005; Pokholok et al., 2005; Rao et al., 2005). In contrast to the activating properties of methylation in budding yeast, in higher eukaryotes and fission yeast (*Schizosaccharomyces pombe*) methylation on H3K9, H4K20 and H3K27 is associated with gene silencing and formation of heterochromatin (Kirmizis et al., 2007; Min et al., 2003; Nakayama et al., 2001; Yu et al., 2006).

Until recently it was believed that methylation is an irreversible histone mark. To date already several demethylase enzymes have been described in mammals and some in budding yeast. For example, demethylation of H3K4 by yeast trimethyl demethylase Jhd2 antagonises active transcription and has been shown to repress telomeric silencing (Liang et al., 2007; Shi, 2007).

1.1.1.3. Other post-translational modifications on histones

Although phosphorylation is an abundant protein modification, it is relatively rare on histone molecules. Phosphorylation takes place on serines, tyrosines and threonines and predominantly on the N-terminal part of histones but not exclusively. This modification is added and removed by kinases and phosphatases, respectively. As in other processes, phosphorylation of histones corresponds to changes in extracellular environment. For example, phosphorylation of H3 serine 10 promotes transcription (by influencing acetylation on H3K14) in response to the change in carbon source (Lo et al., 2001). As phosphorylation adds negative charge to the histone, it undoubtedly influences chromatin structure. In case of H3T118 *in vitro* studies have shown that phosphorylation of this residue dramatically decreases DNA-histone octamer binding and increases nucleosome sliding (North et al., 2011). Phosphorylation of serine 1 on H4 is required for the efficient recruitment of the SWI/SNF chromatin remodelling complexes (Schwabish and Struhl, 2007). Other defined phosphorylation sites on histones include H3S28, H3Y41, H2BS10, H2AS122, H2AT126, H2AS129 and have been linked to diverse processes in the cell (Fuchs et al., 2009).

Large changes to the amino-acid side chains on histones are caused by ubiquitylation and sumoylation whereas other histone modifications cause relatively small molecular changes. Ubiquitylation acts as a signal for protein-protein interactions and in regulating protein stability. In budding yeast only one ubiquitylation site has been found – on histone H2B, lysine 123. This modification leads to the recruitment of Set1 and Dot1 methyltransferases that

are essential for H3K4 and H3K79 methylation, respectively. H2B ubiquitylation also facilitates FACT function and that way stimulates transcript elongation (Foster and Downs, 2009; Pavri et al., 2006). In contrast to ubiquitylation, sumoylation has been reported on all four histone proteins. Nevertheless, specific sumoylation sites have been mapped only on H2A and H2B. The precise role of sumoylation in transcription is largely unknown but in general it is believed to be a repressive mark antagonizing histone acetylation (Nathan et al., 2006).

Proline isomerization is the only non-covalent post-translational modification on histones and the change in proline conformation can cause a significant change in protein structure. If proline 38 on histone H3 is mutated, it affects the ability of Set2 to methylate nearby lysine 36, therefore influencing transcription elongation (Nelson et al., 2006).

In mammalian cells histones are known to be mono- and poly-ADP ribosylated on arginine and glutamate residues. Poly-ADP ribosylated histones have been correlated with less strained chromatin structure, probably as the consequence of the negative charge caused by the modification. Also, this modification increases the acetylation levels of the core histones (Cohen-Armon et al., 2007; Hassa et al., 2006). Conversion of arginine to citrulline neutralizes the positive charge of arginine and therefore has the potential to affect chromatin structure (Cuthbert et al., 2004).

1.1.2. Histone variants

Besides the four highly conserved histones (H2A, H2B, H3, H4), nucleosomes encompass different histone variants that have specific roles in many processes, including DNA repair, chromosome segregation, meiotic recombination and transcription initiation. Compared to their canonical relatives, histone variants are much less conserved, their expression is not coupled to DNA replication and they are found in distinct DNA regions, facilitating specific cellular processes. The structural differences of histone variants alter the structure of nucleosomes and through that change the dynamics of chromatin (Talbert and Henikoff, 2010).

Centromeric histone H3 variants (CenH3; Cse4 in *S. cerevisiae*, CENP-A in humans) are important in the formation of kinetochore and special in their way of forming remarkably smaller nucleosomes by size and with lower capacity to protect DNA from nuclease attack (Dalal et al., 2007). In addition they seem to cause the DNA wrap around the histone octamer in right-handed manner opposed to the common left-handed wrap (Furuyama and Henikoff, 2009).

H3.3 differs from the canonical H3 by only four amino acid substitutions and its assembly to chromatin in *D. melanogaster* is replication-independent (Ahmad and Henikoff, 2002). In human and fruit fly cells H3.3 is assembled into chromatin of transcribed genes, gene regulatory elements and promoters (Schwartz and Ahmad, 2005). Ascomycetes, like *S. cerevisiae*, do not have this

extra version of H3 and where needed, the canonical H3 is incorporated to nucleosomes replication-independently (Mousson et al., 2007).

From the viewpoint of transcription, H2A variant Htz1 (H2A.Z in humans) has a crucial role. This histone variant is conserved from yeast to humans and in yeast comprises about 5% of the total H2A in cells, being broadly but not uniformly distributed throughout the chromosomes. Htz1 can be found in nucleosomes on both sides of the nucleosome free region (NFR), at transcription start sites, promoting efficient RNAPII recruitment and interacting with the components of the transcription machinery (Adam et al., 2001). Deletion of Htz1 in *S. cerevisiae* is not lethal but does make cells grow significantly slower. Htz1 is incorporated to the promoter regions of repressed *GAL1-10* and *PHO5* genes. Although gene activation is not impaired on repressed genes when *HTZ1* is deleted, double mutants with chromatin remodelling complex SWI/SNF or histone modifying complex SAGA cause significant defects in gene induction (Santisteban et al., 2000). In general, nucleosomes containing Htz1 version have high turnover rates and are less stable than H2A containing nucleosomes. Being susceptible to loss they expose promoter DNA to regulatory proteins and that might be the mechanism behind their ability to promote gene transcription (Zhang et al., 2005). Htz1 also locates to subtelomeric regions, restricting the spread of heterochromatin in yeast chromosomes (Meneghini et al., 2003). In transcription elongation Htz1 is important for the normal distribution of elongating RNAPII. In *htz1*Δ cells elongation complexes have different composition compared to wild-type cells. For example, in mutant cells abundance of elongation factor Spt5 was detected on *GAL1* gene. Hence, establishment or maintenance of the normal RNAPII elongation complex might be facilitated by chromatin containing the Htz1 variant. Also, in mutant cells RNAPII transcription rate is approximately 24% slower than in wild type cells, probably caused by the increase in nucleosome occupancy in *htz1*Δ strain (Santisteban et al., 2011).

In DNA repair another version of H2A, H2A.X, is represented. This version of H2A has a specific phosphorylation site coming handy when DNA repair machinery needs to be recruited. In budding yeast all H2A molecules have this additional phosphorylation site and that is the canonical H2A for this organism (Mannironi et al., 1989; van Attikum and Gasser, 2009; West and Bonner, 1980).

1.2. Formation and dynamics of heterochromatin

A classical view of transcriptional silencing states that the highly condensed heterochromatin structure elicits its repressive effects by sterically hindering the access of sequence specific regulatory factors required for binding of transcription machinery and therefore blocking the whole process (Kornberg and Lorch, 1991).

In *S. cerevisiae* heterochromatin formation requires the silent information regulator (SIR) complex. SIR proteins are involved in silencing three main regions – telomeres, ribosomal DNA (rDNA) locus and silent mating type loci (HML and HMR). Classical understanding for SIR complex recruitment is a nucleation-polymerisation model – SIR proteins spreading from silencers, flanking the silent cassettes, along chromatin in a stepwise manner (Rusche et al., 2003).

The mating type, α or a, of a yeast cell is determined by the allele of the mating type locus *MAT*. In addition to the expressed allele in *MAT* locus, *Saccharomyces cerevisiae* strains have silenced copies of mating type genes at HML and HMR loci that contain cryptic copies of *MATα1/α2* or *MATa1/a2* genes, respectively. The HM loci are flanked by silencers termed *E* and *I*. (Donze et al., 1999; Loo and Rine, 1994; Sekinger and Gross, 1999). To establish silenced chromatin, SIR proteins Sir1p, Sir4p, Sir2p and Sir3p have to be recruited to silencer sequences. This recruitment process is hierarchical where one event (the recruitment of a protein) leads to another (Rusche et al., 2003).

The HMR-E silencer consists of binding sites for three essential factors – origin recognition complex (ORC), Rap1 and Abf1. All these proteins have affinity for one or more SIR proteins and facilitate their recruitment to silencer. After the binding of essential factors Sir1 interacts with Orc1 protein and enhances the recruitment of other SIR proteins. Next step in the formation of heterochromatin is the binding of Sir4 to Sir1 and Rap1 proteins. Sir4 is likely responsible for bringing also Sir2 along. Sir3 protein is recruited by its binding to Sir4 and Abf1 proteins. After the assembly, SIR proteins spread from the silencer to their target region. The spreading occurs by the binding of Sir3 and Sir4 proteins to the tails of histones H3 and H4. Sir3 and Sir4 bind more efficiently hypoacetylated histone tails and therefore they need the deacetylase activity of Sir2 for this interaction. The onward binding of Sir4 and Sir3 helps to recruit additional Sir2 proteins and that way facilitate further binding of Sir3 and Sir4 to adjacent nucleosomes (Carmen et al., 2002; Rusche et al., 2002; Rusche et al., 2003; Zhang et al., 2002).

HMR-E silencer promotes assembly of silent chromatin bi-directionally but has an orientation preference, silencing reporter genes more efficiently on Abf1 binding side (adjacent to the *MAT A* genes) than on the side that binds ORC. This property is caused by the nucleosomal positioning on the binding sites of those proteins, promoting spreading mainly in one direction (Zou et al., 2006). HMR-E works also when inserted to other chromosomal contexts. An interesting notion is the ability of the silencer to promote SIR protein spreading without the classical recruitment mechanism described previously. Recently it was shown that HMR-E promotes association of SIR proteins also with distant nucleosomes (even more than 2 kb away) and not only adjacent ones. In the work by Lynch and Rusche simultaneous (and not step-wise) appearance of SIR proteins was detected on regions around the silencer. Also the speed of SIR protein spreading seems to be dependent on the chromatin structure of the

heterochromatin nucleation site as SIR complex spreading was faster from the HMR-E silencer than on telomeric regions (Lynch and Rusche, 2009).

In a recent article, employing genome-wide deep sequencing, it was shown that besides silencers the SIR proteins bind also other sites in the genome. Sir2-dependent binding of Sir3 was seen to DNA regions close to seripauperin (PAU) genes. The PAU genes seem to act as additional recruitment sites to silencing proteins and facilitate the spreading of silenced chromatin near telomeres (Radman-Livaja et al., 2011). One explanation for the findings of Sir3 binding in unexpected chromosomal sites might be the fact that SIR complex can itself promote the formation of higher structures of chromatin through "Sir3/distant nucleosome" interactions caused by H4K16 deacetylation. In a reversed version, chromatin folded into higher-order state can account for Sir3 association with a number of nucleosomes away from the concrete silencer region (Johnson et al., 2009; Shogren-Knaak et al., 2006).

For the cell it is crucial to prevent heterochromatin from spreading beyond the desired regions or assemble to wrong places. For that, barrier elements exist at some junctions between silenced and active chromatin. One naturally occurring barrier near the HMR locus is the $tRNA^{Thr}$ gene (Donze et al., 1999). However, no barrier elements have been identified at most transition sites from eu- to heterochromatin. For example, in telomeric region competition between active and silenced chromatin is proposed. Active chromatin is characterised by changes in chromatin structure, encompassing a set of histone modifications that reduce the affinity of the silencing proteins for nucleosomes, that way limiting the spread of heterochromatin. Presence of specific proteins involved in modifying chromatin, assures that the equilibrium between active and silenced chromatin is reached. Some of the important determinants at the self-forming barriers are HAT Sas2, bromodomain protein Bdf1, histone variant Htz1, histone methyltransferases Dot1 and Set1 as in case of their absence, SIR proteins tend to spread a bit farther at telomeres (Kimura et al., 2002a; Krogan et al., 2002; Ladurner et al., 2003; Meneghini et al., 2003; Suka et al., 2002; van Leeuwen et al., 2002). In addition, only in the case of Sir3 over-expression significant increase in heterochromatin spreading is detected. This suggests that actually the amount of Sir3 proteins themselves (but not Sir4 or Sir2) is the limiting factor for the spread of heterochromatin (Hecht et al., 1996; Strahl-Bolsinger et al., 1997).

1.3. Higher-order structure of chromatin

In addition to heterochromatin formation, other higher-order structures of DNA have been described. The nucleosomal structure needed for the formation of heterochromatin is referred to as the "beads on a string" fibre and its discovery is obliged to the inhabitants of the coop on the roof of the Department of Biophysics in the heart of London's theatre district in early 1970s. Namely the sophisticated London chicken and their donation of erythrocytes led to the

electron micrographs revealing the beautiful structure of nuclear DNA (Chen and Li, 2010; Olins and Olins, 1974; Olins and Olins, 2003). In "beads on a string" fibre the adjacent nucleosomes are attached through linker DNA, the length of which varies among cells and species and is between 20 to 80 base pairs (bps). In higher eukaryotes the linker DNA is associated with nuclear histone proteins H1 or H5 (found only in avian erythrocytes), lacking in *S. cerevisiae*.

The secondary structure of chromatin is a 30 nm fibre. Due to experimental difficulties the exact structure of this compaction of chromatin fibres is not yet clear. So far two different models have been proposed. The solenoid (one-start helix) model states that successive nucleosomes are packed side-to-side in a left-hand helix with bended linker DNA (Widom and Klug, 1985). The Zig-Zag model (two-start helix) proposes the explanation where an essentially straight linker DNA connects nucleosomes on opposite sides of the 30 nm fibre and also has a left-handed turn (Chen and Li, 2010; Robinson and Rhodes, 2006; Schalch et al., 2005; Williams et al., 1986; Woodcock et al., 1984). The 30 nm fibre plays an important role in the formation of heterochromatin and in transcription regulation. Other higher-level chromatin structures probably form by compaction of 30 nm fibres but so far the possible uniform structure of further construction of the chromatin has not been defined yet.

It is clear that chromatin exhibits a very dynamic balance between the open conformation ("beads on the string") and the compacted 30 nm structure. Modifying this equilibrium allows gene expression control on chromatin level.

2. RNA polymerase II dependent transcription

The encounter of two war refugees, François Jacob and Jacque Monod, at the Pasteur Institute in Paris in 1950 was the starting point for the studies of transcription and discovery of mRNA. Seeing the same mechanism behind the lysogeny of bacteriophage lambda induced by ultraviolet light and the ability of *Escherichia coli* to make an enzyme that digests lactose only when the cell encounters that sugar established the base for the future research in the field of gene regulation and resulted in Nobel Prize in Physiology or Medicine for the men involved (Gann, 2011; Jacob and Monod, 1961).

Transcription is the first and highly regulated step in gene expression control. Whilst bacteria have single RNA polymerase consisting of 6 subunits, most eukaryotes have three polymerases (some plants have four) specialised for producing distinct RNA species. RNA polymerase I localises to the nucleolus where it synthesises ribosomal RNA (rRNA). RNA polymerase III is responsible for making tRNAs, 5S rRNA and 7S RNA. The fourth polymerase, discovered in plants, has been shown to have role in heterochromatin formation and gene silencing. The task of RNA polymerase II is to transcribe all protein-coding genes and produce small nuclear RNAs.

2.1. Structure of the RNAPII

RNA polymerase II complex consists of 12 subunits with the whole mass of 550 kDa. The subunits assemble into a structure that is roughly spherical with a cleft (comprised of the two largest subunits – Rpb1 and Rpb2) that is wide enough to accommodate the DNA template (Figure 3). DNA enters the positively charged cleft from down the middle of the enzyme, passing between a pair of mobile elements termed "jaws". In the cleft the two biggest subunits clamp the 8 bp RNA:DNA hybrid and downstream DNA duplex. These interactions make it possible for the elongating RNAPII to slide along DNA and RNA during elongation and also go backwards. Kornberg and colleagues have proposed a transcription cycle in which bending of the bridge helix (F-bridge, subdomain of Rpb1) at the 3' face of the RNA:DNA duplex induces translocation of the nucleic acid by one nucleotide within the RNAPII, while following relaxation of the bridge region opens the binding site for the next complementary NTP (Cramer et al., 2001; Gnatt et al., 2001; Komissarova and Kashlev, 1997).

In general the 12 different subunits participate in the formation of 4 distinct RNAPII modules. The previously mentioned Rpb1 and Rpb2 comprise the "core" module, which forms the active centre. Around the active centre subunits Rpb3 and Rpb10, Rpb11 and Rpb12 are important for RNAPII assembly. Along the sides of the DNA binding cleft lie additional modules – the "jaw-lobe" module that is responsible for clamping the DNA downstream of the active site (Rpb1, Rpb9, Rpb2), the "shelf" module (Rpb5, Rpb6, "foot" and "cleft" regions of Rpb1), and the "clamp" module (Rpb1, Rpb2). RNAPII subunits Rpb4 and Rpb7 were crystallised later and they constitute a tail-like sub-complex to the whole enzyme (Figure 3) (Armache et al., 2005; Cramer et al., 2001; Gnatt et al., 2001). Of all the 12 subunits only Rpb4 and Rpb9 are non-essential, although their deletion causes temperature sensitivity and problems in elongation fidelity (Hemming et al., 2000; Miyao et al., 2001).

A very interesting structural domain is the C-terminus (CTD) of Rpb1. It is composed of tandem repeats of the hydrophilic heptapeptide $Y_1SPTSPS_7$ sequences. In yeast there are 26 repeats, in humans 52. An important feature of CTD is its phosphorylation. Phosphorylation occurs at Serine-2 (Ser-2), Ser-5 and Ser-7 residues and is characteristic to the different phases of transcription. Ser-2 phosphorylation level is higher on an elongating polymerase, whereas during initiation Ser-5 phosphorylation is more abundant. Ser-7 phosphorylation profiles are gene specific, phosphorylated RNAPII peaking either in the 5' or 3' ends of genes or both. Ser-7 phosphorylated RNAPII is specifically enriched over introns, maybe playing a role in splicing. The major function of the phosphorylated CTD is to be the binding site for specific nuclear factors that control transcription (Kim et al., 2011; Phatnani and Greenleaf, 2006).

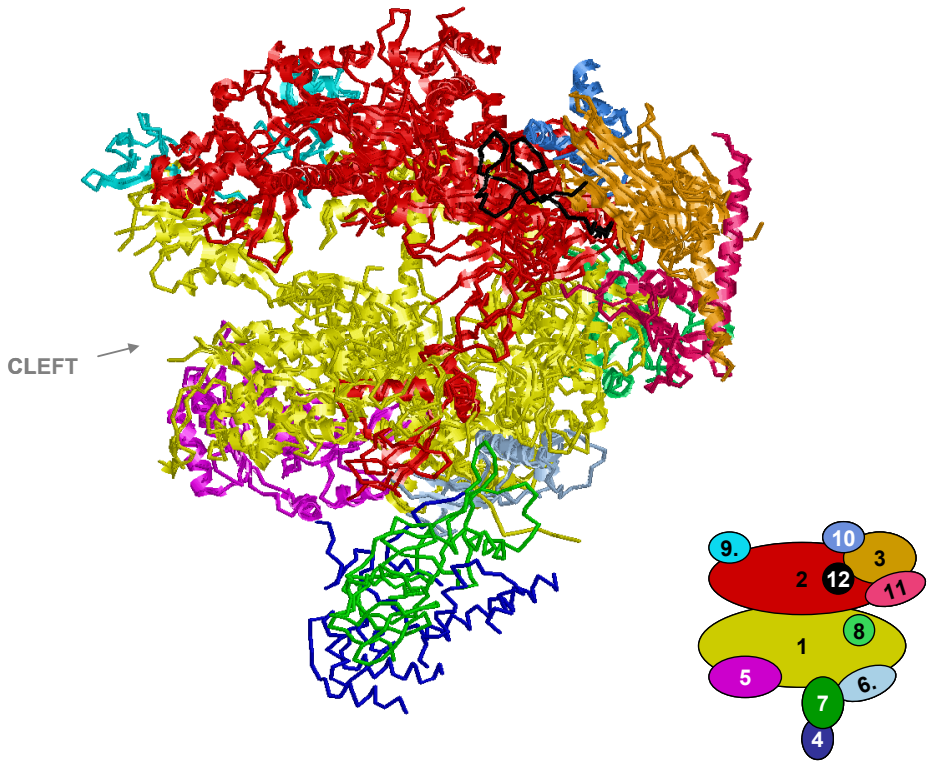

Figure 3. Structure of the RNA polymerase II. Ribbon representation of the 12 subunits of the RNAPII complex. The polymerase subunits (Rpb1-Rpb12) are coloured according to the diagram. *Source*: Protein Data Bank ID: 1NIK (Bushnell and Kornberg, 2003).

2.2. Phases of transcription

Transcription is divided into three main phases: initiation, elongation and termination. All these phases have their own "sub-divisions" that allow exact modulation of gene expression.

During transcription initiation RNAPII is recruited to the promoter regions of protein coding genes. First an activator binds to the promoter and depending on the characteristics of the promoter, recruits chromatin remodellers to evict nucleosomes from around the TSS. Some promoter regions are kept nucleosome free all the time and do not need the same cofactors for transcription. After activator binding general transcription factors (GTF) start to appear. Needed co-activators will bind and the pre-initiation complex (PIC) starts to form ~30–50 nt upstream of the TSS. PIC consists of different GTFs that bind promoter region in a sequential and coordinated way (Nechaev and Adelman, 2011).

The first to bind the promoter region is the TFIID complex containing TATA-binding protein TBP. TFIID complex also contains a set of TBP-

associated factors that serve as targets for further activation of transcription. This complex is a binding site for additional positive and negative regulators. TFIIA binding stabilizes DNA-TBP interaction and restricts the binding of repressors that could stop the formation of initiation complex. Next factor to bind is TFIIB that establishes the spacing between the TATA box and the TSS (Fuda et al., 2009; Nechaev and Adelman, 2011).

RNAPII enters into the pre-initiation complex in association with the Mediator and TFIIF. TFIIF stabilizes the interaction between RNAPII and TFIIB, preventing associations with non-promoter DNA. The final general factors to arrive are TFIIH and its stimulatory factor TFIIE. With this arrival DNA double helix is unfold by the helicase activity of TFIIH and an open complex is formed. RNAPII initiates transcription, attached stably to both, DNA and nascent mRNA and transcribes the first 20–50 bps of mRNA, escaping the promoter. During transcription initiation the CTD is mostly in unphosphorylated state. Lack of modification on the CTD keeps the PIC proteins attached to RNAPII. TFIIH (its Cdk7 subunit) is responsible for phosphorylating Ser-5 and facilitating promoter escape (Nechaev and Adelman, 2011; Phatnani and Greenleaf, 2006).

The early elongation is a slow process and the RNAPII complex has a tendency to pause, arrest and even terminate transcription. In human cells only 1% of the transcription initiation processes complete the whole cycle and give a full-length transcript. This underlines that the transition from the initiation to elongation is a crucial step in gene expression regulation (Darzacq et al., 2007; Marshall and Price, 1992; Nechaev and Adelman, 2011).

Recruitment of P-TEFb kinase triggers transition into productive elongation by phosphorylation of Ser-2 on the CTD. Ser-2 phosphorylation reaches its peak levels 600-1000 nt downstream of TSS. Genome-wide occupancy profiles of RNAPII elongation complex show that all actively transcribed genes in proliferating yeast cells are associated with the whole complex of RNAPII elongation factors. Elongation factors enter into the complex downstream of the TSS within 50 nt. All the elongation factors show characteristic distribution over transcribed genes and can be divided into 3 distinct groups. Group 1 of elongation factors (Spt4, Spt5 and Spt6) are more abundant in the 5’ and 3’ ends of the gene, group 2 factors (Elf1 and Spn1) peak in the 3’ end of gene and group 3 factors (Bur1, Ctk1, Paf1 and Spt16) have a uniform distribution over the coding region. In case of elongation termination elongation factors exit the complex in a two-step manner. As the first step group 3 factors exit upstream of the polyA site, factors from group 1 and 2 tend to exit further downstream probably being present during RNA 3’ end formation and transcription termination (Mayer et al., 2010).

2.2.1. Transcription elongation

The initial model for RNA polymerase II elongation predicted that the entire RNAPII molecule advances along the DNA template at steady speed, translocating as each new nucleotide is added to the mRNA. Nevertheless, DNA footprinting studies showed that at certain short sequence intervals RNAPII was fixed on the template while the RNA chain increased by few nucleotides (Krummel and Chamberlin, 1992a; Krummel and Chamberlin, 1992b). These findings led to the common understanding that during transcription elongation RNA polymerase is moving back and forth, oscillitating between catalytically active and inactive state (Komissarova and Kashlev, 1997). As of now a Brownian ratchet-pawl mechanism for transcript elongation has been accepted by the wide community. It is a model by which no energy other than provided by thermal fluctuations is needed for RNAP translocation. By this mechanism one structural unit of the RNAPII (F-bridge) acts as a reciprocating pawl, pushing RNAPII forward in relation to the nucleic acid scaffold, while the incoming substrate acts as a second, stationary pawl, preventing RNAP from slipping backwards (Bar-Nahum et al., 2005). This model allows polymerase to move rapidly forward, but also to move backward for several nucleotides. This way the newly formed RNA 3' terminus would come out of alignment with the enzymes active site. By the ratchet movement the 3' terminus could be brought back to the active site or polymerase performs endonucleolytic cleavage of the transcript, resuming elongation. All different phases of elongation with the multiple equilibriums between different enzyme states are a good target for regulation by cofactors. The cleavage of RNA by paused RNAPII is highly stimulated by the elongation factor TFIIS (Izban and Luse, 1992). Also general elongation factors TFIIF, Elongin and ELL promote elongation by shifting the equilibriums in favour of transcript formation rather than pausing and termination (Herbert et al., 2008).

2.2.1.1. Polymerase speed and occupancy

The estimated average speed of RNA polymerase II elongation in higher eukaryotes is 18-40 nt per second (Kimura et al., 2002b; O'Brien and Lis, 1993; Tennyson et al., 1995). However, in human cells the maximal speed is up to 70 bases per second, explained by the warmer environment (Darzacq et al., 2007). Most of the constitutively expressed genes are transcribed at low levels but transcriptional bursts occur on genes that are up-regulated by various environmental signals. Also some essential proteins (histones, ribosomal proteins, glycolytic enzymes) have high transcription levels. For such genes the maximal transcriptional initiation rate in yeast cells is estimated to be one new mRNA molecule in every 6-8 seconds (Iyer and Struhl, 1996).

The genome of *S.cerevisiae* encodes for approximately 5900 proteins. First estimations of the number of mRNAs per one gene in yeast cell predicted it to be 1–2 molecules (Struhl and Davis, 1981). Rather recent calculations show that one yeast cell contains approximately 36 000 mRNA molecules under nutrient

rich conditions. But also much higher numbers have been proposed reaching up to 60 000 mRNA molecules (Miura et al., 2008; Zenklusen et al., 2008). Genome-wide studies predict that the median RNAPII density is 0.096 molecules per gene and average transcription rates between 2–30 mRNAs per hour (Pelechano et al., 2010). More recent data, obtained by a newly established method of dynamic transcriptome analysis, also reveals that only a few copies of mRNA of most genes are produced per cell during a cell cycle and the median half-life for mRNA is 11 minutes (Miller et al., 2011).

Global RNAPII ChIP-Chip array at high resolution shows that in general transcription activity and RNAPII occupancy values on genes correlate rather well. Nevertheless, there are exceptions as RNAPII signal was also obtained from the silent mating type loci, telomeric sequences that are not transcribed and from the genes with very low transcription activity (as shown previously by (Holstege et al., 1998)). Therefore genes that are not producing stable transcripts are associated with a significant amount of RNAPII (Steinmetz et al., 2006). Genome-wide RNA abundance profiling confirms the correlation between RNAPII distribution and transcription levels but also emphasises the importance of non-productive/regulatory transcription in the control of gene expression (Kim et al., 2010). Gene specific studies in colorectal carcinoma cells show that the distribution of RNAPII on different genes varies. For some genes more RNAPII is found on the 5'-end, for some on 3'-end and uniform distribution is also possible (Glover-Cutter et al., 2008). As ChIP-chip analysis is not giving specific data whether the polymerase on the gene is elongation competent or not, run-on technique is used to distinctly determine the presence of elongating RNAPII. By comparing RNAPII signals in the end of the gene and in the beginning, run-on assays reveal that the distribution of polymerases on different genes is an intrinsic characteristic that does not correlate with gene length or expression level. When performing ChIP experiments and comparing them with data obtained by run-on method, the discrepancy was evident in most cases. This observation supports previous studies stating that polymerases tend to become transcriptionally inactive and pause/arrest during elongation (Rodriguez-Gil et al., 2010).

2.3. Preparing the chromatin scene for RNAPII dependent transcription

2.3.1. Nucleosome positioning

Besides the known genetic code that directs the translation of the DNA sequence into amino-acids and thereupon into proteins, DNA codes for far more. First it codes for motifs for different DNA binding proteins. This code can be rather flexible where a change in couple of nucleotide does not necessarily mean getting lost in translation (Pabo and Nekludova, 2000). Second, by its nucleotide sequence DNA codes for the access to information – for the

position of nucleosomes. In other words, having DNA in a nucleosome makes obtaining information from this DNA region more difficult.

Sharp bending of the DNA double-helix is needed to form nucleosomes. Bending properties of the DNA come from its nucleotide sequence (Luger et al., 1997). DNA sequences that support nucleosome formation (nucleosome positioning sequence – NPS) are usually enriched with AA dinucleotides, spaced about 10 bp apart resulting in the deficiency of TT nucleotides at the same location. This trend is reversed 5–6 nucleotides in either direction where the complementary strand faces the histone core (Ioshikhes et al., 1996). When looking for NPSs over the genome, it appeared that different genes classes have very specific NPSs in the vicinity of their promoters, indicating their importance in gene regulation (Ioshikhes et al., 2006). Tiled microarray experiments revealed that over 69% of nucleosomal DNA is in well-positioned nucleosomes. What more, at RNAPII promoters a nucleosome free region (NFR) can be found approximately 200 bp upstream from the start codon. The NFR, however, is flanked on both sides by positioned nucleosomes (Yuan et al., 2005).

Using somewhat different approaches, in 2006 two research groups simultaneously presented computational models for predicting nucleosome positioning according to DNA sequence. When comparing the predicted positioning to the data obtained by bench experiments, the concurrence was surprising. Both models were able to predict *in vivo* locations of almost half of the nucleosomes. The results showed that the transcription factor binding sequences are located in regions where nucleosome formation is not predicted to be favourable (AT-rich tracts). When making the prediction model using data from chicken DNA or a synthesized DNA, the model was able to predict nucleosome positions also in yeast genome, indicating that the general features of DNA that influence nucleosome positioning are universal (Ioshikhes et al., 2006; Segal et al., 2006).

In yeast only approximately 20% of RNAPII promoters are considered to contain a TATA box. By analyzing data from nucleosome prediction studies, different types of promoter regulation by nucleosomal structure appear. On promoters without TATA-box, nucleosomes seem to have a positive regulatory effect through a more uniform "nucleosome – NFR – nucleosome" structure. Keeping the NFR means that complexes needed for transcription initiation can be accommodated even without additional loss of nucleosomes. For TATA-box containing promoters nucleosome loss is facilitated through different transcription activators as not so defined structure of nucleosome positioning next to TSS is seen (Ioshikhes et al., 2006; Zanton and Pugh, 2006).

2.3.2. Chromatin remodelling

Chromatin remodellers support correct packaging of the genome but also help to grant access to densely packed DNA, which hides the *cis* elements needed for DNA-binding factors.

There are four known families of chromatin remodellers – SWI/SNF, ISWI, CHD and INO80. All four use the ATP hydrolysis energy to alter the structure of chromatin by moving, ejecting or restructuring nucleosomes. Nevertheless all four participate in specific biological context and differ from each other by the unique composition of their subunits and differences in the domains that regulate the catalytic ATPase. Common to all remodellers is their DNA sequence-independent affinity to nucleosomes, which is facilitated by the recognition of covalent histone modifications. All possess also domains or proteins that recognise other chromatin or transcription factors (Clapier and Cairns, 2009; Ferreira et al., 2007). In the regulation of gene expression chromatin remodellers have dual properties. The antagonism between chromatin organisers and disorganisers sets up a dynamic balance between nucleosome assembly and disassembly.

Two principally different models have been proposed for chromatin remodelling. The first one, based on *in vitro* experiments with chromatin from *Drosophila* embryos, argues that nucleosomes are constantly remodelled (moved back and forth) to allow access to DNA by incoming factors (Varga-Weisz et al., 1995). Second model arises from *in vivo* experiments with human ISWI complex, suggesting that remodelling complexes sample nucleosomes constantly but transiently without causing remodelling. Stable interaction and remodelling would take place only when a specific sign is recognized. This sign can be post-translational modification on histones or a targeting molecule (Erdel et al., 2010).

For example, the ISWI complex has both positive and negative effect on gene expression depending on the ATPase subunit (Isw1 or Isw2) and its counterparts that confer distinct properties to the ATPase. Isw2 displaces basal transcription machinery to repress or silence transcription. Isw1, component of two distinct complexes Isw1a and Isw1b, represses initiation of transcription by specific positioning of promoter proximal dinucleosome or by localizing to the coding region and controlling the amount of RNAPII released into productive elongation (Mellor and Morillon, 2004; Morillon et al., 2003; Simic et al., 2003). In the fruit fly (*Drosophila melanogaster*), ISWI is part of the NURF complex and facilitates transcription by catalyzing nucleosome sliding (Badenhorst et al., 2002).

2.3.3. The dynamics of nucleosomes during transcription

For transcription elongation the higher structures of chromatin are highly repressive although RNAPII can cope with the obstacle consisting of one nucleosome. It has been shown *in vitro* that on chromatin template human RNAPII or SP6 RNAP were not able to initiate transcription from a promoter that is wrapped in a nucleosome, but once on their way and elongating, both polymerases were capable of reading through one nucleosome. This process caused displacement of histones (Kireeva et al., 2002; Lorch et al., 1987).

Nevertheless, a consistent nucleosomal array is a very strong obstacle for RNAPII and additional factors are required to facilitate chromatin transcription (Bondarenko et al., 2006; Kireeva et al., 2005; Orphanides et al., 1998). In the cells, where elongation factor TFIIS is deleted, the RNAPII pause sites correlate well with the positions of nucleosomes showing that nucleosomes are the primary cause for RNAPII pausing *in vivo* (Churchman and Weissman, 2011). Interestingly, the barrier formed by nucleosomes does not represent a uniform hindrance to transcript elongation but varies in respect of transcriptional orientation and depends on the location of the high-affinity DNA region within the nucleosome. Polymerase transcribes less efficiently nucleosomes where the high-affinity DNA region lies just at the entry into the H3/H4 tetramer compared to nucleosomes, where the histone affinity region is located distal to the nucleosome dyad. Hence, the nucleosomal barrier to transcription is determined by the combination of the DNA sequence and the position of this sequence in the nucleosomal structure (Bondarenko et al., 2006).

Lowly transcribed regions are typically not stripped off nucleosomes as the lack of nucleosomes would permit intrinsic transcription initiation. Rather, nucleosomes are chaperoned around elongating RNAPII. Occasionally some nucleosomes are ejected but this ejection is balanced by reassembly by chromatin remodelling factors. For example, yeast Chd1 (from the CHD remodellers family) interacts with elongation factors and is localized to transcribed regions and helps to restore the structure of chromatin. Also several histone chaperons are needed in both disassembly and reassembly of nucleosomes. The H3-H4 chaperon Asf1 (anti-silencing function 1) is essential for the activation of *PHO5* and *PHO8* genes but also participates with Hir1 chaperon in the reassembly of *PHO5* locus. Asf1 is also needed for the removal of histone octamers from the *HO* promoter in concert with the SWI/SNF remodelling complex (Adkins et al., 2004; Gkikopoulos et al., 2009; Schermer et al., 2005). In a complex with chaperon Vps75, Asf1 facilitates histone H3 acetylation by Rtt109 at the position K56. This modification prevents the formation of higher structures of DNA (Bowman et al., 2011; Kaplan et al., 2008; Keck and Pemberton, 2011; Miller et al., 2008). Another H3-H4 chaperon Spt6 is responsible for maintaining chromatin structure and mediates chromatin reassembly in the rear of RNAPII. Spt6 mediated chromatin reformation is essential for transcriptional repression and in the absence of competing chromatin assembly (deletion of Spt6), there is no need for transcription activators to reinitiate the *PHO5* gene (Adkins and Tyler, 2006; Bortvin and Winston, 1996; Kaplan et al., 2003). H2A-H2B dimers are chaperoned by the FACT complex (Belotserkovskaya et al., 2003; Saunders et al., 2003; Schwabish and Struhl, 2004). FACT subunit Spt16 has been shown to be selectively required for gene activation. Genes that have positioned nucleosomes on the transcribed region need FACT to facilitate transcription more than genes with random nucleosome structure (Jimeno-Gonzalez et al., 2006).

The structure of the nucleosome itself has an effect on the dynamics of chromatin in transcription elongation. H2A-H2B and H3-H4 dimers have different

affinity for DNA and thereby their turnover rates vary. Studies in yeast, in slime mold (*Physarum polycephalum*) and also humans have shown that H2B assembles randomly into chromatin (promoters and also coding regions) while genes are transcribed and also while they are inactive. During transcription H2A-H2B moves from nucleosomes faster than H3-H4 and the exchange of H3-H4 is more dependent on transcription as incorporation of H3 is seen only in active genes and mainly within the promoter region (Jamai et al., 2007; Kimura and Cook, 2001; Thiriet and Hayes, 2005).

Evidence from genome wide studies and investigations of single genes show that gene activation causes loss of nucleosomes from the promoter and also from the coding region of actively transcribed genes. Nevertheless, eviction of nucleosomes is not presumption for effective transcription. For example, for genes whose normal expression requires HATs Gcn5 and Elp3 for their activation, no eviction of nucleosomes from the coding region was detected. For counterpoise, on *GAL* genes eviction of nucleosomes was not dependent on the acetylation status of histones (Boeger et al., 2003; Boeger et al., 2004; Kristjuhan and Svejstrup, 2004; Lee et al., 2004; Schwabish and Struhl, 2004). Also, in case of *PHO8* and *PHO5* genes, only promoter nucleosomes were lost after activation, not coding region nucleosomes (Adkins et al., 2004). As the rate of replication-independent H3 exchange between genes varies a lot and does not always correlate with the rate of transcription, it might be that the amount of nucleosomes that stay associated with DNA during transcription varies widely between genes (Gat-Viks and Vingron, 2009).

AIMS OF THE STUDY

During transcription elongation RNAPII has to pass the barrier formed of higher-order structures of chromatin. The mechanism behind traversing the nucleosomes probably depends on the preprogrammed transcription level of the gene. On highly transcribed genes eviction of whole nucleosomes has been reported. The first objective of this study was to determine the area of nucleosome loss during transcription elongation and whether already existing or newly synthesised histones are reassembled into chromatin after transcription repression. Heterochromatic structures have been considered stronger obstacles for RNAPII than nucleosomes. Nevertheless, formation of PIC to promoters in silenced regions has been observed. The second goal of the current research was to investigate whether elongating RNAPII can successfully cope with the heterochromatic structures in the coding region of the transcribed gene. Additionally we aimed to determine the distribution of elongating polymerases on a highly transcribed region on single cell level as plethora of studies on the distribution of transcriptional machinery address the question on whole population level.

II. RESULTS AND DISCUSSION

1. Description of the experimental system

In all the experiments budding yeast *Saccharomyces cerevisiae*, strain W303, was used as the model organism (Thomas and Rothstein, 1989). Genetic manipulations have been performed to construct yeast strains suitable for our experimental set-up. In budding yeast studying transcription coupled chromatin modifications is complicated by the compact genome and short genes (average size 1.6 kb) making it hard to distinguish between chromatin modifications on the coding region and on the promoter. Therefore, in the centre of our studies is a long (9433 nt) non-essential gene *VPS13*, indicated to be involved in sporulation, vacuolar protein sorting and protein-Golgi retention (http://www.yeastgenome.org).

In our experimental system, *GAL10* promoter has been inserted in front of the *VPS13* open reading frame (Figure 4A) to enable the control of transcription activation and inhibition by changing the carbon source in yeast growth media, raffinose and glucose representing the repressed state, galactose activated state (Kristjuhan and Svejstrup, 2004). For studying the mechanism of transcription, foreign DNA sequences of different origin and diverse function have been inserted in the coding region of the *GAL10-VPS13* gene (Figure 4B-D).

2. Loss of nucleosomes in the coding region is determined by elongating RNAPII (Ref. I)

In addition to the opening of short promoter regions, eviction of nucleosomes from the coding region of transcribed genes has been detected (Kristjuhan and Svejstrup, 2004; Schwabish and Struhl, 2004; Zhao et al., 2005). However, the extent of nucleosome loss after transcription activation and the factors determining the nucleosome-free area on a specific locus are not fully known. Chromatin remodelling complex FACT has been shown to facilitate transcription elongation by inducing disassembly of the H2A-H2B dimer and recruiting transcriptional co-activators (Belotserkovskaya et al., 2003; Schwabish and Struhl, 2004; Takahata et al., 2009). Also elongation factor TFIIS, by promoting RNAPII progression from poised state, might contribute to nucleosome eviction (Kireeva et al., 2005). Still there is no direct evidence indicating whether RNAPII itself is able to displace the nucleosomes or some additional factors are needed.

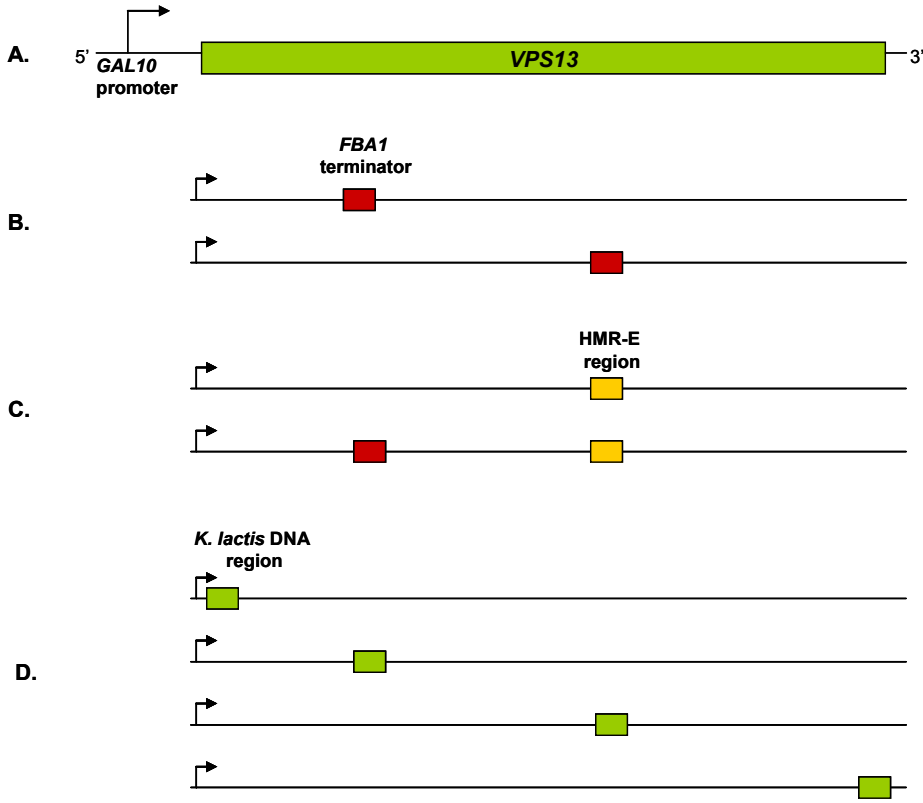

Figure 4. Modifications on the *VPS13* gene. A. Scheme of the *GAL-VPS13* gene showing the *GAL10* promoter in the 5' end. **B-D**. Schematic representations of the *GAL-VPS13* gene from A, with the modifications in the coding region according to the strains used in the experiments. *FBA1* terminator region (red rectangle) 3 kb and 6 kb from the *VPS13* promoter (B, C); HMR-E region (yellow rectangle) 6 kb from the promoter (C); *K. lactis* DNA insertion (green rectangle) 0.1 kb, 3 kb, 6 kb and 9 kb from the *VPS13* promoter (D).

Previous study using ChIP and qPCR had shown that the induction of transcription of *GAL-VPS13* with galactose results in nucleosome loss from the promoter and coding region of the gene (Kristjuhan and Svejstrup, 2004). In our experiments we expanded the detection area of nucleosomes and RNAPII occupancy beyond *GAL-VPS13*, designing primers that recognised a sequence in the *SDH2* gene located downstream of *GAL-VPS13*. As shown in Ref. I, Fig. 1B, contrary to *GAL-VPS13*, no loss of nucleosomes or recruitment of RNAPII on *SDH2* was detected, implying that the eviction of nucleosomes does not spread beyond the coding region of the induced gene. We hypothesized that the possible spread of nucleosome eviction is terminated by the promoter region of *SDH2* or the terminator region of *GAL-VPS13*.

In order to clarify the role of elongating polymerase in determining the nucleosome-free area, we modified the *GAL10-VPS13* model gene by inserting a strong transcription termination sequence into its coding region. We constructed two strains with the strong *FBA1* RNAPII terminator sequence inserted either 3 kb or 6 kb apart from the *VPS13* promoter (Figure 4B). These strains were grown in inducing conditions over-night and distribution of RNAPII and nucleosomes was determined by chromatin immunoprecipitation. The results were clear – no removal of nucleosomes was detected after the terminator sequence in neither strains correlating with the loss of RNAPII signal (Ref. I, Fig. 1C,D). Nucleosomes were efficiently removed only from the regions upstream of the terminator sequences. This result confirms that drastic nucleosome loss occurs only on the transcribed region and does not spread beyond. We also determined more precisely the loss of nucleosomes in the close vicinity of the terminator region using the GAL-VPS13-3kb-term strain. By evaluating the amounts of nucleosomes and RNAPII bound to DNA immediately up- and downstream from the terminator sequence we saw that the area of nucleosome loss indeed overlapped with the terminator site (Ref. I, Fig. 1E). From these results we could conclude that histones are evicted only from the transcribed DNA sequence and the nucleosome-free area does not spread independently from the ongoing transcription. This suggests that nucleosome eviction is tightly coupled to elongating RNAPII moving through the transcribed region.

3. New histones are incorporated to nucleosomes during chromatin reassembly (Ref. I)

After transcription repression with glucose, chromatin structure on the transcribed locus is restored within few minutes (Ref. I, Fig. 2) indicating that this process is replication independent. Reloading of nucleosomes rapidly after transcription repression has been detected also on the *GAL10* and *HSP82* genes (Schwabish and Struhl, 2004; Schwabish and Struhl, 2006; Zhao et al., 2005).

Our next question was, whether the histones that are reloaded to DNA come from the free pool of histone proteins or pre-existing nucleosomes are transferred from in front of the RNAPII to the already transcribed area?

To distinguish between newly synthesised and pre-existing nucleosomes we constructed a new yeast strain based on the GAL-VPS13-6kb-term strain. In addition to constitutively expressed histone H3 with an E2 epitope, an additional copy of histone H3 with a myc tag was inserted to the *HIS3* locus. Expression of H3-myc was under the control of galactose-inducible promoter as in case of *GAL-VPS13* (Ref. I, Fig. 3A). Therefore, after the shift of cells to galactose-containing medium, the transcription of both – *GAL-VPS13-6kb-term* reporter gene and *H3-myc*, was induced.

During the experiment cells were first grown in raffinose containing medium (repressed condition for galactose-inducible genes), then shifted to galactose-

containing medium for 80 minutes and transcription was stopped by the addition of glucose to the growth medium. Western blotting confirmed that the expression of H3-myc was successfully induced in galactose containing medium and a significant amount of H3-myc was still present after 30 minutes of glucose inhibition (Ref. I, Fig. 3B). This result encouraged us to move on to ChIP experiments to determine the possible incorporation of newly synthesised histones into nucleosomes after transcription shut-down. If only old nucleosomes are incorporated to the transcribed locus after transcription, no H3-myc signal should be detected on the gene as H3-myc did not exist before the induction of transcription. If random (new and old) histones are incorporated to the reassembled chromatin, incorporation of H3-myc in addition to H3-3F12 is highly likely and we should be able to detect assembly of newly synthesised histones.

As indicated on Ref. I, Fig. 3C, only low quantities of newly synthesised histones were detected in a non-coding region of chromosome VIII and in the telomeric region of chromosome VI. These signals represent the background incorporation of new histones due to DNA replication and repair-dependent exchange of histones during the whole experiment. Similar low level of incorporation was also detected on the 8.5 kb region of *GAL-VPS13-6kb-term* gene indicating that no significant exchange of nucleosomes took place on the non-transcribed region of the induced gene. But interestingly, considerable incorporation of new histones was observed 3.5 kb downstream of the promoter on the model gene, designating the region that is actively transcribed. These results demonstrate that indeed, old histones are evicted and newly synthesised histones are incorporated to nucleosomes assembled after transcription shut-down.

Similar dynamics of nucleosomes has been detected also on the promoter region of the *PHO5* gene, where nucleosomes from the soluble histone pool, rather than from the chromatin fraction, were used to build up nucleosomes after transcription inhibition (Schermer et al., 2005).

Probably the removal of nucleosomes from actively transcribed loci is a common mechanism for achieving high levels of gene expression. Other studies have shown that H2A-H2B dimers are constantly exchanged during transcription (Jamai et al., 2007) and removal of H3 and H4 has been reported to take place on highly transcribed loci and on promoter regions (Dion et al., 2007; Katan-Khaykovich and Struhl, 2011; Rufiange et al., 2007). It is possible that for genes with lower expression levels no eviction of nucleosomes takes place. In that case nucleosome traversal by RNAPII, facilitated with the assistance of TFIIF and also TFIIS, has been proposed (Luse and Studitsky, 2011).

In our experimental system we can't exclude the possibility that both, old and new histones are incorporated into reassembled chromatin. What we do see is that new histones are also incorporated and a mixture of old and new histones forming nucleosomes can exist. This notion is supported by a recent paper by Katan-Khaykovich and Struhl, where they demonstrated that at loci with rapid H3 exchange, both old and new H3 are used in reassembly of nucleosomes. The

observed splitting of H3-H4 tetramer and generation of chimeric old-new tetramers was not an extensive feature but observed only at highly dynamic chromatin regions (Katan-Khaykovich and Struhl, 2011). High transcription level and nucleosome exchange is also the case in our experimental system, thus we could as well predict incorporation of both, new and also some old histones. This suggests a mechanism for so called "transcriptional memory", where a transcribed region is marked for future action as old histones may carry histone modification signals. That would be an interesting topic to look into in the future.

4. Elongating RNAPII overcomes the barrier of heterochromatin (Ref. II)

It has been long established that in *in vivo* conditions RNAPII is capable of surmounting the physical obstacle to transcription created by the nucleosomal structure of chromatin. Additional factors are needed to assure the efficiency of this process but in general, nucleosomes do not seem to be the biggest problem for the polymerase. Nontheless, heterochromatic structure of chromatin is also an important level in gene expression control. The classical view states that by creating physical barrier, heterochromatin blocks the access to DNA for sequence-specific regulatory factors required for the execution of DNA related processes (Kornberg and Lorch, 1991). Recent studies argue that actually heterochromatic structures are permissive to constitutive binding of PIC components, transcription activators and even RNAPII to the promoter regions of repressed genes (Sekinger and Gross, 1999; Sekinger and Gross, 2001). Therefore another step besides initiation is repressed by heterochromatin. Indeed, in the work of Gao and Gross (Gao and Gross, 2008) it was established that mainly the transition point between RNAPII initiation and elongation is targeted by silenced chromatin as the recruitment of 5'-capping enzymes and elongation factors is disturbed. Considering that data, our next question was whether RNAPII that has already overcome the transition point and entered the elongation phase of transcription can contend with heterochromatic structures in the coding region of a gene?

In order to assemble a heterochromatic structure at a location where we would be able to control transcription activation and elongation, a yeast HMR-E silencer was inserted into the coding region of our model gene *GAL-VPS13* (Figure 4C; Ref. II Fig. 1A). The insertion site was located 6 kb downstream from the beginning of the ORF of *VPS13* and first we controlled if heterochromatin formed on the desired location. Successfully, in the repressed state of transcription, SIR proteins were efficiently recruited to the HMR-E sequence on the model gene (Ref. II Fig. 1B) leaving the promoter and the beginning of the gene free from heterochromatin. As a control we also constructed a strain with the same insertion of the silencer into *GAL-VPS13*, but with the additional deletion of the *SIR4* gene that is needed for the formation of heterochromatic

structures. In GAL-VPS13-HMR-E-*sir4* strain no recruitment of SIR proteins to the silencer was detected (Ref. II Fig. 1B).

Remarkably, upon induction of *GAL-VPS13-HMR-E* transcription, SIR complexes were removed from the entire locus of the model gene (Ref. II Fig. 1B). To confirm normal transcriptional activity we also determined the distribution of RNAPII and nucleosomes on the *GAL-VPS13-HMR-E* locus in comparison to *GAL-VPS13* (Ref. II Fig. 2A, B). Surprisingly RNAPII was recruited to the *GAL-VPS13-HMR-E* locus upon transcription activation and nucleosomes were evicted on the same level as on the locus without the silencer.

Nevertheless, no RNAPII signal or nucleosome removal was detected downstream of the silencer region although the SIR proteins were removed from the entire coding region (Ref. II Fig. 2A, B). The same dynamics of RNAPII and nucleosomes was also seen in the *sir4* strain, establishing the HMR-E sequence as a strong terminator for transcription (Ref. II Fig. 2C). Reverse transcriptase PCR (RT-PCR) experiments (Ref. II Fig. 5) also failed to detect *VPS13* transcripts beyond the HMR-E sequence, affirming the previous result.

Even though the removal of SIR complexes from the model gene was concurrent with the appearance of elongating polymerase in the coding region, it remained possible that disassembly of heterochromatin was triggered by transcriptional activation itself and not by elongating RNAPII. To test this possibility we constructed a yeast strain bearing a *FBA1* terminator sequence followed by the silencer sequence at different positions in the coding region of the *GAL-VPS13* gene (Ref. II Fig. 4A). Such a strain enabled us to determine whether SIR complexes were displaced also from the region where the activity of elongating polymerase did not extend. Upon transcription activation no removal of SIR proteins was detected (Ref. II Fig. 4B). This result confirmed our notion that, as in case of the removal of nucleosomes from the coding region of the *GAL-VPS13* gene, elongating RNAPII is required for the displacement of SIR complexes. Promoter activation event alone is not enough to trigger the removal of silencing complexes.

Having confirmed that RNAPII is capable of pervading heterochromatic structures, the next step was to determine if competing with these structures changes the kinetics of transcription. To test this conception we monitored the accumulation of *VPS13* mRNA after the induction of the *GAL-VPS13-HMR-E* and *GAL-VPS13* genes. RNA samples were collected at after galactose induction and further analyzed by RT-PCR. As a result we saw practically identical kinetics of *VPS13* mRNA accumulation in both GAL-VPS13-HMR-E and GAL-VPS13 strains (Ref. II Fig. 5), indicating that SIR complexes did not have an effect on the kinetics of gene induction and transcript elongation through the region.

4.1. Histone H3 lysine 56 acetylation is needed for effective elongation through heterochromatin on the coding region (Ref. II)

Next we wanted to know which factors are needed for the successful transcription through heterochromatin. In order to clarify this issue we analyzed the efficiency of *GAL-VPS13-HMR-E* mRNA induction in 12 yeast strains with deletions of genes required for different transcription-coupled chromatin modifications. Our main focus was on enzymes that facilitate post-translational histone modifications and therefore control the dynamics of chromatin. Also the effect of deletion of chromatin remodeling factor *RCS1*, histone variant *HTZ1* and histone chaperon *ASF1* genes was investigated.

Deletion of *BRE1* (abolishing transcription coupled H2B K123 monoubiquitylation and also subsequent methylations of histone H3 residues K4 and K79 (Wood et al., 2003)); H3 methyltransferase genes *SET1*, *SET2*, and *DOT1*; chromatin remodeling factor gene *RSC1*; or histone deacetylase gene *RPD3* had no inhibitory effect on kinetics of *GAL-VPS13-HMR-E* induction. We reproducibly detected slightly slower induction of *GAL-VPS13-HMR-E* mRNA in *sas2*Δ and *gcn5*Δ strains (Ref. II Fig. 6A), confirming the earlier results that both histone acetyltransferases play a role in the control of heterochromatin spreading (Kimura et al., 2002a; Kristjuhan et al., 2003; Suka et al., 2002).

Of all the tested strains, severe impairment of *GAL-VPS13-HMR-E* induction was observed in *rtt109*Δ, *asf1*Δ and *htz1*Δ strains. Intriguingly, Rtt109 and Asf1 are required for histone H3 lysine 56 acetylation both *in vivo* and *in vitro*, Rtt109 possessing the acetyltransferases activity and Asf1 chaperoning 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 H2A alternative form incorporated into chromatin near the promoters and at subtelomeric regions. It has also been shown to restrict the spreading of SIR complex (Li et al., 2005; Meneghini et al., 2003; Zhang et al., 2005).

Our next aim was to determine whether the observed defects in transcript formation in *rtt109*Δ, *asf1*Δ and *htz1*Δ deletion strains were due to hindrance by heterochromatin or had an overall impact on *GAL10* promoter-driven transcription. To understand the nature of impairment of transcript formation we determined the efficiency of mRNA production on the endogenous heterochromatin free *GAL10* gene in the same strains (Ref. II Fig. 6B). Induction of endogenous *GAL10* was severely delayed only in the *htz1*Δ strain. It is also possible that in deletion strains transcription of long genes is impaired due to general defects in transcription elongation. To test this idea we introduced the same deletions into strains carrying *GAL-VPS13* without the HMR-E sequence. While Rtt109 and Asf1 deletions affected specifically only the *GAL-VPS13-HMR-E* locus, *htz1*Δ strain showed remarkable delay in *GAL-VPS13* induction (Ref. II Fig. 6C). These results indicate that Asf1 and Rtt109 are crucial for transcription of the heterochromatic locus while deletion of Htz1 causes an overall defect in transcription on *GAL10* promoter derived genes. The effect of *htz1*Δ was rather expected as previous studies have also observed slow

induction of inducible genes (including *GAL-VPS13*) in *htz1*Δ background and decreased RNAPII occupancy (Adam et al., 2001; Santisteban et al., 2011; Santisteban et al., 2000).

Interestingly, Rtt109, Arf1 and Htz1 are required only for the normal, quick induction of the heterochromatin-covered gene as after overnight growth in galactose-containing medium the mRNA of the model gene was induced in all strains (Ref. II Fig. 6, lane O/N). The probable explanation for this phenomenon might be that during the long-term growth under inducing conditions heterochromatic structures are removed from the gene due to replication, enabling transcription elongation to take place.

To confirm the requirement of H3 K56 acetylation for RNAPII elongation through heterochromatin we also constructed a strain where lysine 56 in histone H3 was mutated into arginine, mimicking the deacetylated state of this residue. As in case of *rtt109*Δ and *asf1*Δ strains, transcript production from the heterochromatic locus *GAL-VPS13-HMR-E* was severely impaired (Ref. II Fig. 6D) while induction of the endogenous *GAL10* or heterochromatin free *GAL-VPS13* loci was unchanged when compared to the wild-type strain (Ref. II Fig. 6). We also confirmed that transcription of the heterochromatic locus was not impaired in strains where H3K56 deacetylases Hst3 and Hst4 were deleted, causing constitutive acetylation of K56. In addition, no impairment of heterochromatic locus transcription was observed in the strain where lysine on position 56 was replaced with glutamine (H3K56Q), also mimicking the permanently acetylated state of this residue (Ref. II Fig. 6E). More importantly, when the H3K56Q mutation was combined with the deletion of Rtt109, the inhibitory effect of *rtt109*Δ was reversed indicating that H3K56 acetylation and not Rtt109 protein itself was needed for the disturbance of heterochromatic complexes (Ref. II Fig. 6E).

H3K56 acetylation is cell cycle regulated and appears predominantly in S phase when nucleosomes are re-formed after DNA replication (Masumoto et al., 2005). Therefore we speculated that progression through the S-phase might be needed for the effective transcription of heterochromatic *GAL-VPS13-HMR-E* locus. To test this hypothesis we induced cell cycle arrest in G1 phase with yeast peptide pheromone α-factor and analysed the kinetics of *GAL-VPS13* and *GAL-VPS13-HMR-E* mRNA induction. Severe inhibition of mRNA synthesis on *GAL-VPS13-HMR-E* appeared again only in the strain with H3 K56R mutant background (Ref. II Fig. 7). These results indicated that no progression through the S-phase is needed for the effective RNAPII progression through heterochromatin but acetylation of H3 K56 is crucial for the process.

As a final confirmation for the need of K56 acetylation we performed a ChIP experiment directly detecting K56 acetylation mark on nucleosomes in the transcribed locus before, during and after transcriptional induction. In both, GAL-VPS13 and GAL-VPS13-HMR-E strains elevated K56 acetylation levels were seen at the 5.5 kb region of the model gene 120 min after switching to transcription permissive conditions and shortly after transcription inhibition (Ref. II Fig. 8). No fluctuation of the acetylation mark was detected in GAL-

VPS13-HMR-E strain on 6.4 kb region, confirming that H3 acK56 was brought to the locus in transcription-dependent manner as the 6.4 kb region was not transcribed in this strain (Ref. II Fig. 6).

In general, H3 K56 acetylation mark that occurs only before incorporation of H3 into chromatin, is removed by Hst3/4 deacetylases after the DNA synthesis is completed (Celic et al., 2006; Maas et al., 2006; Yang et al., 2008). Replication independent appearance of K56 acetylation mark has been reported mainly on promoter proximal nucleosomes and also in the coding region of highly expressed genes (Rufiange et al., 2007; Schneider et al., 2006). As H3 K56 acetylation is characteristic to newly synthesised histones, nucleosomes bearing this mark confirm transcription-coupled incorporation of new histones into highly transcribed loci.

What might be the role of K56 acetylation in displacement of SIR proteins facilitated by RNAPII? Residue 56 is located in the globular domain of histone H3 extending towards the DNA major groove at the entry and exit points of the nucleosome core particle (Figure 1) (Hyland et al., 2005; Ozdemir et al., 2005). This position makes it a very attractive candidate for regulation of the histone octamer-DNA and nucleosome-nucleosome interactions. In the presence of K56 acetylation nucleosomes are more loosely bound to DNA and could be removed more easily. Therefore acetylation of K56 might facilitate removal of nucleosomes during transcription elongation. As the elongating RNAPII progresses through the transcribed locus, it displaces nucleosomes from its way. New histones with K56 acetylation will be loaded to chromatin when RNAPII moves away and that might be an advantage for the next polymerases transcribing the same locus (Kaplan et al., 2008; Rufiange et al., 2007; Schneider et al., 2006).

Another possibility is that K56 acetylation inhibits re-association of SIR complexes with the transcribed locus and that way helps to open the heterochromatic structures. In the presence of K56 acetylation, formation of SIR complexes is disrupted on telomeres. Also, in silenced chromatin H3 K56 is mainly in hypoacetylated state (Miller et al., 2008; Xu et al., 2007; Yang et al., 2008). As implied by the unequal distribution of SIR complexes on our model gene (Ref. II Fig. 1B), the border of heterochromatic region is probably not strictly defined but rather in a constantly changing equilibrium between SIR protein binding and free chromatin. As the nucleosomes are constantly interchanged in transcribed locus, the new histone-DNA complexes would contain K56 acetylated H3 that acts as a hindrance to SIR complex formation (Xu et al., 2007; Yang et al., 2008). Therefore, as a result of K56 acetylation the border between eu- and heterochromatin could be shifted in favour of euchromatin. In the absence of K56 acetylation such mechanism is abolished, new nucleosomes do not restrict SIR complex spreading and the opening of the heterochromatic locus is slower than in wild type cells.

5. RNAPII occupancy on a transcribed locus (Ref. III)

The amount of mRNA produced is generally reflected by the level of RNAPII recruitment to the gene. However, uneven distribution of RNAPII on different genes has been reported previously by genome-wide studies. In some loci RNAPII signal has been found to be more abundant in the 3'-end of the gene, in others in 5' end and on some loci elevated levels of RNAPII have been detected on both ends of the gene (Glover-Cutter et al., 2008; Kim et al., 2010; Rodriguez-Gil et al., 2010; Steinmetz et al., 2006).

Previous studies on the model gene *GAL-VPS13* revealed that the distribution of the elongating RNAPII along the investigated locus was not uniform (Kristjuhan and Svejstrup, 2004). We repeatedly detected considerably higher amount of RNAPII in the 5' than in the 3' region of the induced gene (Ref. I, Fig. 1B; Ref. III, Fig. 1). This would be an expected pattern of polymerase distribution in the initial stage of gene activation when transcription starts in the majority of cells simultaneously. Nevertheless, we could see the same pattern also after a long period in cells grown over-night when the expression of galactose-induced gene should be already stabilized after the initial burst of transcription.

In case of our model gene the gradual loss of RNAPII signal towards the end of the gene poses two possible explanations. Firstly, the elongation rate of RNAPII might accelerate towards the end of the gene, creating a longer distance between adjacent RNAPII molecules. Secondly, as our studies have been made on whole cell population, it might be that only a fraction of cells express full-length transcript while others abort transcription on random positions in the transcribed locus. Such a situation would create a deceptive RNAPII ChIP enrichment signal in the beginning of the transcribed gene as most of the cells in the population have polymerases at the beginning of the gene but only some of them have RNAPII also at the end.

To further analyse the actual distribution of elongating RNAPII we first reaffirmed that the decrease of the amount of RNAPII signal towards the end of the gene was not caused by uneven efficiency of qPCR primers used to analyse different regions of co-precipitated *VPS13* DNA. To analyse the presence of RNAPII at different regions of *GAL-VPS13* without primer bias, we made a panel of yeast strains containing insertions of a 700 bp DNA sequence (originating from *Kluyveromyces lactis VPS13* gene) at different distances (0.1 kb, 3 kb, 6 kb and 9 kb) from the promoter of the *GAL-VPS13* gene (Figure 4D; Ref. III, Fig. 2A). ChIP analysis of these strains showed that after over-night induction of the *GAL-VPS13* gene, the relative amount of RNAPII on the DNA insert was again dependent on the distance of the inserted DNA from the promoter of the gene (Ref. III, Fig. 2B). RNAPII signal was approximately 3 times higher in the beginning of the gene compared to the 3' end. As we were operating with 4 separate yeast strains we also controlled the identical induction of the galactose-regulated genes in all those strains to rule out any fluctuations of RNAPII level due to the differences in gene induction. RNAPII recruitment

to the *GAL10* control gene was analysed and the results confirmed that RNAPII levels in all strains were very similar (Ref. III, Fig. 2C). Therefore we concluded that the elevated levels of RNAPII in the beginning of GAL-VPS13 were not detected due to primer bias and reflected a real decrease on RNAPII level towards the 3' region of the gene.

5.1. Uniform distribution of RNAPII on an active gene determined by re-ChIP (Ref. III)

In previous studies *in situ* hybridisation and measuring real-time GFP-bound mRNA synthesis show remarkable variability of gene expression in individual yeast cells (Larson et al., 2011; Zenklusen et al., 2008). Such experiments fit well for estimating the number of actively transcribing polymerases in a single locus but they do not describe the distribution of these polymerases along the transcribed gene. The conventional ChIP method reflects the average density of RNAPIIs in cell population and therefore is not suitable to distinguish whether the higher RNAPII signal in the 5' end of the gene is due to higher density of polymerases in all cells or, alternatively, more polymerase is detected due to the initiation of *GAL-VPS13* in most cells, while only in a fraction of them RNAPII completes the transcription cycle reaching the end of the gene. Hence we needed a more sensitive method to detect multiple transcribing RNAPII complexes on a single *GAL-VPS13* locus and to determine the distribution of these complexes.

We constructed new yeast strains where the Rpb3 subunit of RNAPII was tagged with E2 epitope in its genomic locus and in addition, two extra copies of *RPB3* genes, carrying either E4- or myc-tags, were inserted into *HIS3* and *LEU2* loci. As RNAPII complex contains only one Rpb3 subunit, every RNAPII in this strain was tagged either with E2-, E4- or myc-tag. ChIP data confirmed that all differently tagged Rpb3 subunits were equally incorporated into functional RNAPII complexes as all of them were efficiently recruited to *GAL-VPS13* locus after galactose induction (Ref. III, Fig. 3).

The constructed strains enabled us to perform sequential ChIP (re-ChIP) experiments. At least two rounds of chromatin immunoprecipitation were performed after the induction of *GAL-VPS13* gene. In the first round the antibody recognising E2-tag was used, pulling down RNAPII complexes carrying this epitope (one third of all polymerases in the cell extract). In the second round we re-precipitated the eluate with another antibody against another Rpb3 tag. RNAPII signals from the second round of ChIP were analysed. No signal in the second round meant that only one RNAPII molecule carrying the E2-tag was bound to the studied DNA region. A detectable RNAPII signal in the second round of the ChIP indicated that we had at least two differently tagged polymerases attached to a single DNA fragment. Importantly, successful recovery of DNA in the re-ChIP assay confirms that the whole RNAPII-DNA complex originated from the same cell, because multiple polymerases were

bound to the same DNA fragment. Therefore we have an opportunity to detect the presence of multiple transcribing polymerases on a single chromatin fragment.

We compared the recovery of RNAPII in the second round of re-ChIP with different antibodies on the entire *GAL-VPS13* locus. The 4H8 antibody recognising Rpb1 subunit of RNAPII was used to detect the general efficiency of RNAPII immunoprecipitation in the second round of the re-ChIP. 4H8 antibody recognises all RNAPII complexes regardless of their epitope tag, therefore the signal detected with this antibody, was defined as the maximal possible recovery of RNAPII complexes. Expectedly, the signal recovery with E4 or myc antibodies was less efficient than with 4H8, remaining to 30–40 per-cent of the maximal possible recovery (Ref. III, Fig. 4A). To our surprise we did not observe major differences in the efficiency of RNAPII recovery in different regions of the *GAL-VPS13* gene hence in the distribution of the polymerase as detected previously with conventional ChIP. The recovery of differently tagged polymerases was uniform all over the *GAL-VPS13* locus regardless of the distance from the promoter. This suggests that the uneven distribution of RNAPII on the *GAL-VPS13* gene detected by conventional ChIP assay was deceptive and reflects the heterogeneity of the cell population rather than the different spacing of polymerases in individual cells.

When applying re-ChIP to genes that are less intensively transcribed we were unable to detect DNA in the second round of re-ChIP. This might result from the low sensitivity of the re-ChIP assay, or, only one polymerase might be transcribing lowly expressed genes and in that case no signal from the re-ChIP would be expected. The latter possibility is also supported by several over-genome studies showing that transcription of most genes is a rare event and occurs only few times during the cell cycle (Holstege et al., 1998; Nagalakshmi et al., 2008; Pelechano et al., 2010). Also the *in situ* hybridisation studies showed that most constitutively expressed genes are transcribed by single polymerase (Zenklusen et al., 2008).

Next we were interested in the number of polymerases, which are present in a single precipitated DNA-protein complex. We created a formula describing the probable relation between the recovery of a differently tagged polymerase in the second round of re-ChIP and the corresponding number of polymerases (Ref. III, Fig. 4B). The probability curve for multiple RNAPII complex detection gives the minimal number of RNAPII complexes bound to the same DNA fragment. When fitting our experimentally obtained re-ChIP values to the probability curve we saw that there are at least 2 to 2.5 polymerase complexes on the same DNA fragment in the GAL-VPS13 locus. As the average length of the DNA fragments in our experiments was around 500 bp as determined by DNA gel-electrophoresis, it makes minimally one RNAPII complex per 250 bps.

As our results indicated there seems to be two possibilities to achieve the uniform distribution of RNAPII complexes on transcribed genes. Either a portion of all the transcribing polymerases abort transcription in a coordinated

way or the leading polymerase elongates slower than the following polymerases. In the latter case the "holes" caused by premature spontaneous termination of transcription would be filled by faster polymerases that catch up with the leading polymerase. Probably both possibilities are true. DNA damage and the subsequent DNA repair mechanisms may cause displacement and ubiquitylation of elongating polymerases in the coding region of genes (Somesh et al., 2005; Woudstra et al., 2002). The leading polymerase, on the other hand, might be slowed down by the encounter of relatively more obstacles on its way compared to the following polymerases. Often pausing and backtracking of the elongating polymerase occur (Churchman and Weissman, 2011; Sigurdsson et al., 2010). During transcription elongation RNAPII complex has to open the structure of chromatin and displace the histones in the coding region on highly transcribed genes (Kristjuhan and Svejstrup, 2004; Lee et al., 2004; Schwabish and Struhl, 2004). These processes are good candidates for slowing down the leading polymerase whereas the following polymerases can take advantage of the opened chromatin. Or even if the nucleosomes are reassembled behind the first RNAPII, they might be more loosely attached as a result of H3K56 acetylation occurring on the newly synthesised histones.

Also, leading polymerases might be more prone to abortions than the followers. Many regulated genes are expressed as transcriptional bursts, where rapid induction of the gene and transcription of multiple mRNAs is followed by relative inactivity of the promoter (Chubb et al., 2006; Pare et al., 2009; Raj et al., 2006; Zenklusen et al., 2008). In this case a number of polymerases transcribe the same gene and if the first one aborts, its place is taken over by the next RNAPII, preserving the uniform distribution of polymerases as seen in our experiments. This scenario suggests a situation where the absolute number of transcribing polymerases decreases towards the end of the transcribed gene, nevertheless full-length transcript is synthesised if there are enough polymerases and at least some of them reach the end of the gene. Such mechanism might be the most efficient strategy for gene expression control as it enables the cooperation of individual polymerases in competing with the barrier of nucleosomes on a template (Jin et al., 2010; Kulaeva et al., 2010).

In the light of our results it would be wise to interpret whole-genome studies with caution. In these studies the higher signal of RNAPII in the 5' end of genes is often interpreted as the promoter-proximal pausing of polymerases. In the context of our study the actual distribution of RNAPII might be much more uniform and therefore the so-called paused polymerases actually represent termination of RNAPII in early steps of transcription.

CONCLUSIONS

In the current work budding yeast was used as a model system to study the mechanisms of RNAPII-dependent transcription elongation. To understand better the process of nucleosome eviction from the coding region of highly transcribed genes we defined the area of nucleosome removal and showed its dependence on elongating RNAPII. In addition we investigated the dynamics of histones after transcription repression and detected the incorporation of newly synthesised histone molecules to reassembled nucleosomes. We also found that elongating RNAPII is capable of traversing heterochromatic structures, so far considered impenetrable for the transcription machinery. When looking for the factors facilitating the displacement of silencing complexes, we identified histone H3 acetylation at lysine residue 56 as the post-translational modification needed for this process.

While studying the whole cell population we repeatedly detected higher levels of RNAPII signal in the 5' end of the model gene than in the 3' end. Hence we decided to analyze the distribution of RNAPII also on single chromatin fragment. Interestingly, we discovered that on single cell level elongating RNAPII complexes are uniformly distributed throughout the entire length of the gene.

Shortly, the following conclusions can be drawn from the current study:

1. Elongating RNAPII determines the area of nucleosome loss in transcribed locus.
2. Newly synthesised histones are incorporated into nucleosomes after transcription inhibition.
3. Elongating RNAPII displaces silencing complexes from the coding region of the gene.
4. Acetylation of H3 K56 is required for RNAPII elongation through heterochromatin.
5. Elongating RNAPII is uniformly distributed on transcribed locus.

SUMMARY IN ESTONIAN

RNA polümeraas II-sõltuva transkriptsiooni elongatsiooni mehhanismide uurimine

Kõigis eukarüootsetes rakkudes on DNA seotud histoonivalkudega, moodustades nukleosoome. DNA ja valkude kompleksi nimetatakse kromatiiniks ning see struktuur on takistuseks kõigile DNA-ga toimuvatele protsessidele. Käesolevas töös uurisime, kuidas tuleb selle takistusega toime DNA-lt informatsiooni kopeeriv ensüüm RNA polümeraas II. Mudelorganismina kasutasime katsetes pagaripärmi (*Saccharomyces cerevisiae*).

Protsessi, mille käigus DNA-l oleva informatsiooni põhjal sünteesitakse mRNA, nimetatakse transkriptsiooniks. Trankriptsioon jagatakse kolmeks erinevaks etapiks – initsiatsioon, elongatsioon ja terminatsioon. DNA kõrgem kokkupakitud olek mõjutab eelkõige elongatsiooniprotsessi, mille jooksul transkribeeriv RNA polümeraas peab läbima DNA-st ja histoonidest moodustunud nukleosoomid. Varasematest töödest on teada, et geenidelt, mida aktiivselt transkribeeritakse, eemaldatakse nukleosoomid transkriptsiooni käigus täielikult ning nukleosoomne struktuur taastub pärast protsessi lõppu. Minu töö üheks eesmärgiks oli uurida, kui suures ulatuses eemaldatakse DNA-lt nukleosoomid ning kas kromatiini struktuuri taastamisel kasutatakse samu histooni molekule, mis sealt transkriptsiooni käigus eemaldati. Leidsime, et selle ala ulatuse, millelt nukleosoomid eemaldatakse, määrab ära elongeeriv polümeraas ning taastatud kromatiini struktuuris detekteerisime ka „uusi“ histooni molekule. Seega on just RNA polümeraas peamiseks kromatiini struktuuri muutusi esilekutsuvaks komponendiks ning kromatiini struktuuri taastamisel kasutatakse nii „vanu“ kui „uusi“ histoone.

Lisaks nuklesoomsele struktuurile on RNA polümeraas II jaoks takistavaks teguriks ka nn vaigistatud kromatiin ehk heterokromatiin. Vaigistatud kromatiini puhul on nukleosoomid seotud represseerivate valgukompleksidega, mida pagaripärmis nimetatakse SIR valkudeks. Katsetes õnnestus meil tekitada olukord, kus SIR kompleksid paiknesid ka meie mudelgeenil. Tänu sellele saime uurida heterokromatiinsete komplekside mõju transkribeerivale RNA polümeraas II-le. Selgus, et juba elongeeriv polümeraas suudab SIR valgud DNA-lt eemaldada ning edukalt mRNAd sünteesida. Selgitasime ka välja, et heterokromatiini lõhkumiseks on vaja, et mõned nuklesoomis olevatest histooni valkudest oleks keemiliselt modifitseeritud. Nimelt peab histoon H3 56. positsioonis olev lüsiinijääk olema atsetüleeritud, et RNA polümeraas saaks heterokromatiinsete kompleksidega kaetud ala transkribeerida.

Mitmetest katsetest selgus, et indutseerides mudelgeenil transkriptsiooni, jaotus RNA polümeraas II geenil ebaühtlaselt – nägime, et polümeraasi komplekse on rohkem geeni 5’ otsas kui 3’ otsas. Kuna meie katsed olid tehtud kasutades materjali kogu rakupopulatsioonist, huvitas meid, kas polümeraas on transkribeeritaval geenil samamoodi jaotunud ka ühe raku tasemel. Kasutades järjestikust kromatiini immuunopretsipitatsiooni leidsime, et transkribeeritaval

geenilookusel on RNA polümeraas II-e molekulid jaotunud ühtlaselt. Seega on polümeraasi populatsioonisisese ebaühtlase jaotuse põhjuseks pigem see, et mõnedes rakkudes transkriptsiooni küll alustatakse, kuid see katkeb. Neis rakkudes, kus polümeraas viib protsessi lõpuni, on ta jaotunud aga ühtlaselt üle kogu trankribeeritava piirkonna.

Antud tööst saadud tulemused on oluliseks täienduseks baasteadmistele transkriptsiooni elongatsioonist ning rikastavad arusaama RNA polümeraas II-sõltuva transkriptsiooni elongatsiooniprotsessist.

REFERENCES

Adam, M., Robert, F., Larochelle, M. and Gaudreau, L. (2001) H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Mol Cell Biol*, **21**, 6270–6279.

Adkins, M.W., Howar, S.R. and Tyler, J.K. (2004) Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast PHO5 and PHO8 genes. *Mol Cell*, **14**, 657–666.

Adkins, M.W. and Tyler, J.K. (2006) Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. *Mol Cell*, **21**, 405–416.

Ahmad, K. and Henikoff, S. (2002) The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol Cell*, **9**, 1191–1200.

Ahn, S.H., Diaz, R.L., Grunstein, M. and Allis, C.D. (2006) Histone H2B deacetylation at lysine 11 is required for yeast apoptosis induced by phosphorylation of H2B at serine 10. *Mol Cell*, **24**, 211–220.

Allfrey, V.G., Faulkner, R. and Mirsky, A.E. (1964) Acetylation and Methylation of Histones and Their Possible Role in the Regulation of Rna Synthesis. *Proc Natl Acad Sci U S A*, **51**, 786–794.

Armache, K.J., Mitterweger, S., Meinhart, A. and Cramer, P. (2005) Structures of complete RNA polymerase II and its subcomplex, Rpb4/7. *J Biol Chem*, **280**, 7131–7134.

Badenhorst, P., Voas, M., Rebay, I. and Wu, C. (2002) Biological functions of the ISWI chromatin remodeling complex NURF. *Genes Dev*, **16**, 3186–3198.

Bannister, A.J. and Kouzarides, T. (2011) Regulation of chromatin by histone modifications. *Cell Res*, **21**, 381–395.

Bar-Nahum, G., Epshtein, V., Ruckenstein, A.E., Rafikov, R., Mustaev, A. and Nudler, E. (2005) A ratchet mechanism of transcription elongation and its control. *Cell*, **120**, 183–193.

Barth, T.K. and Imhof, A. (2010) Fast signals and slow marks: the dynamics of histone modifications. *Trends Biochem Sci*, **35**, 618–626.

Bedford, M.T. and Richard, S. (2005) Arginine methylation an emerging regulator of protein function. *Mol Cell*, **18**, 263–272.

Belotserkovskaya, R., Oh, S., Bondarenko, V.A., Orphanides, G., Studitsky, V.M. and Reinberg, D. (2003) FACT facilitates transcription-dependent nucleosome alteration. *Science*, **301**, 1090–1093.

Boeger, H., Griesenbeck, J., Strattan, J.S. and Kornberg, R.D. (2003) Nucleosomes unfold completely at a transcriptionally active promoter. *Mol Cell*, **11**, 1587–1598.

Boeger, H., Griesenbeck, J., Strattan, J.S. and Kornberg, R.D. (2004) Removal of promoter nucleosomes by disassembly rather than sliding in vivo. *Mol Cell*, **14**, 667–673.

Bondarenko, V.A., Steele, L.M., Ujvari, A., Gaykalova, D.A., Kulaeva, O.I., Polikanov, Y.S., Luse, D.S. and Studitsky, V.M. (2006) Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol Cell*, **24**, 469–479.

Bortvin, A. and Winston, F. (1996) Evidence that Spt6p controls chromatin structure by a direct interaction with histones. *Science*, **272**, 1473–1476.

Bowman, A., Ward, R., Wiechens, N., Singh, V., El-Mkami, H., Norman, D.G. and Owen-Hughes, T. (2011) The histone chaperones Nap1 and Vps75 bind histones H3 and H4 in a tetrameric conformation. *Mol Cell*, **41**, 398–408.

Briggs, S.D., Bryk, M., Strahl, B.D., Cheung, W.L., Davie, J.K., Dent, S.Y., Winston, F. and Allis, C.D. (2001) Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae. *Genes Dev*, **15**, 3286–3295.

Bushnell, D.A. and Kornberg, R.D. (2003) Complete, 12-subunit RNA polymerase II at 4.1-A resolution: implications for the initiation of transcription. *Proc Natl Acad Sci U S A*, **100**, 6969–6973.

Carmen, A.A., Milne, L. and Grunstein, M. (2002) Acetylation of the yeast histone H4 N terminus regulates its binding to heterochromatin protein SIR3. *J Biol Chem*, **277**, 4778–4781.

Carrozza, M.J., Li, B., Florens, L., Suganuma, T., Swanson, S.K., Lee, K.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell*, **123**, 581–592.

Celic, I., Masumoto, H., Griffith, W.P., Meluh, P., Cotter, R.J., Boeke, J.D. and Verreault, A. (2006) The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation. *Curr Biol*, **16**, 1280–1289.

Chen, P. and Li, G. (2010) Dynamics of the higher-order structure of chromatin. *Protein Cell*, **1**, 967–971.

Chubb, J.R., Trcek, T., Shenoy, S.M. and Singer, R.H. (2006) Transcriptional pulsing of a developmental gene. *Curr Biol*, **16**, 1018–1025.

Churchman, L.S. and Weissman, J.S. (2011) Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature*, **469**, 368–373.

Clapier, C.R. and Cairns, B.R. (2009) The biology of chromatin remodeling complexes. *Annu Rev Biochem*, **78**, 273–304.

Cohen-Armon, M., Visochek, L., Rozensal, D., Kalal, A., Geistrikh, I., Klein, R., Bendetz-Nezer, S., Yao, Z. and Seger, R. (2007) DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: a link to histone acetylation. *Mol Cell*, **25**, 297–308.

Cote, J. and Richard, S. (2005) Tudor domains bind symmetrical dimethylated arginines. *J Biol Chem*, **280**, 28476–28483.

Cramer, P., Bushnell, D.A. and Kornberg, R.D. (2001) Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science*, **292**, 1863–1876.

Cuthbert, G.L., Daujat, S., Snowden, A.W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P.D., Tempst, P., Bannister, A.J. and Kouzarides, T. (2004) Histone deimination antagonizes arginine methylation. *Cell*, **118**, 545–553.

Dalal, Y., Wang, H., Lindsay, S. and Henikoff, S. (2007) Tetrameric structure of centromeric nucleosomes in interphase Drosophila cells. *PLoS Biol*, **5**, e218.

Darzacq, X., Shav-Tal, Y., de Turris, V., Brody, Y., Shenoy, S.M., Phair, R.D. and Singer, R.H. (2007) In vivo dynamics of RNA polymerase II transcription. *Nat Struct Mol Biol*, **14**, 796–806.

Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K. and Zhou, M.M. (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature*, **399**, 491–496.

Dion, M.F., Kaplan, T., Kim, M., Buratowski, S., Friedman, N. and Rando, O.J. (2007) Dynamics of replication-independent histone turnover in budding yeast. *Science*, **315**, 1405–1408.

Donze, D., Adams, C.R., Rine, J. and Kamakaka, R.T. (1999) The boundaries of the silenced HMR domain in Saccharomyces cerevisiae. *Genes Dev*, **13**, 698–708.
Driscoll, R., Hudson, A. and Jackson, S.P. (2007) Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science*, **315**, 649–652.
Erdel, F., Schubert, T., Marth, C., Langst, G. and Rippe, K. (2010) Human ISWI chromatin-remodeling complexes sample nucleosomes via transient binding reactions and become immobilized at active sites. *Proc Natl Acad Sci U S A*, **107**, 19873–19878.
Feng, Q., Wang, H., Ng, H.H., Erdjument-Bromage, H., Tempst, P., Struhl, K. and Zhang, Y. (2002) Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr Biol*, **12**, 1052–1058.
Ferreira, H., Flaus, A. and Owen-Hughes, T. (2007) Histone modifications influence the action of Snf2 family remodelling enzymes by different mechanisms. *J Mol Biol*, **374**, 563–579.
Flemming, W. (1882) *Zellsubstanz, Kern und Zelltheilung*. F.C.W. Vogel, Leipzig.
Foster, E.R. and Downs, J.A. (2009) Methylation of H3 K4 and K79 is not strictly dependent on H2B K123 ubiquitylation. *J Cell Biol*, **184**, 631–638.
Fuchs, S.M., Laribee, R.N. and Strahl, B.D. (2009) Protein modifications in transcription elongation. *Biochim Biophys Acta*, **1789**, 26–36.
Fuda, N.J., Ardehali, M.B. and Lis, J.T. (2009) Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature*, **461**, 186–192.
Furuyama, T. and Henikoff, S. (2009) Centromeric nucleosomes induce positive DNA supercoils. *Cell*, **138**, 104–113.
Gann, A. (2011) Jacob and Monod: from operons to EvoDevo. *Curr Biol*, **20**, R718–723.
Gao, L. and Gross, D.S. (2008) Sir2 silences gene transcription by targeting the transition between RNA polymerase II initiation and elongation. *Mol Cell Biol*, **28**, 3979–3994.
Gat-Viks, I. and Vingron, M. (2009) Evidence for gene-specific rather than transcription rate-dependent histone H3 exchange in yeast coding regions. *PLoS Comput Biol*, **5**, e1000282.
Gkikopoulos, T., Havas, K.M., Dewar, H. and Owen-Hughes, T. (2009) SWI/SNF and Asf1p cooperate to displace histones during induction of the saccharomyces cerevisiae HO promoter. *Mol Cell Biol*, **29**, 4057–4066.
Glover-Cutter, K., Kim, S., Espinosa, J. and Bentley, D.L. (2008) RNA polymerase II pauses and associates with pre-mRNA processing factors at both ends of genes. *Nat Struct Mol Biol*, **15**, 71–78.
Gnatt, A.L., Cramer, P., Fu, J., Bushnell, D.A. and Kornberg, R.D. (2001) Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 A resolution. *Science*, **292**, 1876–1882.
Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev*, **11**, 1640–1650.
Han, J., Zhou, H., Horazdovsky, B., Zhang, K., Xu, R.M. and Zhang, Z. (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science*, **315**, 653–655.

Hassa, P.O., Haenni, S.S., Elser, M. and Hottiger, M.O. (2006) Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? *Microbiol Mol Biol Rev*, **70**, 789–829.

Hecht, A., Strahl-Bolsinger, S. and Grunstein, M. (1996) Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature*, **383**, 92–96.

Hemming, S.A., Jansma, D.B., Macgregor, P.F., Goryachev, A., Friesen, J.D. and Edwards, A.M. (2000) RNA polymerase II subunit Rpb9 regulates transcription elongation in vivo. *J Biol Chem*, **275**, 35506–35511.

Herbert, K.M., Greenleaf, W.J. and Block, S.M. (2008) Single-molecule studies of RNA polymerase: motoring along. *Annu Rev Biochem*, **77**, 149–176.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S. and Young, R.A. (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell*, **95**, 717–728.

http://www.yeastgenome.org. Saccharomyces Genome Database

Hyland, E.M., Cosgrove, M.S., Molina, H., Wang, D., Pandey, A., Cottee, R.J. and Boeke, J.D. (2005) Insights into the role of histone H3 and histone H4 core modifiable residues in Saccharomyces cerevisiae. *Mol Cell Biol*, **25**, 10060–10070.

Iizuka, M. and Smith, M.M. (2003) Functional consequences of histone modifications. *Curr Opin Genet Dev*, **13**, 154–160.

Ioshikhes, I., Bolshoy, A., Derenshteyn, K., Borodovsky, M. and Trifonov, E.N. (1996) Nucleosome DNA sequence pattern revealed by multiple alignment of experimentally mapped sequences. *J Mol Biol*, **262**, 129–139.

Ioshikhes, I.P., Albert, I., Zanton, S.J. and Pugh, B.F. (2006) Nucleosome positions predicted through comparative genomics. *Nat Genet*, **38**, 1210–1215.

Izban, M.G. and Luse, D.S. (1992) The RNA polymerase II ternary complex cleaves the nascent transcript in a 3'–5' direction in the presence of elongation factor SII. *Genes Dev*, **6**, 1342–1356.

Iyer, V. and Struhl, K. (1996) Absolute mRNA levels and transcriptional initiation rates in Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A*, **93**, 5208–5212.

Jackson, V., Shires, A., Tanphaichitr, N. and Chalkley, R. (1976) Modifications to histones immediately after synthesis. *J Mol Biol*, **104**, 471–483.

Jacob, F. and Monod, J. (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol*, **3**, 318–356.

Jacobs, S.A. and Khorasanizadeh, S. (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science*, **295**, 2080–2083.

Jamai, A., Imoberdorf, R.M. and Strubin, M. (2007) Continuous histone H2B and transcription-dependent histone H3 exchange in yeast cells outside of replication. *Mol Cell*, **25**, 345–355.

Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science*, **293**, 1074–1080.

Jimeno-Gonzalez, S., Gomez-Herreros, F., Alepuz, P.M. and Chavez, S. (2006) A gene-specific requirement for FACT during transcription is related to the chromatin organization of the transcribed region. *Mol Cell Biol*, **26**, 8710–8721.

Jin, J., Bai, L., Johnson, D.S., Fulbright, R.M., Kireeva, M.L., Kashlev, M. and Wang, M.D. (2010) Synergistic action of RNA polymerases in overcoming the nucleosomal barrier. *Nat Struct Mol Biol*, **17**, 745–752.

Johnson, A., Li, G., Sikorski, T.W., Buratowski, S., Woodcock, C.L. and Moazed, D. (2009) Reconstitution of heterochromatin-dependent transcriptional gene silencing. *Mol Cell*, **35**, 769–781.

Kaplan, C.D., Laprade, L. and Winston, F. (2003) Transcription elongation factors repress transcription initiation from cryptic sites. *Science*, **301**, 1096–1099.
Kaplan, T., Liu, C.L., Erkmann, J.A., Holik, J., Grunstein, M., Kaufman, P.D., Friedman, N. and Rando, O.J. (2008) Cell cycle- and chaperone-mediated regulation of H3K56ac incorporation in yeast. *PLoS Genet*, **4**, e1000270.
Katan-Khaykovich, Y. and Struhl, K. (2011) Splitting of H3-H4 tetramers at transcriptionally active genes undergoing dynamic histone exchange. *Proc Natl Acad Sci U S A*, **108**, 1296–1301.
Keck, K.M. and Pemberton, L.F. (2011) Interaction with the Histone Chaperone Vps75 Promotes Nuclear Localization and HAT Activity of Rtt109 In Vivo. *Traffic*.
Kim, H., Erickson, B., Luo, W., Seward, D., Graber, J.H., Pollock, D.D., Megee, P.C. and Bentley, D.L. (2011) Gene-specific RNA polymerase II phosphorylation and the CTD code. *Nat Struct Mol Biol*, **17**, 1279–1286.
Kim, T.S., Liu, C.L., Yassour, M., Holik, J., Friedman, N., Buratowski, S. and Rando, O.J. (2010) RNA polymerase mapping during stress responses reveals widespread nonproductive transcription in yeast. *Genome Biol*, **11**, R75.
Kimura, A., Umehara, T. and Horikoshi, M. (2002a) Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat Genet*, **32**, 370–377.
Kimura, H. and Cook, P.R. (2001) Kinetics of core histones in living human cells: little exchange of H3 and H4 and some rapid exchange of H2B. *J Cell Biol*, **153**, 1341–1353.
Kimura, H., Sugaya, K. and Cook, P.R. (2002b) The transcription cycle of RNA polymerase II in living cells. *J Cell Biol*, **159**, 777–782.
Kireeva, M.L., Hancock, B., Cremona, G.H., Walter, W., Studitsky, V.M. and Kashlev, M. (2005) Nature of the nucleosomal barrier to RNA polymerase II. *Mol Cell*, **18**, 97–108.
Kireeva, M.L., Walter, W., Tchernajenko, V., Bondarenko, V., Kashlev, M. and Studitsky, V.M. (2002) Nucleosome remodeling induced by RNA polymerase II: loss of the H2A/H2B dimer during transcription. *Mol Cell*, **9**, 541–552.
Kirmizis, A., Santos-Rosa, H., Penkett, C.J., Singer, M.A., Vermeulen, M., Mann, M., Bahler, J., Green, R.D. and Kouzarides, T. (2007) Arginine methylation at histone H3R2 controls deposition of H3K4 trimethylation. *Nature*, **449**, 928–932.
Komissarova, N. and Kashlev, M. (1997) RNA polymerase switches between inactivated and activated states By translocating back and forth along the DNA and the RNA. *J Biol Chem*, **272**, 15329–15338.
Kornberg, R.D. and Lorch, Y. (1991) Irresistible force meets immovable object: transcription and the nucleosome. *Cell*, **67**, 833–836.
Kouzarides, T. (2007) Chromatin modifications and their function. *Cell*, **128**, 693–705.
Kristjuhan, A. and Svejstrup, J.Q. (2004) Evidence for distinct mechanisms facilitating transcript elongation through chromatin in vivo. *Embo J*, **23**, 4243–4252.
Kristjuhan, A., Wittschieben, B.O., Walker, J., Roberts, D., Cairns, B.R. and Svejstrup, J.Q. (2003) Spreading of Sir3 protein in cells with severe histone H3 hypoacetylation. *Proc Natl Acad Sci U S A*, **100**, 7551–7556.
Krogan, N.J., Dover, J., Khorrami, S., Greenblatt, J.F., Schneider, J., Johnston, M. and Shilatifard, A. (2002) COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. *J Biol Chem*, **277**, 10753–10755.

Krummel, B. and Chamberlin, M.J. (1992a) Structural analysis of ternary complexes of Escherichia coli RNA polymerase. Deoxyribonuclease I footprinting of defined complexes. *J Mol Biol*, **225**, 239–250.

Krummel, B. and Chamberlin, M.J. (1992b) Structural analysis of ternary complexes of Escherichia coli RNA polymerase. Individual complexes halted along different transcription units have distinct and unexpected biochemical properties. *J Mol Biol*, **225**, 221–237.

Kulaeva, O.I., Hsieh, F.K. and Studitsky, V.M. (2010) RNA polymerase complexes cooperate to relieve the nucleosomal barrier and evict histones. *Proc Natl Acad Sci U S A*, **107**, 11325–11330.

Kuo, M.H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature*, **383**, 269–272.

Kurdistani, S.K. and Grunstein, M. (2003) Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol*, **4**, 276–284.

Ladurner, A.G., Inouye, C., Jain, R. and Tjian, R. (2003) Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Mol Cell*, **11**, 365–376.

Larson, D.R., Zenklusen, D., Wu, B., Chao, J.A. and Singer, R.H. (2011) Real-time observation of transcription initiation and elongation on an endogenous yeast gene. *Science*, **332**, 475–478.

Latham, J.A. and Dent, S.Y. (2007) Cross-regulation of histone modifications. *Nat Struct Mol Biol*, **14**, 1017–1024.

Lee, C.K., Shibata, Y., Rao, B., Strahl, B.D. and Lieb, J.D. (2004) Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet*, **36**, 900–905.

Li, B., Pattenden, S.G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., Gerton, J. and Workman, J.L. (2005) Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proc Natl Acad Sci U S A*, **102**, 18385–18390.

Liang, G., Klose, R.J., Gardner, K.E. and Zhang, Y. (2007) Yeast Jhd2p is a histone H3 Lys4 trimethyl demethylase. *Nat Struct Mol Biol*, **14**, 243–245.

Liu, C.L., Kaplan, T., Kim, M., Buratowski, S., Schreiber, S.L., Friedman, N. and Rando, O.J. (2005) Single-nucleosome mapping of histone modifications in S. cerevisiae. *PLoS Biol*, **3**, e328.

Lo, W.S., Duggan, L., Emre, N.C., Belotserkovskya, R., Lane, W.S., Shiekhattar, R. and Berger, S.L. (2001) Snf1--a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. *Science*, **293**, 1142–1146.

Loo, S. and Rine, J. (1994) Silencers and domains of generalized repression. *Science*, **264**, 1768–1771.

Lorch, Y., LaPointe, J.W. and Kornberg, R.D. (1987) Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell*, **49**, 203–210.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, **389**, 251–260.

Luse, D.S. and Studitsky, V.M. (2011) The mechanism of nucleosome traversal by RNA polymerase II: Roles for template uncoiling and transcript elongation factors. *RNA Biol*, **8**.

Lynch, P.J. and Rusche, L.N. (2009) A silencer promotes the assembly of silenced chromatin independently of recruitment. *Mol Cell Biol*, **29**, 43–56.
Maas, N.L., Miller, K.M., DeFazio, L.G. and Toczyski, D.P. (2006) Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. *Mol Cell*, **23**, 109–119.
Mann, R.K. and Grunstein, M. (1992) Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. *Embo J*, **11**, 3297–3306.
Mannironi, C., Bonner, W.M. and Hatch, C.L. (1989) H2A.X. a histone isoprotein with a conserved C-terminal sequence, is encoded by a novel mRNA with both DNA replication type and polyA 3' processing signals. *Nucleic Acids Res*, **17**, 9113–9126.
Marshall, N.F. and Price, D.H. (1992) Control of formation of two distinct classes of RNA polymerase II elongation complexes. *Mol Cell Biol*, **12**, 2078–2090.
Masumoto, H., Hawke, D., Kobayashi, R. and Verreault, A. (2005) A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature*, **436**, 294–298.
Mayer, A., Lidschreiber, M., Siebert, M., Leike, K., Soding, J. and Cramer, P. (2010) Uniform transitions of the general RNA polymerase II transcription complex. *Nat Struct Mol Biol*, **17**, 1272–1278.
Mellor, J. and Morillon, A. (2004) ISWI complexes in Saccharomyces cerevisiae. *Biochim Biophys Acta*, **1677**, 100–112.
Meneghini, M.D., Wu, M. and Madhani, H.D. (2003) Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell*, **112**, 725–736.
Miller, A., Yang, B., Foster, T. and Kirchmaier, A.L. (2008) Proliferating cell nuclear antigen and ASF1 modulate silent chromatin in Saccharomyces cerevisiae via lysine 56 on histone H3. *Genetics*, **179**, 793–809.
Miller, C., Schwalb, B., Maier, K., Schulz, D., Dumcke, S., Zacher, B., Mayer, A., Sydow, J., Marcinowski, L., Dolken, L., Martin, D.E., Tresch, A. and Cramer, P. (2011) Dynamic transcriptome analysis measures rates of mRNA synthesis and decay in yeast. *Mol Syst Biol*, **7**, 458.
Min, J., Zhang, Y. and Xu, R.M. (2003) Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes Dev*, **17**, 1823–1828.
Miura, F., Kawaguchi, N., Yoshida, M., Uematsu, C., Kito, K., Sakaki, Y. and Ito, T. (2008) Absolute quantification of the budding yeast transcriptome by means of competitive PCR between genomic and complementary DNAs. *BMC Genomics*, **9**, 574.
Miyao, T., Barnett, J.D. and Woychik, N.A. (2001) Deletion of the RNA polymerase subunit RPB4 acts as a global, not stress-specific, shut-off switch for RNA polymerase II transcription at high temperatures. *J Biol Chem*, **276**, 46408–46413.
Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N. and Mellor, J. (2003) Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. *Cell*, **115**, 425–435.
Mousson, F., Ochsenbein, F. and Mann, C. (2007) The histone chaperone Asf1 at the crossroads of chromatin and DNA checkpoint pathways. *Chromosoma*, **116**, 79–93.
Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M. and Snyder, M. (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science*, **320**, 1344–1349.

Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D. and Grewal, S.I. (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science*, **292**, 110–113.

Nathan, D., Ingvarsdottir, K., Sterner, D.E., Bylebyl, G.R., Dokmanovic, M., Dorsey, J.A., Whelan, K.A., Krsmanovic, M., Lane, W.S., Meluh, P.B., Johnson, E.S. and Berger, S.L. (2006) Histone sumoylation is a negative regulator in Saccharomyces cerevisiae and shows dynamic interplay with positive-acting histone modifications. *Genes Dev*, **20**, 966–976.

Nechaev, S. and Adelman, K. (2011) Pol II waiting in the starting gates: Regulating the transition from transcription initiation into productive elongation. *Biochim Biophys Acta*, **1809**, 34–45.

Nelson, C.J., Santos-Rosa, H. and Kouzarides, T. (2006) Proline isomerization of histone H3 regulates lysine methylation and gene expression. *Cell*, **126**, 905–916.

Ng, H.H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y. and Struhl, K. (2002) Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev*, **16**, 1518–1527.

North, J.A., Javaid, S., Ferdinand, M.B., Chatterjee, N., Picking, J.W., Shoffner, M., Nakkula, R.J., Bartholomew, B., Ottesen, J.J., Fishel, R. and Poirier, M.G. (2011) Phosphorylation of histone H3(T118) alters nucleosome dynamics and remodeling. *Nucleic Acids Res*.

O'Brien, T. and Lis, J.T. (1993) Rapid changes in Drosophila transcription after an instantaneous heat shock. *Mol Cell Biol*, **13**, 3456–3463.

Olins, A.L. and Olins, D.E. (1974) Spheroid chromatin units (v bodies). *Science*, **183**, 330–332.

Olins, D.E. and Olins, A.L. (2003) Chromatin history: our view from the bridge. *Nat Rev Mol Cell Biol*, **4**, 809–814.

Orphanides, G., LeRoy, G., Chang, C.H., Luse, D.S. and Reinberg, D. (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell*, **92**, 105–116.

Ozdemir, A., Spicuglia, S., Lasonder, E., Vermeulen, M., Campsteijn, C., Stunnenberg, H.G. and Logie, C. (2005) Characterization of lysine 56 of histone H3 as an acetylation site in Saccharomyces cerevisiae. *J Biol Chem*, **280**, 25949–25952.

Pabo, C.O. and Nekludova, L. (2000) Geometric analysis and comparison of protein-DNA interfaces: why is there no simple code for recognition? *J Mol Biol*, **301**, 597–624.

Pare, A., Lemons, D., Kosman, D., Beaver, W., Freund, Y. and McGinnis, W. (2009) Visualization of individual Scr mRNAs during Drosophila embryogenesis yields evidence for transcriptional bursting. *Curr Biol*, **19**, 2037–2042.

Parthun, M.R. (2007) Hat1: the emerging cellular roles of a type B histone acetyltransferase. *Oncogene*, **26**, 5319–5328.

Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A. and Reinberg, D. (2006) Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell*, **125**, 703–717.

Pelechano, V., Chavez, S. and Perez-Ortin, J.E. (2010) A complete set of nascent transcription rates for yeast genes. *PLoS One*, **5**, e15442.

Phatnani, H.P. and Greenleaf, A.L. (2006) Phosphorylation and functions of the RNA polymerase II CTD. *Genes Dev*, **20**, 2922–2936.

Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolsheimer, E., Zeitlinger, J., Lewitter, F.,

Gifford, D.K. and Young, R.A. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell*, **122**, 517–527.
Radman-Livaja, M., Ruben, G., Weiner, A., Friedman, N., Kamakaka, R. and Rando, O.J. (2011) Dynamics of Sir3 spreading in budding yeast: secondary recruitment sites and euchromatic localization. *Embo J*, **30**, 1012–1026.
Raj, A., Peskin, C.S., Tranchina, D., Vargas, D.Y. and Tyagi, S. (2006) Stochastic mRNA synthesis in mammalian cells. *PLoS Biol*, **4**, e309.
Rao, B., Shibata, Y., Strahl, B.D. and Lieb, J.D. (2005) Dimethylation of histone H3 at lysine 36 demarcates regulatory and nonregulatory chromatin genome-wide. *Mol Cell Biol*, **25**, 9447–9459.
Recht, J., Tsubota, T., Tanny, J.C., Diaz, R.L., Berger, J.M., Zhang, X., Garcia, B.A., Shabanowitz, J., Burlingame, A.L., Hunt, D.F., Kaufman, P.D. and Allis, C.D. (2006) Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis. *Proc Natl Acad Sci U S A*, **103**, 6988–6993.
Richmond, T.J. and Davey, C.A. (2003) The structure of DNA in the nucleosome core. *Nature*, **423**, 145–150.
Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D. and Klug, A. (1984) Structure of the nucleosome core particle at 7 A resolution. *Nature*, **311**, 532–537.
Robinson, P.J. and Rhodes, D. (2006) Structure of the '30 nm' chromatin fibre: a key role for the linker histone. *Curr Opin Struct Biol*, **16**, 336–343.
Rodriguez-Gil, A., Garcia-Martinez, J., Pelechano, V., Munoz-Centeno Mde, L., Geli, V., Perez-Ortin, J.E. and Chavez, S. (2010) The distribution of active RNA polymerase II along the transcribed region is gene-specific and controlled by elongation factors. *Nucleic Acids Res*, **38**, 4651–4664.
Rufiange, A., Jacques, P.E., Bhat, W., Robert, F. and Nourani, A. (2007) Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell*, **27**, 393–405.
Rundlett, S.E., Carmen, A.A., Kobayashi, R., Bavykin, S., Turner, B.M. and Grunstein, M. (1996) HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc Natl Acad Sci U S A*, **93**, 14503–14508.
Rusche, L.N., Kirchmaier, A.L. and Rine, J. (2002) Ordered nucleation and spreading of silenced chromatin in Saccharomyces cerevisiae. *Mol Biol Cell*, **13**, 2207–2222.
Rusche, L.N., Kirchmaier, A.L. and Rine, J. (2003) The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. *Annu Rev Biochem*, **72**, 481–516.
Sakabe, K., Wang, Z. and Hart, G.W. (2010) Beta-N-acetylglucosamine (O-GlcNAc) is part of the histone code. *Proc Natl Acad Sci U S A*, **107**, 19915–19920.
Santisteban, M.S., Hang, M. and Smith, M.M. (2011) Histone Variant H2A.Z and RNA Polymerase II Transcription Elongation. *Mol Cell Biol*, **31**, 1848–1860.
Santisteban, M.S., Kalashnikova, T. and Smith, M.M. (2000) Histone H2A.Z regulats transcription and is partially redundant with nucleosome remodeling complexes. *Cell*, **103**, 411–422.
Saunders, A., Werner, J., Andrulis, E.D., Nakayama, T., Hirose, S., Reinberg, D. and Lis, J.T. (2003) Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. *Science*, **301**, 1094–1096.
Schalch, T., Duda, S., Sargent, D.F. and Richmond, T.J. (2005) X-ray structure of a tetranucleosome and its implications for the chromatin fibre. *Nature*, **436**, 138–141.

Schermer, U.J., Korber, P. and Horz, W. (2005) Histones are incorporated in trans during reassembly of the yeast PHO5 promoter. *Mol Cell*, **19**, 279–285.
Schneider, J., Bajwa, P., Johnson, F.C., Bhaumik, S.R. and Shilatifard, A. (2006) Rtt109 is required for proper H3K56 acetylation: a chromatin mark associated with the elongating RNA polymerase II. *J Biol Chem*, **281**, 37270–37274.
Schwabish, M.A. and Struhl, K. (2004) Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol Cell Biol*, **24**, 10111–10117.
Schwabish, M.A. and Struhl, K. (2006) Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. *Mol Cell*, **22**, 415–422.
Schwabish, M.A. and Struhl, K. (2007) The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. *Mol Cell Biol*, **27**, 6987–6995.
Schwartz, B.E. and Ahmad, K. (2005) Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev*, **19**, 804–814.
Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thastrom, A., Field, Y., Moore, I.K., Wang, J.P. and Widom, J. (2006) A genomic code for nucleosome positioning. *Nature*, **442**, 772–778.
Sekinger, E.A. and Gross, D.S. (1999) SIR repression of a yeast heat shock gene: UAS and TATA footprints persist within heterochromatin. *Embo J*, **18**, 7041–7055.
Sekinger, E.A. and Gross, D.S. (2001) Silenced chromatin is permissive to activator binding and PIC recruitment. *Cell*, **105**, 403–414.
Shi, Y. (2007) Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet*, **8**, 829–833.
Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R. and Peterson, C.L. (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science*, **311**, 844–847.
Sigurdsson, S., Dirac-Svejstrup, A.B. and Svejstrup, J.Q. (2010) Evidence that transcript cleavage is essential for RNA polymerase II transcription and cell viability. *Mol Cell*, **38**, 202–210.
Simic, R., Lindstrom, D.L., Tran, H.G., Roinick, K.L., Costa, P.J., Johnson, A.D., Hartzog, G.A. and Arndt, K.M. (2003) Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *Embo J*, **22**, 1846–1856.
Somesh, B.P., Reid, J., Liu, W.F., Sogaard, T.M., Erdjument-Bromage, H., Tempst, P. and Svejstrup, J.Q. (2005) Multiple mechanisms confining RNA polymerase II ubiquitylation to polymerases undergoing transcriptional arrest. *Cell*, **121**, 913–923.
Steinmetz, E.J., Warren, C.L., Kuehner, J.N., Panbehi, B., Ansari, A.Z. and Brow, D.A. (2006) Genome-wide distribution of yeast RNA polymerase II and its control by Sen1 helicase. *Mol Cell*, **24**, 735–746.
Strahl-Bolsinger, S., Hecht, A., Luo, K. and Grunstein, M. (1997) SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes Dev*, **11**, 83–93.
Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature*, **403**, 41–45.
Strahl, B.D., Grant, P.A., Briggs, S.D., Sun, Z.W., Bone, J.R., Caldwell, J.A., Mollah, S., Cook, R.G., Shabanowitz, J., Hunt, D.F. and Allis, C.D. (2002) Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. *Mol Cell Biol*, **22**, 1298–1306.

Struhl, K. and Davis, R.W. (1981) Transcription of the his3 gene region in Saccharomyces cerevisiae. *J Mol Biol*, **152**, 535–552.
Suka, N., Luo, K. and Grunstein, M. (2002) Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat Genet*, **32**, 378–383.
Suka, N., Suka, Y., Carmen, A.A., Wu, J. and Grunstein, M. (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. *Mol Cell*, **8**, 473–479.
Takahata, S., Yu, Y. and Stillman, D.J. (2009) FACT and Asf1 regulate nucleosome dynamics and coactivator binding at the HO promoter. *Mol Cell*, **34**, 405–415.
Talbert, P.B. and Henikoff, S. (2010) Histone variants – ancient wrap artists of the epigenome. *Nat Rev Mol Cell Biol*, **11**, 264–275.
Taverna, S.D., Ilin, S., Rogers, R.S., Tanny, J.C., Lavender, H., Li, H., Baker, L., Boyle, J., Blair, L.P., Chait, B.T., Patel, D.J., Aitchison, J.D., Tackett, A.J. and Allis, C.D. (2006) Yng1 PHD finger binding to H3 trimethylated at K4 promotes NuA3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. *Mol Cell*, **24**, 785–796.
Tennyson, C.N., Klamut, H.J. and Worton, R.G. (1995) The human dystrophin gene requires 16 hours to be transcribed and is cotranscriptionally spliced. *Nat Genet*, **9**, 184–190.
Thiriet, C. and Hayes, J.J. (2005) Replication-independent core histone dynamics at transcriptionally active loci in vivo. *Genes Dev*, **19**, 677–682.
Thomas, B.J. and Rothstein, R. (1989) Elevated recombination rates in transcriptionally active DNA. *Cell*, **56**, 619–630.
Tsubota, T., Berndsen, C.E., Erkmann, J.A., Smith, C.L., Yang, L., Freitas, M.A., Denu, J.M. and Kaufman, P.D. (2007) Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. *Mol Cell*, **25**, 703–712.
van Attikum, H. and Gasser, S.M. (2009) Crosstalk between histone modifications during the DNA damage response. *Trends Cell Biol*, **19**, 207–217.
van Leeuwen, F., Gafken, P.R. and Gottschling, D.E. (2002) Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell*, **109**, 745–756.
Varga-Weisz, P.D., Blank, T.A. and Becker, P.B. (1995) Energy-dependent chromatin accessibility and nucleosome mobility in a cell-free system. *Embo J*, **14**, 2209–2216.
West, M.H. and Bonner, W.M. (1980) Histone 2A, a heteromorphous family of eight protein species. *Biochemistry*, **19**, 3238–3245.
Widom, J. and Klug, A. (1985) Structure of the 300A chromatin filament: X-ray diffraction from oriented samples. *Cell*, **43**, 207–213.
Williams, S.P., Athey, B.D., Muglia, L.J., Schappe, R.S., Gough, A.H. and Langmore, J.P. (1986) Chromatin fibers are left-handed double helices with diameter and mass per unit length that depend on linker length. *Biophys J*, **49**, 233–248.
Wood, A., Schneider, J., Dover, J., Johnston, M. and Shilatifard, A. (2003) The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *J Biol Chem*, **278**, 34739–34742.
Woodcock, C.L., Frado, L.L. and Rattner, J.B. (1984) The higher-order structure of chromatin: evidence for a helical ribbon arrangement. *J Cell Biol*, **99**, 42–52.
Woudstra, E.C., Gilbert, C., Fellows, J., Jansen, L., Brouwer, J., Erdjument-Bromage, H., Tempst, P. and Svejstrup, J.Q. (2002) A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. *Nature*, **415**, 929–933.

Xu, F., Zhang, K. and Grunstein, M. (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell*, **121**, 375–385.

Xu, F., Zhang, Q., Zhang, K., Xie, W. and Grunstein, M. (2007) Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. *Mol Cell*, **27**, 890–900.

Yang, B., Miller, A. and Kirchmaier, A.L. (2008) HST3/HST4-dependent deacetylation of lysine 56 of histone H3 in silent chromatin. *Mol Biol Cell*, **19**, 4993–5005.

Yang, X.J. and Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene*, **26**, 5310–5318.

Yu, M.C., Lamming, D.W., Eskin, J.A., Sinclair, D.A. and Silver, P.A. (2006) The role of protein arginine methylation in the formation of silent chromatin. *Genes Dev*, **20**, 3249–3254.

Yuan, G.C., Liu, Y.J., Dion, M.F., Slack, M.D., Wu, L.F., Altschuler, S.J. and Rando, O.J. (2005) Genome-scale identification of nucleosome positions in S. cerevisiae. *Science*, **309**, 626–630.

Zanton, S.J. and Pugh, B.F. (2006) Full and partial genome-wide assembly and disassembly of the yeast transcription machinery in response to heat shock. *Genes Dev*, **20**, 2250–2265.

Zenklusen, D., Larson, D.R. and Singer, R.H. (2008) Single-RNA counting reveals alternative modes of gene expression in yeast. *Nat Struct Mol Biol*, **15**, 1263–1271.

Zhang, H., Roberts, D.N. and Cairns, B.R. (2005) Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell*, **123**, 219–231.

Zhang, Z., Hayashi, M.K., Merkel, O., Stillman, B. and Xu, R.M. (2002) Structure and function of the BAH-containing domain of Orc1p in epigenetic silencing. *Embo J*, **21**, 4600–4611.

Zhao, J., Herrera-Diaz, J. and Gross, D.S. (2005) Domain-wide displacement of histones by activated heat shock factor occurs independently of Swi/Snf and is not correlated with RNA polymerase II density. *Mol Cell Biol*, **25**, 8985–8999.

Zou, Y., Yu, Q. and Bi, X. (2006) Asymmetric positioning of nucleosomes and directional establishment of transcriptionally silent chromatin by Saccharomyces cerevisiae silencers. *Mol Cell Biol*, **26**, 7806–7819.

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To: gena family (kadrijan; kaja; kkss; marinel; monikaka; piiaserk; pilleh; toniso; seitse; siims; vadja)
Subject: 6 down, 6 to go

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PS. I propose to meet next at Kristiina's place. What do you think? Or visit Kristiina at Monika's place?

I also thank all my friends outside the labs, Estonian and international ones. Again, hope to use a more personal approach in doing it properly.

My family and relatives have always been a solid support to me. Two great women, my mother and grandmother, have provided me with endless love and support in whatever I do. I hope some day I will be their worthy, carrying on the unconditional goodness, caring and, last but not least, art of making wonderful food to all the people around me. I am very proud of my brothers Siim and Arno for all their achievements. And I am sure that our father would also be. Ilze, Anna and Hugo have brought a lot of joy and intercultural learning to our family. My aunts Laine and Merike and uncle Arvo have provided me with 13 cool cousins being a big part of my life. To my "genetic half-sisters" – just bear with me :).

To Balázs: "*-A kutyanyalogatás tényleg jó, de ha egy galamb nyal meg, as már nem annyira.- …. -A nagypapának csak egy kutyája van, de nézd meg, a galambok milyen sokan vannak! Képzeld csak el, ha mind egyszerre jönnének, és megnyalnának! Fúj, gusztustalan! – … – De a galamb röptében is megnyalhatana. Átrepülne fölötted, kidugná a nyelvét, és sutty, végighúzná a fejeden!*" (Kivirähk 2008).

To everybody:

tõusen istuli
et vaadata tähti
pisut lähemalt

(in translation: i sit up; to look at the stars; a bit more closely)
(Pauklin 2011)

References:
Kivirähk, A., (2008) Sári, Samu és a titkok. Cerkabella Könyvkiadó
Pauklin, S., (2011) Aheldatud Jõgeva. JI

PUBLICATIONS

CURRICULUM VITAE

Signe Värv

Date of Birth: 17.05.1980
Nationality: Estonian
Contact: University of Tartu, Intitute of Molecular and Cell Biology, 23 Riia Street, 51010, Tartu Estonia
e-mail: signe.varv@ut.ee

Education and professional employment

2004–...	University of Tartu, *Ph. D* student, researcher at the Institute of Molecular and Cell Biology, Department of Cell Biology (supervisor Arnold Kristjuhan)
2002–2004	University of Tartu, *M.Sc* in Gene Technology, field of transgenic technology (supervisor Dina Lepik)
1998–2002	University of Tartu, *B. Sc* in Gene Technology, field of molecular diagnostics (supervisor Dina Lepik)
1987–1998	Miina Härma Gymnasium (graduated with honors)

Special courses and conferences

2010	EMBO conferene on „Gene Transcription in Yeast", San Feliu de Guixols, Spain
2010	35th FEBS Congress „Molecules of Life", Göteborg, Sweden
2009	EMBO conference on „Chromatin and Epigenetics", Heidelberg, Germany
2009	CSH Laboratory Conference „Mechanisms of eukaryotic transcription", Cold Spring Harbor, USA
2009	4th HBGS Student Council Symposium „Epigenetic Mechanisms in Disease and Development", Helsinki, Finland
2008	EMBO conference on „Gene Transcription in Yeast", San Feliu de Guixols, Spain
2007	Palmse mõis Summer School in Molecular Biology „Chromosomes, chromatin and epigenetics", Palmse, Estonia
2007	EMBO conference „Chromatin and Epigenetics", Heidelberg, Germany
2007	32th FEBS Congress „Molecular Machines", Vienna, Austria
2006	a course on 7900HT Real Time Instrument, Tartu, Estonia

Professional organisations

Member of the Estonian Biochemical Society

Scientific work

The focus of my work is on gene expression and my aim is to elucidate the mechanisms of eukaryotic transcription elongation with the emphasis on chromatin remodelling and modifying during this process.

List of publications

Lepik D, Jaks V, Kadaja L, Värv S, Maimets T. (2003) Electroporation and carrier DNA cause p53 activation, cell cycle arrest, and apoptosis. *Anal Biochem* 318(1):52–9.

Värv, S., Kristjuhan, K., Kristjuhan, A. (2007) RNA polymerase II determines the area of nucleosome loss in transcribed gene loci. *Biochem Biophys Res Commun* 358(2):666–71.

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.

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 Biol Chem* 285(51):40004–11.

Peil, K., Värv, S., Lõoke, M., Kristjuhan, K., Kristjuhan, A. (2011) Uniform distribution of elongating RNA polymerase II complexes in transcribed gene locus. *J Biol Chem* 286(27):23817–22.

Social activities

- Volunteer in NGO Sunwheel, mentor for youngsters with lesser opportunities
- Biology tutor in the Pysics, Chemisty and Biology work-shops program by the Science School of Tarty University

CURRICULUM VITAE

Signe Värv

Sünniaeg:	17.05.1980
Kodakondsus:	Eesti
Kontaktandmed:	Tartu Ülikool, Molekulaar- ja rakubioloogia Instituut, Riia 23, 51010, Tartu, Eesti
e-mail:	signe.varv@ut.ee

Haridus ja erialane teenistuskäik

2004 – ...	Tartu Ülikool, Molekulaar- ja rakubioloogia instituut, doktoriõpingud, teadur
2002–2004	Tartu Ülikool, Molekulaar- ja rakubioloogia instituut, *M.Sc* geenitehnoloogias, transgeense tehnoloogia erialal
1998–2002	Tartu Ülikool, Molekulaar- ja rakubioloogia instituut *B.Sc* geenitehnoloogias, molekulaardiagnostika erialal
1987–1998	Miina Härma Gümnaasium (hõbemedal)

Erialane enesetäiendus

2010	EMBO konverents „Gene Transcription in Yeast", San Feliu de Guixols, Hispaania
2010	35. FEBS kongress „Molecules of Life", Göteborg, Rootsi
2009	EMBO konverents „Chromatin and Epigenetics", Heidelberg, Saksamaa
2009	CSH konverents „Mechanisms of eukaryotic transcription", Cold Spring Harbor, USA
2009	4. HBGS Sümpoosium „Epigenetic Mechanisms in Disease and Development", Helsingi, Soome
2008	EMBO konverents „Gene Transcription in Yeast", San Feliu de Guixols, Hispaania
2007	Palmse mõisa suvekool molekulaarbioloogias „Chromosomes, chromatin and epigenetics", Palmse, Eesti
2007	EMBO konverents „Chromatin and Epigenetics", Heidelberg, Saksamaa
2007	32. FEBS kongress „Molecular Machines", Viin, Austria
2006	kursus 7900HT kvantitatiivse PCR-i masina kasutamisest, Tartu, Eesti

Teadusorganisatsioonid

Eesti Biokeemia Seltsi liige

Teadustöö

Minu uurimustöö põhisuunaks on geenide ekspressiooni uurimine. Põhitähelepanu on pööratud transkriptsiooni elongatsioonile ning selle seostele kromatiini struktuuri muutuste ning modifitseerimisega.

Teaduspublikatsioonid

Lepik D, Jaks V, Kadaja L, Värv S, Maimets T. (2003) Electroporation and carrier DNA cause p53 activation, cell cycle arrest, and apoptosis. *Anal Biochem* 318(1):52–9.

Värv, S., Kristjuhan, K., Kristjuhan, A. (2007) RNA polymerase II determines the area of nucleosome loss in transcribed gene loci. *Biochem Biophys Res Commun* 358(2):666–71.

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.

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 Biol Chem* 285(51):40004–11.

Peil, K., Värv, S., Lõoke, M., Kristjuhan, K., Kristjuhan, A. (2011) Uniform distribution of elongating RNA polymerase II complexes in transcribed gene locus. *J Biol Chem* 286(27):23817–22.

Ühiskondlik tegevus

- Vabatahtlik MTÜ-s Päikeseratas, mentor vähemate võimalustega noortele
- Bioloogia õpikodade juhendaja Füüsika, keemia ja bioloogia õpikodade programmis

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