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Study on the importance of histone acetylation in the survival of *Saccharomyces cerevisiae*

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Study on the importance of histone acetylation in the survival of *S.cerevisiae*

Abstract: In eukaryotes, chromosomal DNA is packaged into chromatin. The basic unit of chromatin is a nucleosome, consisting of DNA wrapped around a histone octamer. Histones can be modified by several post-translational modifications, such as acetylation, methylation, phosphorylation, etc. In *S. cerevisiae*, Taf14 protein associates with a number of different complexes, such as TFIID and TFIIF, the chromatin remodeling complexes SWI/SNF and INO80, and the histone acetyl transferase NuA3. It was found, that yeast cells lacking Taf14 protein can tolerate mutant histone H3, even when all five acetylatable lysine residues are changed to non-acetylatable arginines. However, mutant H4 histones that had two or more lysine replacements to arginines were not alive. It was also determined, that the presence of Taf14 protein in TFIID or NuA3 complex is not necessary for yeast cells to survive with mutant H4 histones.

Keywords: *Saccharomyces cerevisiae,* histone acetylation, mutant H3 and H4 histones, Taf14 protein, TFIID, NuA3

CERCS: B230 Microbiology, bacteriology, virology, mycology

Histoonide atsetüleerimise olulisus pagaripärmi *S.cerevisiae* elumusele

Lühikokkuvõte: Eukarüootides on kromosomaalne DNA pakitud kromatiiniks. Kromatiini esmaseks pakkimisühikuks on nukleosoom, mis omakorda koosneb ümber histoonide oktameeri keerdunud DNA-st. Histoone on võimalik post-translatsiooniliselt modifitseerida mitmel erineval moel, nagu näiteks atsetüleerimise, metüleerimise, fosforüleermisega. Pagaripärmis *S. cerevisiae* on Taf14 valk mitmete erinevate komplekside, nagu TFIID ja TFIIF, kromatiini remodelleerivate komplekside SWI/SNF ja INO80 ning histooni atsetüültransferaas NuA3 kompleksi, koostisosa. Leiti et pärmirakud, milles puudus Taf14 valk suudavad taluda mutantset H3 histooni isegi juhul, kui kõik viis atsetüleeritavat lüsiinijääki on muudetud mitte-atsetüleeritavateks arginiinijääkideks. Samas kahe või rohkema lüsiinijäägi asendamine arginiiniga H4 histooni puhul oli rakkudele letaalne. Samuti leiti, et mutantsete H4 histoonidega ellujäämiseks ei ole vaja Taf14 valgu funktsioneerimist TFIID või NuA3 kompleksis.

Võtmesõnad: *Saccharomyces cerevisiae,* histoonide atsetüleerimine, mutantsed H3 ja H4 histoonid, Taf14 valk, TFIID, NuA3

CERCS: B230 Mikrobioloogia, bakterioloogia, viroloogia, mükoloogia

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LIST OF ABBREVIATIONS

5FOA	5-Fluoroorotic acid
Acetyl-CoA	acetyl-coenzyme A
C-terminal	carboxyl-terminal
INO80	inositol requiring 80
N-terminal	amino-terminal
NuA3	nucleosome acetyltransferase of histone 3
PTMs	post-transcriptional modifications
RSC	remodelling the structure of chromatin
SAGA	Spt-Ada-Gcn5-acetyltransferase
SWI/SNF	switching defective/sucrose nonfermenting
Taf14	transcription initiation factor TFIID subunit 14
TFIID	transcription factor II D
TFIIF	transcription factor II F
WT	wild-type
YEATS	Yaf9, ENL, AF9, Taf14, Sas5
YPD	yeast extract peptone dextrose

INTRODUCTION

In all eukaryotes, chromosomal DNA is packaged into chromatin. The basic packaging unit is a nucleosome, consisting of DNA and an octamer of core histones. Histones are subjected to a number of post-translational modifications (PTMs). PTMs, such as acetylation, crotonylation, methylation and phosphorylation, influence nucleosome dynamics, having an essential role in the epigenetic regulation of transcription and replication. There are also other numerous protein complexes, enzymes and transcription factors that play an important role in the cell's fate, affecting its phenotype and viability. The budding yeast *Saccharomyces cerevisiae* is an important and commonly used model system for studying eukaryotic molecular biology. Results gained from these studies are applicable in the fields of functional genomics, systems biology, biotechnology and also medicine.

In *Saccharomyces cerevisiae*, Taf14 is a protein that is associated with many multisubunit complexes, such as the general transcription factors TFIID and TFIIF, the chromatin remodeling complexes SWI/SNF and INO80, and the histone acetyl transferase NuA3. Also, Taf14 contains a highly conserved YEATS domain, that serves as a reader of histone PTMs. Although deletion of *TAF14* is not lethal, it causes reduced growth rate, sensitivity to DNA damaging agents, and elevated temperatures.

The current thesis focuses on the study of the importance of different histone acetylations in the survival of mutant yeast strain, lacking Taf14 protein. It was discovered that yeast cells without Taf14 protein tolerate mutant histone H3, even when all five acetylatable lysine residues in the N-terminal tail of H3 are mutated to non-acetylatable arginines. The replacement of wt H4 with any mutant carrying two lysine replacement was already lethal. An attempt to identify the complex, that requires Taf14 protein to tolerate mutant histones was made.

1. LITERATURE OVERVIEW

1.1 Chromatin

In eukaryotic cells chromosomal DNA is packaged into chromatin. Chromatin is a nucleoprotein complex, which consists of DNA and associated histone and nonhistone proteins (Panday and Grove, 2016). The basic packaging unit of chromatin is a nucleosome, consisting of DNA wrapped around a core histone octamer. Histone octamer consists of two copies of H2A- H2B and H3-H4 dimers (Luger et al., 1997). Nucleosomes and the linker DNA between them form the first packaging level, an 11 nm fiber referred to as a “beads on a string” structure (Figure 1). The next level of packaging is a 30 nm fiber. The final condensation level is a mitotic chromosome, with the diameter of about 700 nm (Maeshima and Eltsov, 2008) (Figure 1).

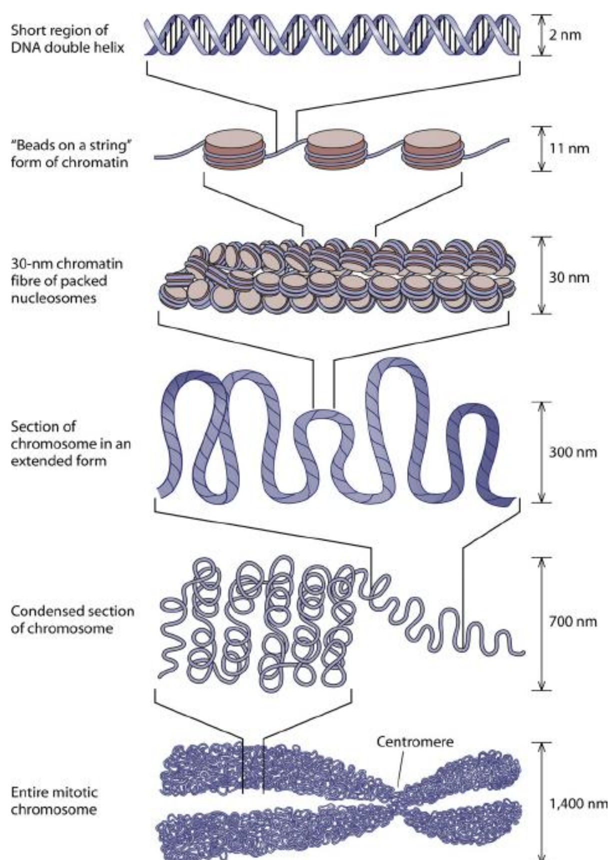

Figure 1. Chromatin structure. DNA is wrapped around a histone octamer forming nucleosomes. The nucleosomes are separated by stretches of linker DNA. This basic structure is folded into a fiber-like structure (30 nm in diameter). These 30-nm fibers are further compacted into higher-order structures (Jansen and Verstrepen, 2011).

1.1.1 Nucleosomes

The basic repeating functional and structural unit of chromatin is the nucleosome - a complex containing DNA and two copies of histone heterodimers (H2A-H2B and H3-H4 histone pairs), which repeats every 160 to 240 bp across the genome and is close to 10 nm in diameter. Histones are positively charged nuclear proteins denoted H1, H2A, H2B, H3, and H4, consisting of functionally different "histone fold" and "histone tail" (Lee and Orr-Weaver, 2001). Since histones are positively charged, it helps them to bind negatively charged DNA very tightly (due to the phosphate groups in DNA's phosphate-sugar backbone). Histones carry different post-translational modifications, that influence the functional abilities of the chromatin. Each nucleosome contains the disk-shaped histone core which consists of two H3 and two H4 proteins forming a tetramer, combing with two H2A/H2B dimers (Lee and Orr-Weaver, 2001) (Figure 2). The addition of the linker histone H1 to the linker DNA forms the nucleosome in higher eukaryotes (Maeshima and Eltsov, 2008).

In *S. cerevisiae* two genes encode each of the canonical histones (Dollard et al., 1994). The eight genes are arranged into four pairs of divergently transcribed loci: HTA1-HTB1 and HTA2-HTB2, each encoding histone proteins H2A and H2B; and HHT1-HHF1 and HHT2-HHF2, each encoding histone proteins H3 and H4 (Dollard et al., 1994). Due to this redundancy, deletion of any one histone locus does not result in lethality (Dollard et al., 1994). The H3-H4 protein dimers interact at the H3 C-termini through a four-helix bundle, and the H2A-H2B dimers bind to the resulting central H3-H4 tetramer through a similar four-helix bundle interaction between the H2B and H4 C-termini (Luger et al., 1997). No classical linker histones have been found to be connected within yeast chromatin in a stoichiometric manner (White et al., 2001). There has been found a gene (*HHO*) encoding a protein with linker histone homology (Hho1p), and it was proposed that this gene product may act as a linker histone in yeast (White et al., 2001). Deletion of this gene has no growth or mating defects, suggesting that this protein does not have a significant role in yeast chromatin organization (Patterton et al., 1998).

Figure 2. The crystal structure of yeast nucleosome core particle. Depicted down the superhelical axis. H2A is colored yellow, H2B red, H3 blue and H4 green. The DNA is colored in turquoise. α-helices and the location of the N- and C-terminal tails are also shown. The position of the molecular dyad axis is marked by Φ (White et al., 2001).

The X-ray crystal structure of the nucleosome core particle was resolved in 1997 (Luger et al, 1997). The core histones have a structure of a histone fold domain, which consists of three α-helices (α1, α2 and α3) separated by two loops (L1 and L2) (Arents et al., 1995), and an unstructured N-terminal tail, which appears outside the core (Lee and Orr-Weaver, 2001).

The residues of N-terminal tails of the four core histones are targets for post-translational modifications such as acetylation, methylation, and phosphorylation, that correspond with changes in gene activity (Davie, 1998). Histone tail acetylation, which occurs on lysins, is typically proposed to result in a change in chromatin structure (Wolffe and Kurumizaka, 1998). Unlike histones H3 and H4, H2A and H2B histones contain both a N-terminal tail and a C-terminal tail, and those tails are critical for maintaining nucleosome function and genome stability (Jiang et al., 2017).

The chemical nature of histones can also be changed at the protein sequence level, where the canonical histones can be substituted by histone variants. Besides the four canonical histones (H2A, H2B, H3 and H4), there exist histone variants that have crucial roles in chromosome segregation, transcriptional regulation, and DNA repair (Talbert and Henikoff, 2010). In budding yeast, the only histone variants that are found are H2A.Z (a close variant of H2A) and Cse4 (a centromeric H3-like protein) (Eriksson et al., 2012). H2A.Z plays an important role in gene activation and silencing, nucleosome turnover, DNA repair, heterochromatin, boundary element and chromatin fiber formation (Zlatanova and Thakar, 2008). Nucleosomes that

contain the histone H2A.Z variant compact chromatin fibers more readily than those with canonical H2A (Fan et al., 2002). Centromere-specific histone H3 variants are crucial for assembly of the kinetochore (Santaguida and Musacchio, 2009).

There are three principal functions of the nucleosome. First, the nucleosome acts as the fundamental unit of genomic compaction, arranging approximately 200 bp of DNA (McGinty and Tan., 2015). Second, it serves as a signaling hub for chromatin-templated mechanisms by giving a scaffold for the binding of chromatin enzymes and exposing a combinatorial array of post-translational modifications (McGinty and Tan., 2015). This array of post-translational modifications acts as a regulator the induction of chromatin enzymes (Taverna et al., 2007) and provides the higher-order compaction of chromatin and stability of the nucleosome (Luger et al., 2012). Third, the nucleosome has an ability to self-assemble into higher-order structures of chromatin for further compaction of the genome (McGinty and Tan., 2015).

1.1.2 Histone post-translational modifications

The tails of the core histones extend out of the nucleosome and make histones susceptible to a great number of post-translational modifications (PTMs), such as acetylation, crotonylation methylation, phosphorylation, ubiquitylation, sumoylation, proline isomerization, mono- and poly-ADP ribosylation (Figure 3) (Bowman and Poirier, 2015, Smolle and Workman, 2012). Histone PTMs regulate the structure of nucleosome and the function of it, for example during DNA replication, transcription and DNA damage/repair processes. This regulation process is possible as the result of interactions in the nucleosomes between N-terminal tails and DNA or other parts of histones (Arya and Schlick, 2009). Both histone modifications and structure of chromatin influence the presence of nuclear factors presented to the DNA.

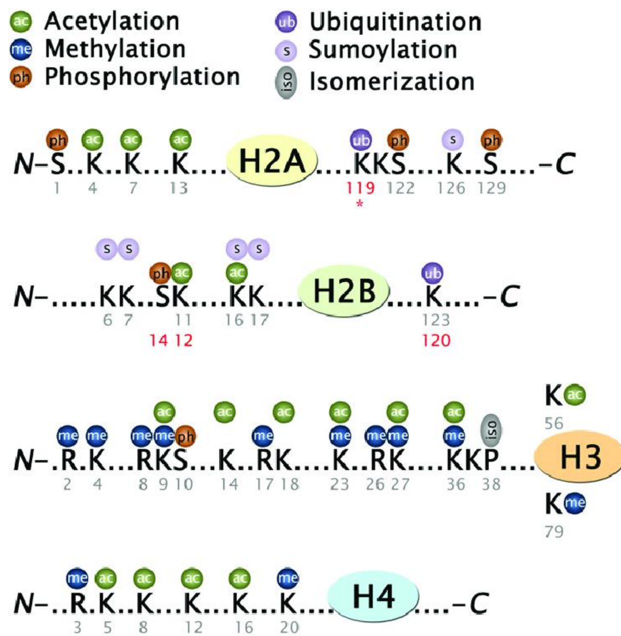

Figure 3. Histone post-translational modifications in yeast. Alternative residue numbers which correspond to mammalian histones are shown in red. *ubiquitination of histone H2A on K119 does not exist in yeast (Smolle and Workman, 2012).

1.1.2.1 Histone acetylation

Histone acetylation is the most common modification on histones. It is catalyzed by a variety of enzymes that transfer the acetyl moiety ($COCH_3$) from acetyl-CoA to the ε-amino group of lysine residues (Fuchs et al., 2008). There are nine histone acetyltransferases (HATs) identified in yeast that act on the four core histones (Kurdistani and Grunstein, 2003). These HAT complexes have various substrate specificities - some recognize only a few sites, while others act on several lysine residues on multiple tails. HATs are usually found as members of larger protein complexes, thereby this substrate specificity is most likely regulated by associations with other complex subunits (Lee and Workman, 2007).

Histone acetylation is generally associated with transcription activation. Acetylation of lysine changes the chemical properties of the amino acid - it converts the basic residue lysine into the neutral charged ε-N-acetyllysine (Fuchs et al., 2008). Studies have shown that acetylation can also reduce the associations between nucleosomes (Lee et al., 1993). Therefore, acetylated nucleosomes are destabilized, promoting both nucleosomal rearrangement by ATP-dependent chromatin remodeling complexes and binding of a number of DNA-binding factors involved in transcription, DNA repair, and many other processes (Nagy and Tora, 2007). Often these

remodeling factors are recruited to chromatin by direct binding to the acetyl mark by bromodomain-containing proteins (Dhalluin et al., 1999).

Whereas histone acetylation generally promotes transcription, there is a competing process of histone deacetylation that acts in a repressive manner (Thiagalingam et al., 2003). There have been found nine histone deacetylase enzymes (HDACs) in yeast (Kurdistani and Grunstein, 2003). HDAC function is associated with repression of remodeling activities and nucleosome stabilization (Fuchs et al., 2008).

Histone acetyltransferases are classified into two specific groups (type A and type B). Type A histone acetyltransferases are nuclear enzymes that can acetylate histones which are packaged into chromatin (Qin and Parthun, 2002). Type B histone acetyltransferases are believed to be involved in the acetylation of newly synthesized histones (Qin and Parthun, 2002). The first type B histone acetyltransferase was isolated from *S. cerevisiae*, and it comprises two subunits, Hat1p and Hat2p (Kleff et al., 1995). In yeast neither deleting the *HAT1* gene nor mutating histone H4 lysine residues 5 and 12 results in cell death (Kleff et al., 1995). The absence of major growth defects associated with these mutations can be explained by a functional redundancy that exists between the acetylation of newly synthesized histone H3 and newly synthesized histone H4 (Qin and Parthun, 2002). The N-terminal tail of histone H3 has five lysine residues (at positions 9, 14, 18, 23, and 27) that are subject to reversible acetylation in yeast. Lysine residues 14 and 23 at histone H3 are crucial for DNA damage repair (Qin and Parthun, 2002). There are four acetylated lysines at the N-terminal tail of histone H4: K5, K8, K12, and K16 (Dion et al., 2005). From these, only K16 is highly associated with a particular regulatory role in yeast - K16 acetylation state controls the extent of silent heterochromatin (Dion et al., 2005). The functions of K5, K8, and K12 are less known.

Histone acetylation/deacetylation has a crucial role in the regulation of many processes taking place in the cell, such as chromatin transcription and dynamics, cell cycle progression, gene silencing, apoptosis, DNA replication, differentiation, DNA repair and nuclear import (Dar and Singh., 2019).

Like histone acetylation, histone lysine **crotonylation** (a newly discovered histone PTM) has been detected from yeast to human and is generally associated with active transcription (Tan et al., 2011). In yeast crotonylation of lysine residues is reversibly catalyzed by acetyltransferases and deacetylases (Andrews et al., 2016). Crotonylation, like acetylation, also occurs on the ε-amino group of lysine, but it differs from acetylation by its planar orientation and four-carbon length (Li et al., 2016). The YEATS domain is an effective reader of histone lysine

crotonylation. YEATS domain of Taf14 protein engages crotonyllysine via a unique π-π-π-stacking mechanism and can recognize the histone mark H3K9ac (Andrews et al., 2016).

1.1.2.2 Histone methylation

Methylation is a significantly more complex modification than acetylation. Several amino acids can be methylated including arginine and lysine residues on histones (Walsh, 2006). Up to two methyl groups can be added to one arginine residue while lysine can accept up to three methyl groups (Bedford and Richard, 2005). In contrast to acetylation, the charge of the amino acid sidechain of lysine or arginine is not changed by methylation (Fuchs et al., 2008). Like acetylation, methylated lysine or arginine residues serve as binding partners for several protein domains including PHD domains and chromodomains (Li et al., 2006).

In budding yeast lysine methylation of histones has been identified at a few locations on the histone H3: H3K4 (Briggs et al., 2001), H3K36 (Strahl et al., 2002) and H3K79 (Feng et al., 2002). Histone methylation plays many diverse roles in transcription. For instance, methylation at H3K4, H3K36, and H3K79 is associated with active transcription (Rando, 2007; Liu et al., 2005). In contrast, methylation at H3K9 in fission yeast and methylation at H3K27 in higher eukaryotes is associated with heterochromatin formation and gene silencing (Nakayama et al., 2001). Arginine methylation has been reported on H3R2 and on H4R3 in yeast (Kirmizis et al., 2007). Over several years, methylation was assumed to be an irreversible process, however, demethylase enzymes have been identified and they act on both methyllysine and methylarginine (Chang et al., 2007; Shi 2007).

1.1.2.3 Histone phosphorylation

One of the most abundant and recognized protein modifications is phosphorylation, however, it is relatively rare on histone molecules (Fuchs et al., 2008). Histone phosphorylation is the transfer of a phosphoryl group to a serine, threonine or tyrosine residue within the N-terminal part of histones (Singh and Gunjan, 2011). This post-translational modification is catalyzed by kinases and phosphatases, which add or remove a phosphoryl group, respectively.

In budding yeast, histone H2AS129 phosphorylation is crucial for the repair of DNA double-stranded breaks (DSB) by involving the INO80 complex (Redon et al., 2003).

1.1.2.4 Other post-translational modifications on histones

There are also other histone post-translational modifications. Histone ubiquitination is a modification, which is regulated by the attachment of ubiquitin (Ub) to the side chain of lysine residue. Ubiquitin is a small regulatory protein of 76 amino acids, and it is widely expressed and highly conserved in eukaryotic organisms (Hayat, 2016). It has been found that in budding yeast *S. cerevisiae* there is only one site of ubiquitination - on histone H2B, at lysine 123, and this modification results in gene activation (Trujillo et al., 2011).

In comparison to ubiquitination modification, less known about histone sumoylation and its function. Sumoylation is the transfer of a "Small Ubiquitin-related MOdifier protein" (SUMO), consisting of approximately 100 amino acids, to lysine residues of target proteins (Vanzan et al., 2017). It is being found that all four core histones can be sumoylated in yeast cells (Nathan et al., 2006). Histone sumoylation generally associates with transcriptional repression, and in fact it antagonizes histone acetylation (Nathan et al., 2006), however, further investigations are needed to clarify the role of sumoylation on cell fate.

1.1.2.5 Protein domains recognizing histone post-translational modifications

As mentioned before, histone modifications have a crucial role in regulation of cellular processes, and therefore affect the cell's fate. Histone modifications, that have been described earlier, are carried out by different protein complexes, such as NuA3, SWI/SNF and INO80. A great number of conserved domains have been found to be essential in establishing various chromatin modifications by these protein complexes (Zhang et al., 2011).

Histone modifications were hypothesized to constitute a "histone code", in which different patterns of histone modifications are "read" by various proteins to produce an effect on gene expression (Strahl and Allis, 2000). Just as there are a great number of PTMs on the histone tails, there are also many protein domains that recognize and bind to specific PTMs on these tails (Lothrop et al, 2013).

The YEATS domain is one of the domains that recognizes chromatin modifications, is involved in transcription and can be found in 59 different eukaryotes ranging from yeasts to humans (Schulze et al., 2009). In budding yeast *S. cerevisiae,* there are three YEATS domain-containing proteins (Taf14, Sas5, and Yaf9), all of which are associated with HAT complexes, chromatin-remodeling complexes or transcription-regulating complexes (Schulze et al., 2009). Sas5, Yaf9 and Taf14 triple null mutants are inviable, suggesting that YEATS domain family

proteins are essential (Zhang et al., 2004). The YEATS domain is present as a single copy in all yeast YEATS-domain-containing proteins and is located at the N-terminus of proteins (Zhang et al., 2011). Moreover, the majority of YEATS-domain-containing proteins do not have any other associated domain, meaning that YEATS domain might have the crucial role in the functions of YEATS-domain-containing proteins (Schulze et al., 2009; Zhang et al., 2011). However, the function and action mechanisms of YEATS domain are not very clear and defined.

The bromodomain is a protein domain of approximately 110 amino acid residues, found in many chromatin-associated proteins and in almost all known histone acetyltransferases (HATs) (Jeanmougin et al., 1997). From yeast genetic studies it has been assumed that bromodomains have an essential role in chromatin remodeling (Brownell and Allis, 1996; Filetici et al., 1998), and it was also found that bromodomains function as acetyl-lysine binding domains (Dhalluin et al., 1999).

Chromodomain is a protein domain of approximately 40–50 amino acid residues found in all eukaryotes (Koonin et al., 1995) that specifically recognizes methylated lysine residues within histones (Sims et al., 2005). It was discovered that the yeast chromodomain protein Chd1 binds directly to di-methyl H3K4 (H3K4me2) (Pray-Grant et al, 2005). *CHD1*-null mutations in yeast are viable but have subtle phenotypes when grown under special conditions, for example, deletion of *CHD1* causes hyper-resistance to a 6-azauracil phenotype (Woodage et al., 1997).

The PHD (plant homeodomain) finger is a protein domain of about 50–80 amino acid residues of diverse sequences that contain a zinc-binding motif that presents in many chromatin-associated proteins and have an important role in in the regulation of gene expression (Aasland et al., 1995). The PHD fingers recognize unmodified or methylated lysine residues on the N-terminal tail of histone H3 (Musselman and Kutateladze, 2011). The majority of those bind to histone H3 tails either methylated at K4 or unmodified in that position (K4me3/2 vs K4me0). (Ali et al., 2014). Several of PHD fingers occur in tandem or next to additional reader domain types (for instance, bromodomains and chromodomains) (Dhar et al., 2012), proposing capabilities of combinatorial interaction.

1.2 Overview of Taf14 protein

Taf14 is one of the three YEATS domain-containing proteins in *S. cerevisiae*. It is a non-essential protein, *TAF14* null mutants are viable, however cells devoid of Taf14 are thermo-

and osmo-sensitive, have aberrant morphology and cytoskeletal defects (Henry et al., 1994), and also have defects in actin organization (Welch et al., 1993). Taf14 protein has also a role in bud morphogenesis, mating projection formation, and might be involved in the negative regulation of chromatin silencing (Welch and Drubin, 1994). Taf14 protein has a length of 244 amino acid and it is a component of several complexes: transcription factors TFIIF and TFIID, the nucleosomal histone H3 acetyltransferase (NuA3), chromatin remodelers INO80 and SWI/SNF (Cairns et al. 1996) (Figure 4). It also interacts with catalytic Sth1 subunit of RSC complex and Gal11 subunit of Mediator complex (Kabani et al., 2005). The role of Taf14 in all of these complexes has remained enigmatic.

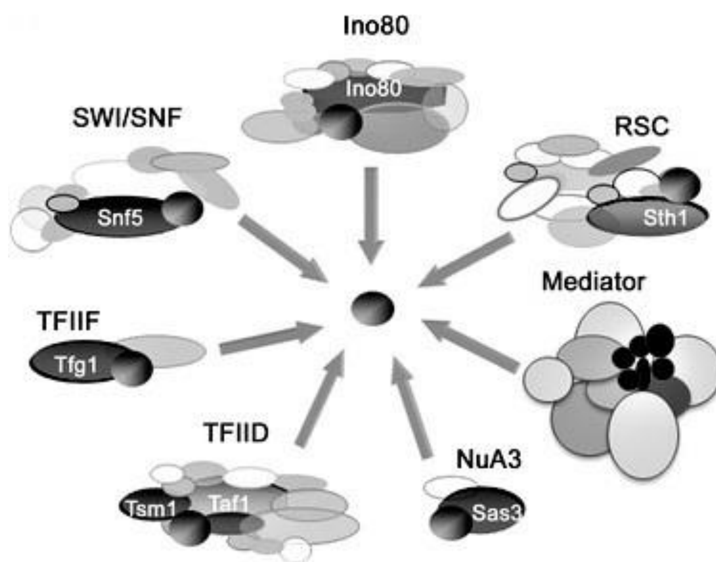

Figure 4. Seven nuclear complexes that contain or bind to Taf14 protein. (Schulze et al., 2010).

Transcription factor TFIID complex

TFIID is a transcription factor complex that is needed for RNAPII-mediated transcription of protein-coding genes and some small nuclear RNAs (Tansey and Herr, 1997). TFIID complex consists of Spt15p (TATA binding protein) and 14 TBP-associated factors (TAFs) (Sanders et al., 2002). Recognition of promoter DNA by the TFIID complex is essential for the formation of the preinitiation complex (PIC) during transcription initiation (Buratowski et al., 1989). The recruitment of TFIID to promoters depends on an upstream activating sequence in the promoter region (Li et al., 2002). A subset of the TAFs (Taf5p, Taf6p, Taf9p, Taf10p, and Taf12p) are subunits of both TFIID and the SAGA transcriptional regulatory complex, that plays role in histone acetylation and in chromatin transcriptional activation or repression (Grant et al., 1998). The studies show that TFIID acts primarily at the TATA-less promoters of stress-repressed

housekeeping genes, introducing about 90% of the yeast genome, whereas SAGA predominates at highly-regulated, stress-responsive TATA box-containing genes, introducing about 10% of the genome (Huisinga and Pugh, 2004). It has been shown that Taf14 interacts with TFIID complex through two binding domains on Taf2 C-terminal tail (Feigerle and Weil, 2016). It was also shown that cells with Taf14-less TFIID complex grew similarly to wt cells, so Taf14 role in TFIID complex remains obscure (Feigerle and Weil, 2016).

Transcription factor TFIIF complex

TFIIF complex participates in both transcription initiation and elongation. TFIIF is one of 3 general factors (TFIIB, TFIIE, and TFIIF) that directly bind RNA polymerase II (Bushnell et al, 1996). TFIIF has a role in open complex formation, promoter escape (Yan et al, 1999), transcription start site selection (Ghazy et al, 2004), stabilization of an RNA–DNA hybrid within RNA polymerase II (Khaperskyy et al, 2008), and in stimulation of elongation by purified RNA polymerase II (Izban and Luse, 1992; Yan et al, 1999). It has been shown *in vitro* that Taf14 has only a stimulatory effect for the functioning of the TFIIF complex, it is also the only non-essential subunit of TFIIF (Henry et al., 1994). So, the role of Taf14 in TFIIF is also undetermined.

NuA3 histone acetyltransferase complex

NuA3 is a Gcn5-independent multi subunit complex of about 400-kDa. It catalyzes the acetylation of histone H3. In *S. cerevisiae* NuA3 complex consists of six subunits, including Taf14 (Howe et al., 2002). No role for Taf14 has been found in functioning of NuA3 complex.

SWI/SNF complex

The first identified ATP-dependent chromatin remodeler was yeast **SWI/SNF** complex (Carlson et al., 1984). The members of the SWI / SNF family are characterized by the presence of an N-terminally located HAS (helicase SANT) domains, which are known for recruiting actin, and C-terminally located bromodomains, characterized to bind to the acetylated lysines of histones (Filippakopoulos and Knapp, 2012). Yeast contains 2 SWI/SNF ATPases, Snf2 and Sth1, that make up 2 complexes - ySWI/SNF and RSC, respectively (Cairns et al., 1996). It has been found that SWI/SNF complexes participate in gene transcriptional regulation (Armstrong and Emerson, 1998). For instance, the cooperative association of ySWI/SNF with histone acetyltransferase complexes results in activation of gene transcription (Roberts and Winston, 1997). However, it has been suggested that the SWI/SNF complexes are also involved in gene repression (Trouche et al., 1997). Additionally, also it has been proposed that SWI/SNF complexes have a role in DNA replication and repair and nucleotide excision repair on

reconstituted nucleosomal substrates *in vitro* (Flanagan and Peterson, 1999). It has been shown that Taf14 is the member of Snf5 module in SWI/SNF complex and it has been proposed that Snf5 module is required for efficient SWI/SNF complex recruitment to chromatin (Sen et al., 2017). However, the precise role of Taf14 protein in the functioning of SWI/SNF complex remains to be determined.

INO80 complex

The INO80 family of remodelers was firstly observed in *S. cerevisiae* - the yeast Ino80 gene product controls inositol-responsive gene expression (Ebbert et al., 1999). In yeast INO80 and SWR1 complexes regulate the genome-wide distribution and dynamics of the histone variant H2A.Z, being able to replace nucleosomal H2A.Z/H2B with free H2A/H2B dimers (Bao and Shen, 2011). In addition, both complexes were reported to take part in telomere regulation and chromosome segregation in yeast (Krogan et al., 2004). The role of Taf14 in Ino80 complex has remained obscure. However, it is known that inactivation of Ino80 complex by knock-out of its catalytic subunit is lethal in *S. cerevisiae* W303 strain background, while knock-out of *TAF14* is not (Shen et al., 2000).

2. EXPERIMENTAL PART

2.1 The aims of the study

1. To analyze whether there are any lysine residues on histone H3 or H4 N-terminal tails, that are important for the survival of *S. cerevisiae* strain lacking Taf14 protein (taf14Δ background).
2. To determine in which complex Taf14 is needed to tolerate mutant histones.

2.2 Materials and methods

2.2.1 Yeast strains, plasmids, media

All materials, yeast strains and plasmids that were used to perform experiments were provided by University of Tartu, Institute of Molecular and Cell Biology.

Yeast strains: All *Saccharomyces cerevisiae* strains were derived from the W303 background. All the used yeast strains are described in supplementary table S1.

Plasmids: Plasmids with HIS3 marker gene: pRS413_H3/H4 (wt), pRS413_H3K9,14,18,23,27R, pRS413_H4K16R, pRS413_H4K5,8,12R, pRS413_H4K5R, pRS413_H4K8R, pRS413_H4K12R.

Plasmids with ADE2 marker gene: pRS412_H3/H4 (wt), pRS412_H4K5R, pRS412_H4K8R, pRS412_H4K12R, pRS412_H4K5,8R, pRS412_H4K5,12R, pRS412_H4K8,12R, pRS412_H4K5,8,12R.

Plates: SC (Synthetic complete) plates (excluding either histidine or adenine) for spot test assay [agar 20 g/l, YNB (Yeast nitrogen base, Applichem) 6,7 g/l, glucose 20 g/l, Drop-out mix 2 g/l].

5FOA plates for selection of the strains of *Saccharomyces cerevisiae* that do not contain the URA3 marker gene [5-FOA 1 mg/ml, YNB 6,7 g/l, agar 2%, glucose 2%, nitrogen bases and amino acids – adenine 20 µg/ml, uracil 20 µg/ml, histidine 40 µg/ml, tryptophan 40 µg/ml, leucine 80 µg/ml, lysine 80 µg/ml]

YPD plates [agar 20 g/l, yeast extract 10 g/l, mycological peptone 20 g/l, glucose 20 g/l]

YPD media [yeast extract 10 g/l, mycological peptone 20 g/l, glucose 20 g/l]

2.2.2 Agarose gel electrophoresis

For analysis of plasmid DNA, agarose gel electrophoresis was used. Agarose was dissolved in 1x TAE buffer (40 mM Tris-acetate; 1 mM EDTA) and ethidium bromide (EtBr) (0,5µg/ml) was added.

2.2.3 Yeast transfection with electroporation.

Electroporation is one of the most efficient methods to perform yeast transfection. Yeast cells were grown overnight in 25 ml YPD medium. Then, they were centrifuged in 50 ml tubes 2000 rpm for 5 minutes. After that yeast cells were resuspended in 9 ml TE buffer + 1 ml 1M LiAC. Then, cells were shacked slowly (100 rpm) for 45 minutes in 30°C. Next, 250 µl 1M DTT was added to the cells and shaking was continued for 15 minutes. Then, cells were placed in 50 ml tube, and 40 ml of mQ water was added. Next, yeast cells were collected by centrifuging 2000 rpm for 5 minutes. After that, cells were washed twice with 50 ml ice-cold mQ water and kept on ice. Then, cells were resuspended in 800 µl 1M sorbitol and collected (centrifuged 5000 rpm for 1 minute). Next, they were resuspended again in approximately 100 µl 1M sorbitol. Then, 5 µl of plasmid DNA (0.1 µg/µl) and 40 µl cells were mixed and placed in electroporation cuvette on ice. Then electroporation procedure was performed (C=25 µF, PC=200 ohm, V=1500 V, 2 mm cuvette): After electroporation, 1ml of 1M sorbitol was added to the cells. After that, cells were placed to the Eppendorf tube and collected with centrifugation for 3 minutes at 3000 rpm. Then most of the sorbitol was removed, and cells were resuspended in remaining 100-200 µl liquid. Finally, yeast cells with plasmid DNA were plated on selection agar plates. Colonies were visible after 3-4 days.

2.2.4 Yeast transfection with LiAc and heat-shock

Yeast transfection with LiAc and heat-shock is one of the transfection methods that is also quick and easy to perform, and it usually chosen for plasmid transfection. Firstly, 3 ml of exponentially growing yeast cells were collected with centrifugation for 1 min at 5000 rpm. Then, the medium was removed, and yeast cells were washed with 500 µl 0.1M LiAC+ TE solution. Cells were collected again with centrifugation for 1 min at 5000 rpm. Next, 40 µl of 0.1M LiAc+ TE and 7 µl of denatured carrier DNA (95°C, 6-7 minutes, cooled quickly on ice) was added. After that, 5 µl plasmid DNA was added, and cells were kept on room temperature (RT) for 5 minutes. Then, 300 µl 0.1M LiAC+TE+ 40% PEG solution was added, mixed with

vortex, and this mixture was kept on RT for 15 minutes. Next, 30 µl of DMSO was added and mixed with vortex. Finally, cells were heat-shocked for 10 minutes on 42°C. In the last step of experiment, cells were collected with centrifugation for 3 minutes at 3000 rpm, supernatant was removed, transfected yeast cells were dissolved in 150 µl sterile water and plated on selection plates.

2.2.5 Yeast spot test assay (yeast growth assay)

Spot test assay is a frequently used method to comparatively analyse growth physiology and viability of yeast cells. This method involves a serial dilution and spotting of yeast cultures on solid media plates – selective synthetic complete (SC) agar plates were being used. First, 400 µl of sterile water was put into Eppendorf tube, and a small amount of yeast cells was added with a sterile toothpick. This mixture was vortexed and sonicated for 5 seconds. Then culture density was measured with Z2 Cell and Particle Counter (Beckman Coulter). The dilution of cells was made so, that in the first dilution there were 10000 cells/µl, then 10-fold serial dilutions of cell suspension were made. For that 10 µl of first dilution was added to 90 µl of water. It was repeated until five dilutions were made (so that in the last one there was 1 cell/µl). Before taking cells and after adding them to a dilution, the tubes were vortexed. Finally, 5 µl of each dilution was spotted onto plates. Plates were incubated at 30 °C for 2-5 days until growth of colonies was visible.

2.2.6 Plasmid exchange system

For analysis of mutant histones, plasmid exchange system is used. In this system both chromosomal copies of the histones H3 and H4 are deleted from the genome and wild-type H3 and H4 histones are expressed from a plasmid with an URA3 marker gene. After transfection with another plasmid expressing different mutant histones and containing either HIS3 or ADE2 marker gene, cells are plated to the 5FOA selection plate. As the enzyme Ura3 converts 5FOA into toxic metabolite 5-fluoro-uracil, only the cells that have lost the initial plasmid expressing wt histones, can survive. This allows easy and fast analysis of several different mutants.

2.3 Results

2.3.1 Transfection of *taf14Δ* yeast strain with plasmids expressing different mutant histones

To know whether there are any histone modifications that become important for survival of yeast cells without Taf14 protein (taf14Δ background), AKY1707 yeast strain was used. In this strain *TAF14* coding region, as well as two copies of histones H3 and H4 are deleted from the genome and H3 and H4 histones are expressed from the plasmid with *URA3* marker gene. This plasmid can be easily replaced with plasmid encoding different mutant histones. For that yeast strain AKY1707 was transfected with electroporation method with different plasmids. **pRS413_H3H4 (wt)**, was used as a positive control. In this case, plasmid with *URA3* marker gene was replaced with plasmid with HIS3 marker, but both histones were still wt. For studying the importance of H3 acetylations in *taf14Δ* background, a fivefold mutant of H3 **pRS413_H3K9,14,18,23,27R** was chosen. In this mutant all five acetylatable lysines in H3 N-terminal tail have been mutated to arginine, so they can no longer be modified. This mutant was chosen, because if cells can grow with this mutant, any single modification is also not important for the survival of the *taf14Δ* yeast strain. For analyzing the importance of H4 acetylations in *taf14Δ* background, two plasmids, **pRS413_H4K16R** and **pRS413_H4K5,8,12R** were used. These two plasmids cover all the acetylatable lysines in H4 N-terminal tail. All the transfections, except the one with **pRS413_H4K5,8,12R** plasmids gave colonies on -HIS plates. The colonies from transfection with either **pRS413_H3H4, pRS413_H3K9,14,18,23,27R** or **pRS413_H4K16R** were streaked out in parallel to -HIS and to 5FOA plates (Figure 5). All the yeast strains, where original plasmid was replaced with different transfected pRS413 plasmids, were able to grow on 5FOA plates (Figure 5). Although yeast strain with a fivefold mutant of histone H3 grew slower than the other two strains, mutating all acetylatable lysines in histone H3 N-terminal tail was not lethal for *taf14Δ* strain.

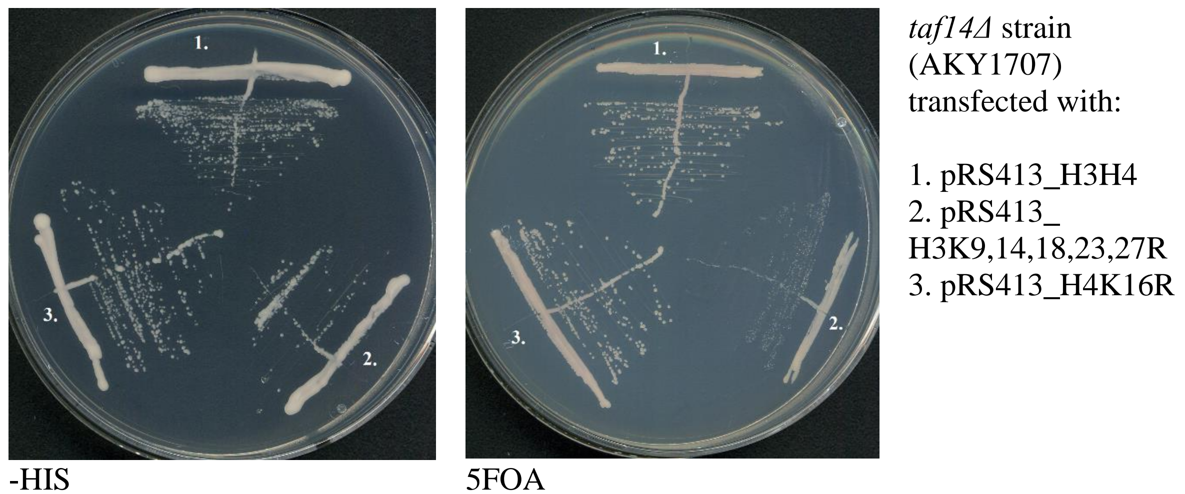

Figure 5. Growth of the *taf14Δ* strain (AKY1707) transfected with plasmids expressing different mutant histones. The left picture shows the growth of the colonies from different transfections on -HIS plates. The right picture shows the growth of these colonies on the 5FOA selection plate.

As there were no colonies on -HIS plate in the case of transfection with **pRS413_H4K5,8,12R** plasmid, the quality of plasmid DNA was checked with agarose gel electrophoresis (Figure 6).

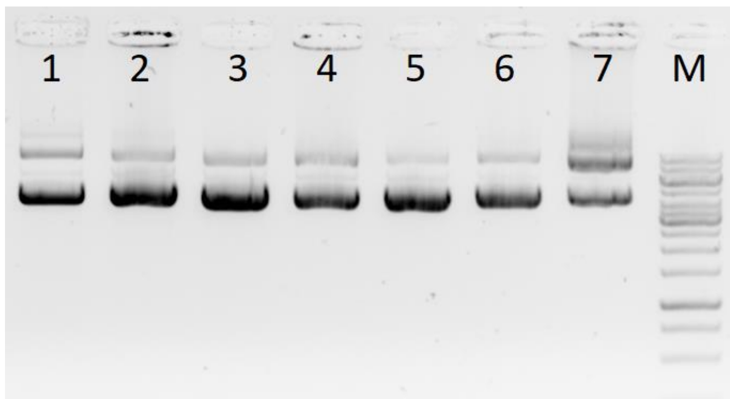

Figure 6. The analysis of different pRS413 plasmids. Lane 1) pRS413_H3H4 2) pRS413_H3K9,14,18,23,27R 3) pRS413_H4K5R 4) pRS413_H48R 5) pRS413_H4K12R 6) pRS413_H4K16R and 7) pRS413_H4K5,8,12R. Lane M is a DNA size marker (GeneRuler 1 kb Ladder, Thermo Scientific)

Compared to the other plasmids, there was slightly less circular form of **pRS413_H4K5,8,12R** plasmid. As for the transfection plasmid DNA is taken in excess, there should have been enough correct form of plasmid DNA for the transfection. Thereby the transfection with

pRS413_H4K5,8,12R was repeated. In addition, three plasmids with single mutations (**pRS413_H4K5R, pRS413_H4K8R** and **pRS413_H4K12R)** were also included. Again, all the transfections, except the one with **pRS413_H4K5,8,12R** plasmids gave colonies on -HIS plates. The colonies from transfection with either **pRS413_H4K5, pRS413_H4K8R** or **pRS413_H4K12R** were streaked out in parallel to -HIS and to 5FOA plates (Figure 7). Although, the yeast strains with single mutations in H4 N-terminal tail, were all able to grow on 5FOA plates, the replacement of K12 residue to arginine, has the strongest growth defect (Figure 7).

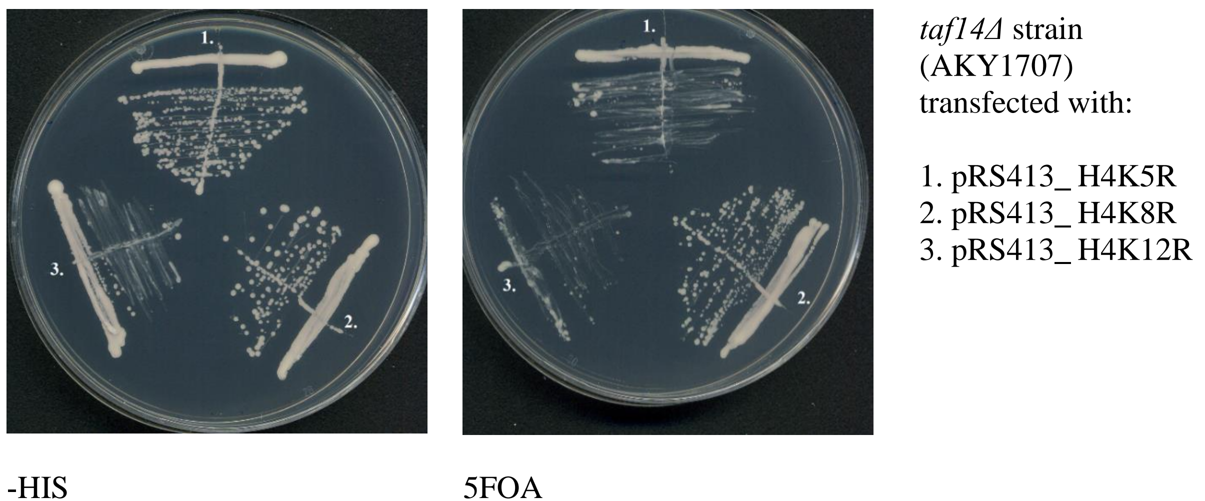

Figure 7. Growth of the *taf14Δ* strain (AKY1707) transfected with plasmids expressing different mutant H4 histones. The left picture shows the growth of the colonies from different transfections on -HIS plates. The right picture shows the growth of these colonies on the 5FOA selection plate.

As the transfection with triple mutant **pRS413_H4K5,8,12R** plasmid failed again, the control transfection to yeast strain expressing wt Taf14 protein and containing histone plasmid exchange system (AKY202) was made. AKY202 strain was transfected with plasmids **pRS413_H3H4, pRS413_H4K12R** and **pRS413_H4K5,8,12R**. Now all the transfections gave colonies on -HIS plates. The colonies from all the transfections were streaked out in parallel to -HIS and to 5FOA plates (Figure 8). All yeast strains with replaced histone plasmids were able to grow on 5FOA plates (Figure 8).

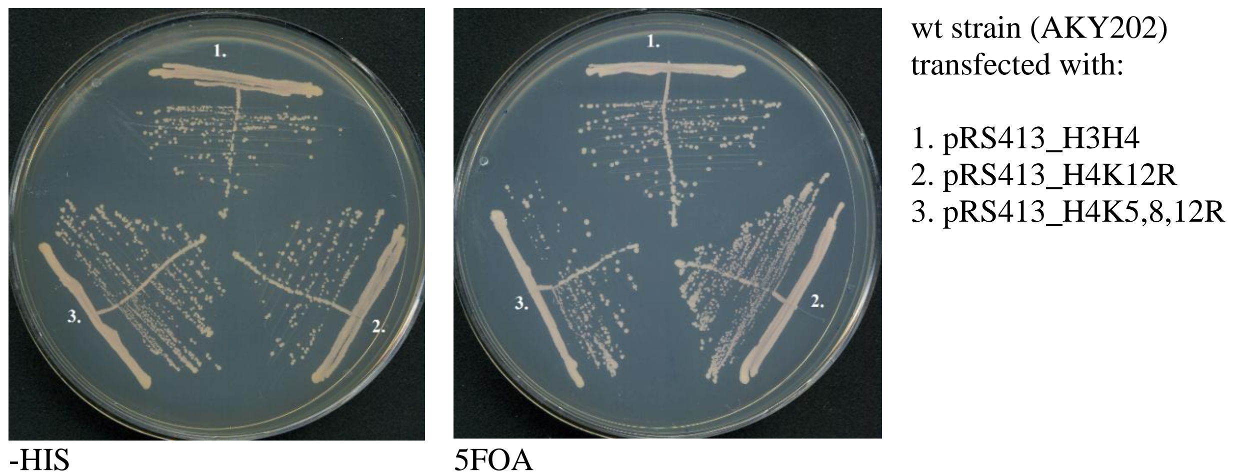

Figure 8. Growth of the wt strain (AKY202) transfected with plasmids expressing different mutant H4 histones. The left picture shows the growth of the colonies from different transfections on -HIS plates. The right picture shows the growth of these colonies on the 5FOA selection plate.

This result shows that for *taf14Δ* yeast strain expression of mutant histone **H4K5,8,12R** is already lethal, even when plasmid with wt H4 is also present. At the same time, cells expressing wt Taf14 protein, are viable with just mutant **H4K5,8,12R** histones.

2.3.2 Transfection of *taf14Δ* yeast strain with plasmids expressing different double mutant H4 histones

As the *taf14Δ* cells could tolerate all single mutations in H4 histone, but triple mutation was lethal, different double mutation combinations were tested next. AKY1707 strain was transfected using LiAc and heat-shock method with the following plasmids: **pRS412_H3/H4, pRS412_H4K12R, pRS412_H4K5,8R, pRS412_H4K5,12R** and **pRS412_H4K8,12R** (in these plasmids *ADE2* marker gene is used instead of *HIS3*). From these transfections, only first three (**H3/H4**, **H4K12R** and **pRS412_H4K5,8R**) gave colonies on -ADE plates. The colonies from these transfections were streaked out in parallel to -ADE and to 5FOA plates (Figure 9). While *taf14Δ* cells expressing either wt H4 or H4K12R histones were alive, cells expressing H4K5,8R double mutant, failed to grow on the 5FOA selection plate (Figure 9). This result shows that although *taf14Δ* cells can tolerate H4K5,8R mutant when wt H4 histone is also present, substitution of wt H4 histone with any double mutant H4 histone is lethal.

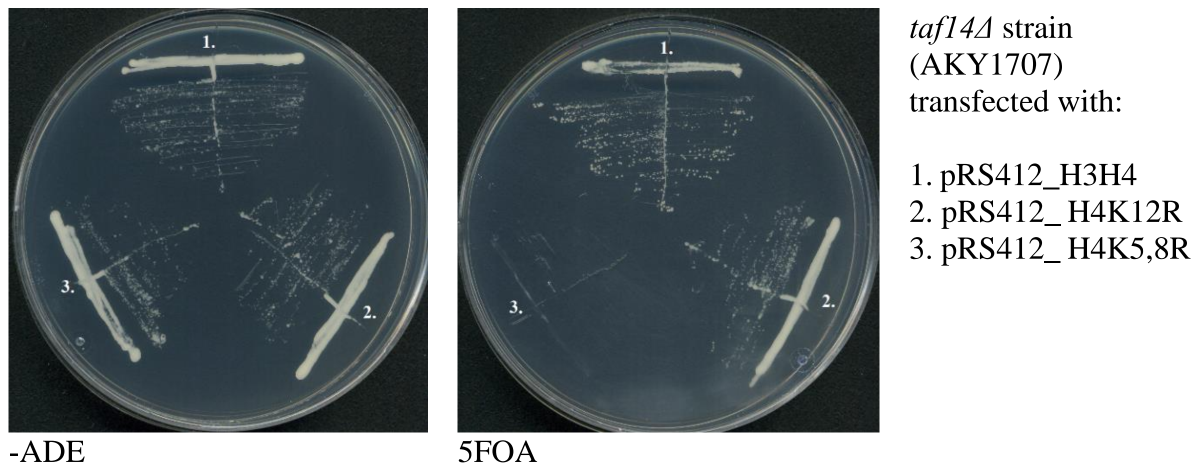

Figure 9. Growth of the *taf14Δ* strain (AKY1707) transfected with plasmids expressing different mutant H4 histones. The left picture shows the growth of the colonies from different transfections on -ADE plates. The right picture shows the growth of these colonies on the 5FOA selection plate.

2.3.3 Growth analysis of *taf14Δ* yeast strain expressing different mutant H4 histones

To analyze the growth of *taf14Δ* cells expressing different H4 mutant histones, transfections with plasmids **pRS412_H4K5R** and **pRS412_H4K8R,** using LiAc and heat-shock method, were also made. After substitution of plasmid expressing wt H4 with plasmids expressing mutant H4 histones, spot test analysis was used to compare the growth of different strains (Figure 10). While the growth of yeast strains with **wt H4**, **H4K5R** and **H4K8R** histones were comparable, the growth of *taf14Δ* strain with **H4K12R** histones was disturbed.

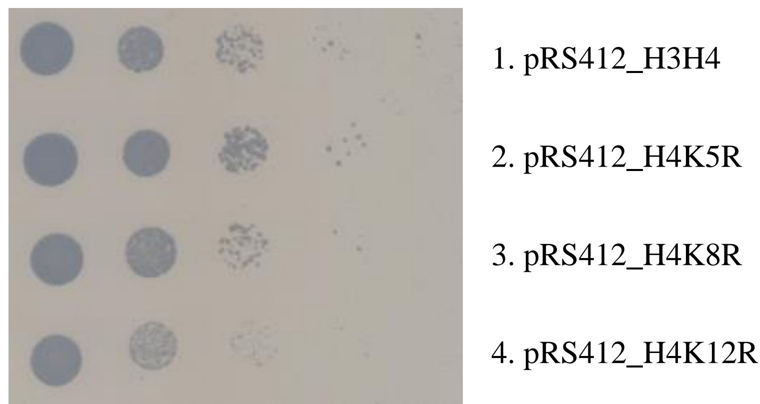

Figure 10. Spot test assay for growth analysis of *taf14Δ* strain with different H4 mutants. 10-fold serial dilutions of *taf14Δ* cells expressing different H4 mutant histones from pRS412 plasmids were spotted onto SC-ADE plates and grown at 30°C for three days. 1. pRS412_H3H4, 2. pRS412_H4K5R, 3. pRS412_H4K8R, 4. pRS412_H4K12R.

2.3.4 Transfection of *taf2ΔC* yeast strain with plasmids expressing different mutant H4 histones

As the Taf14 protein is the member of several chromatin related complexes, the next question was, in which complexes it is needed to tolerate mutant H4 histones. To answer that, the first complex under investigation was TFIID, so transfections to AKY1997 strain were made. In this strain Taf2 protein has a truncation in C-terminus (*taf2ΔC*) + plasmid exchange system for histones. It is known from the literature that Taf14 interacts with TFIID complex through the binding sites on this Taf2 "tail". So, in this mutant strain Taf14 protein is no longer in TFIID complex. AKY1997 strain was transfected with **pRS412_H3H4, pRS412_H4K12R, pRS412_H4K5,8R, pRS412_H4K5,12R, pRS412_H4K8,12R** and **pRS412_H4K5,8,12R** plasmids, using LiAc and heat-shock method, All the transfections gave colonies on -ADE selective plates. The colonies were streaked out in parallel to -ADE and to 5FOA plates (Figure 11). All the yeast strains with replaced histone plasmids were able to grow on 5FOA plates (Figure 11).

Figure 11. Growth of the *taf2ΔC* strain (AKY1997) transfected with plasmids expressing different mutant H4 histones. The left picture shows the growth of the colonies from different transfections on -ADE plates. The right picture shows the growth of these colonies on the 5FOA selection plate.

As the yeast strain, where TFIID complex lacks Taf14 protein grows well with all the tested mutant H4 histones, it is not the TFIID complex, where Taf14 is needed for tolerating mutant H4 histones.

2.3.5 Transfection of *sas3Δ* yeast strain with plasmids expressing different mutant H4 histones

Next the transfections to AKY2069 strain were made (this strain lacks the catalytic Sas3 subunit of NuA3 complex + plasmid exchange system). AKY2069 strain was transfected with **pRS412_H3/H4, pRS412_H4K12R, pRS412_H4K5,8R, pRS412_H4K5,12R, pRS412_H4K8,12R** and **pRS412_H4K5,8,12R** plasmids**,** using LiAc and heat-shock method. All the transfections gave colonies on -ADE selective plates. The colonies were streaked out in parallel to -ADE and to 5FOA plates (Figure 12). All the yeast strains with replaced histone plasmids were able to grow on 5FOA plates (Figure 12).

sas3Δ strain
(AKY2069)
transfected with:

1. pRS412_H3H4
2. pRS412_H4K12R
3. pRS412_H4K5,8R
4. pRS412_H4K5,12R
5. pRS412_H4K8,12R
6. pRS412_H4K5,8,12R

-ADE 5FOA

Figure 12. Growth of the *sas3Δ* strain (AKY2069) transfected with plasmids expressing different mutant H4 histones. The left picture shows the growth of the colonies from different transfections on -ADE plates. The right picture shows the growth of these colonies on the 5FOA selection plate.

As the yeast strain, where NuA3 complex lacks its catalytic subunit and therefore this complex is non-functional, grows well with all the tested mutant H4 histones, it is not the NuA3 complex, where Taf14 is needed for tolerating mutant H4 histones.

2.4 Discussion

The aim of this study was to analyze the importance of different histone acetylations for the survival of the yeast strain without Taf14 protein. As the previous experiments from our group had discovered, the deletion of *YAF9* is lethal in *taf14Δ* background. Yaf9 protein together with Taf14 is one of the three YEATS domain-containing protein in *S. cerevisiae*. As the targets of YEATS domains are considered to be different acetylated/crotonylated lysine residues in histones, the hypothesis was, that it is possible that some histone acetylations are critical for the survival of the *taf14Δ* yeast strain. To test this hypothesis, *taf14Δ* yeast strain with plasmid exchange system for histones was used. In this strain it is possible to replace the original plasmid expressing wt H3 and H4 histones with plasmid expressing histones with different mutations. For the first experiment three different mutants were chosen to cover all the acetylatable lysine residues in H3 and H4 N-terminal tails: for H3 histone five-fold mutant H3K9,14,18,23,27R and for H4 histone H4K5,8,12R and H4K16R mutants were used. To our great surprise, the five-fold mutation of H3 N-terminal tail was not lethal in *taf14Δ* strain, as *in vitro* experiments have proposed that the main target of Yaf9 YEATS domain is acetylated H3K27 (Klein et al., 2018). It was even more unexpected that the triple mutant H4K5,8,12R, and double mutants H4K5,12R and H4K8,12R in the next experiments, were already lethal in cells expressing also wt H4 histones. It suggests that in *taf14Δ* strain some very fundamental chromatin related process is disturbed, but further experiments are needed to clarify which one.

As Taf14 is the member of several different chromatin-related complexes, the next question of the study was, in which complex Taf14 is needed for the cells to survive with mutant H4 histones. There are two ways to answer that question, either to use some mutant strain, where Taf14 is specifically missing from some complex or to use the mutant strain, where the whole Taf14-containing complex is non-functional. The example of the first option is the tested AKY1997 strain, where Taf2 protein has a truncation in C-terminus. As the C-terminal part of Taf2 contains binding sites for Taf14 protein, the lack of it results in yeast strain, where TFIID complex does not contain Taf14 protein. As the survival of AKY1997 strain was not affected, when wt H4 histones were replaced with different H4 mutants (even with the triple H4K5,8,12R mutant), it can be concluded that it is not TFIID complex, where Taf14 protein is needed to survive with mutant H4 histones. The example of the second option is AKY2069 strain, where the coding region of *SAS3*, the catalytic subunit of NuA3 complex, is deleted from the genome, therefore making the NuA3 complex non-functional. When wt H4 histones were replaced with different H4 mutants in AKY2069 strain, it did not affect the survival of the strain, leading to

the conclusion that it is not NuA3 complex either, where Taf14 protein is needed to make it possible for the cells to survive with mutant H4 histones.

For further research it would be interesting to investigate, whether it is the YEATS domain of Taf14, that is important for the survival of the yeast strains with mutant H4 histones. Alternatively, it could be the C-terminal part of Taf14, that is responsible for its interaction of with different complexes, that is needed for tolerating mutant H4 histones.

SUMMARY

The budding yeast *S. cerevisiae* is a great model system for studying eukaryotic molecular biology. In yeast Taf14 protein is associated with several complexes, including transcription factors TFIID and TFIIF, the chromatin remodeling complexes SWI/SNF and INO80, and the histone acetyl transferase NuA3. *TAF14* is a non-essential gene, however, deletion of *TAF14* coding region causes reduced growth rate and sensitivity to DNA damaging agents.

The experiments showed, that for the survival of the budding yeast *S. cerevisiae* strain lacking Taf14 protein (taf14Δ background, strain AKY1707) acetylation of the histone H3 N-terminal tail is not essential. When wt histone H3 was replaced with H3 mutant, where all the acetylatable lysines in N-terminal tail were mutated to arginines (H3K9,14,18,23,27R), *taf14Δ* strain had a growth defect, but cells were alive. However, when two or more acetylatable lysines in H4 histone were mutated to arginines (either H4K5, K8 or K12), it was lethal for *taf14Δ* strain. Moreover, double mutation of either H4K5,12R or H4K8,12R in *taf14Δ* strain was already lethal in combination with wt H4 histone.

The second aim of the study was to determine, in which complex the function of Taf14 is needed, for the cells to survive with the mutant histones. To answer that question, survival of yeast strain, where Taf14 is no longer in TFIID complex (AKY1997 strain, Taf2 lacks the C-terminus, needed for Taf14 interaction with TFIID) was tested with different H4 histone mutants. Replacement of wt H4 histone with different H4 mutants, even with the triple mutant H4K5,8,12R, resulted in the cell growth. This result confirms, that it is not TFIID complex, where Taf14 is needed for tolerating mutant histones. The next complex under investigation was NuA3 acetyltransferase. Survival of the strain, where the Sas3 catalytic subunit of NuA3 complex is missing (AKY2069, *sas3Δ*), was tested with mutant H4 histones. Again, replacement of wt H4 histone with different H4 mutants, resulted in the cell growth. Therefore, it is not NuA3 complex either, where Taf14 is needed for tolerating mutant histones.

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Supplementary Table S1. Yeast strains

Strain	Genotype	Source
AKY202	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX YCp50:hht2-hhf2 (URA3)*	Laboratory collection
AKY1707	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 YCp50:hht2-hhf2 (URA3)*	Laboratory collection
AKY1967	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS413_H3H4 (HIS3)*	This study
AKY1968	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS413_H3K9,14,18,23,27R (HIS3)*	This study
AKY1969	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS413_H4K16R (HIS3)*	This study
AKY1981	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS413_H4K5R (HIS3)*	This study
AKY1982	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS413_H4K8R (HIS3)*	This study
AKY1983	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS413_H4K12R (HIS3)*	This study
AKY1984	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX pRS413_H3H4 (HIS3)*	This study
AKY1985	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX pRS413_H4K12R (HIS3)*	This study
AKY1986	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX pRS413_H4K5,8,12R (HIS3)*	This study
AKY1997	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 YCp50:hht2-hhf2 (URA3)*	Laboratory collection
AKY2035	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS412_H3H4 (ADE2)*	This study
AKY2036	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS412_H4K12R (ADE2)*	This study
AKY2047	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS412_H4K5R (ADE2)*	This study

AKY2048	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf14::natMX6 pRS412_H4K8R (ADE2)*	This study
AKY2059	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 pRS412_H3H4 (ADE2)*	This study
AKY2060	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 pRS412_H4K12R (ADE2)*	This study
AKY2061	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 pRS412_H4K5,8R (ADE2)*	This study
AKY2064	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 pRS412_H4K5,12R (ADE2)*	This study
AKY2065	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 pRS412_H4K8,12R (ADE2)*	This study
AKY2066	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX taf2ΔC::HIS3 pRS412_H4K5,8,12R (ADE2)*	This study
AKY2069	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 YCp50:hht2-hhf2 (URA3)*	Laboratory collection
AKY2077	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 pRS412_H3H4 (ADE2)*	This study
AKY2078	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 pRS412_H4K12R (ADE2)*	This study
AKY2079	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 pRS412_H4K5,8R (ADE2)*	This study
AKY2080	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 pRS412_H4K5,12R (ADE2)*	This study
AKY2081	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 pRS412_H4K8,12R (ADE2)*	This study
AKY2082	*MAT A, hht1-hhf1::LEU2 hht2-hhf2::kanMX sas3::natMX6 pRS412_H4K5,8,12R (ADE2)*	This study

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