Identification of novel histone marks required for the transcriptional stress response

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“impresionante”

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SUMMARY

In nature, ecosystems are in constant change. Temperature fluctuations, water and nutrient availability, increases of osmolytes and toxic agents are some common changes organisms have to cope with to survive and propagate. In this variable context, evolution has equipped budding yeast (*Saccharomyces cerevisiae*) with a wide catalogue of molecular mechanisms designed to neutralize the damaging effects of environmental insults or stresses. One common strategy is to modulate gene expression to generate a new transcriptional program to confront environmental stresses.

Cells tightly control their transcriptional program. A critical step to control gene expression is the regulation of nucleosome and histone dynamics. Histone post-translational modifications are key elements that modulate histone interaction with DNA and other proteins and thus transcription. They exert its effect on transcription depending on which histone residues are modified, the type of modifications and the interplay with other proteins. Despite the high number of histone post-translational modifications described and their known underlying mechanisms, histone dynamic regulation is far from being completely understood.

To evaluate the specific histone residues required for properly inducing transcription upon heat and osmotic stress, we performed a high throughput genetic screening. By assaying a complete library of histone mutants, we were able to extract general conclusions regarding the nature, localization and properties of the histone residues required upon stress. We screened three different stress-activated promoters to establish the specific residues and regions necessary for either heat, osmotic stress or both.

Based on the screening, we selected the histone residues H4 serine 47 (H4-S47) and threonine 30 (H4-T30) for further characterization. We measured gene expression on mutants for both residues with different techniques (fluorescent reporters, northern blot and RNA sequencing). Additionally, we identified and characterized the kinases that modify both residues upon stress. Thus, we described new mechanisms modulating yeast stress-induced transcription through the histone residues H4-S47 and H4-T30.

Following, we characterized and selected from the screening additional histone residues for further analysis, as interesting new candidates to have a role in stress-mediated transcription regulation in yeast.

In summary, results presented in this thesis provide novel insights into histone modifications relevant to respond to heat and osmotic stress.

A la natura, els ecosistemes canvien constantment. Les fluctuacions de temperatura, la disponibilitat d'aigua i de nutrients i l'augment d'osmòlits i d'agents tòxics són alguns dels canvis habituals als què els organismes han de fer front per sobreviure i propagar-se. En aquest context variable, l'evolució ha proporcionat al llevat (*Saccharomyces cerevisiae*) un ampli catàleg de mecanismes moleculars dissenyats per neutralitzar els efectes perjudicials dels estressos ambientals. Una estratègia comuna és modular l'expressió gènica per generar un nou programa de transcripció adequat per fer front a l'estrès ambiental.

Les cèl·lules exerceixen un ferm control sobre el seu programa transcripcional. Un pas crític per controlar l'expressió gènica és la regulació de les dinàmiques de les histones i dels nucleosomes. Les modificacions post-traduccionals d'histones són uns elements clau que modulen la interacció de les histones amb el DNA i altres proteïnes i, en conseqüència, la transcripció. Aquestes exerceixen el seu efecte sobre la transcripció depenen dels residus d'histona modificats, del tipus de modificacions i de les interaccions amb altres proteïnes. Malgrat l'elevat nombre de modificacions post-traduccionals d'histones descrites i els seus mecanismes subjacents coneguts, la regulació dinàmica de les histones encara està lluny de ser completament entesa.

Per avaluar els residus d'histones específicament necessaris per a induir correctament la transcripció en resposta a un estrès tèrmic i osmòtic, hem dut a terme un cribratge genètic a gran escala. Mitjançant l'assaig d'una llibreria completa de mutants d'histones, hem pogut extreure conclusions generals respecte a la naturalesa,

localització i propietats dels residus d'histones necessaris en resposta a estrès. Hem estudiat tres promotors diferents activats per estrès per establir els residus i les regions necessàries específicament per a l'estrès tèrmic, l'estrès osmòtic o ambdós.

Basant-nos en el cribratge, hem seleccionat els residus serina 47 i treonina 30 de la histona H4 (H4-S47 i H4-T30) per a una caracterització més detallada. Mitjançant l'avaluació de l'expressió gènica amb diferents tècniques (reporters fluorescents, northern blot i seqüenciació de RNA), hem caracteritzat els efectes sobre la resposta transcripcional d'estrès de les mutacions en tots dos residus. A més, hem identificat i caracteritzat les quinases que modifiquen els dos residus en resposta a estrès. D'aquesta manera, hem descrit nous mecanismes que modulen la transcripció induïda per l'estrès en llevats, a través dels residus H4-S47 i H4-T30.

Finalment, hem caracteritzat i seleccionat residus d'histona addicionals, com a nous candidats interesants per tenir un paper en la regulació de la transcripció durant estrès en llevat.

En resum, els resultats presentats en aquesta tesi proporcionen nova informació sobre modificacions d'histona rellevants per respondre a l'estrès tèrmic i osmòtic.

PREFACE

Upon environmental stresses such as osmotic or heat shock, the yeast *Saccharomyces cerevisiae* activates several intracellular signaling pathways in order to survive and adapt. Upon these conditions, stressed cells drastically reconfigure their gene expression program, up-regulating a set of stress-responsive genes while down-regulating the stress-repressed ones, aiming to better cope with the extracellular insult (Gasch *et al.*, 2000; Nadal-Ribelles *et al.*, 2012). This transcriptional response is extensively regulated by several mechanisms, from messenger RNA (mRNA) biogenesis and chromatin remodeling, to mRNA modification, export and translation (de Nadal, Ammerer and Posas, 2011).

In order to generate a transcriptional outburst, nucleosomes need to be removed from stress-responsive genes allowing RNA-polymerase machinery to bind to chromatin and initiate transcription. Histone regulation is often mediated by post-translational modifications (PTMs). Since early 60s, when histone acetylation and methylation were first detected (Allfrey, Faulkner and Mirsky, 1964), hundreds of new modifications have been described on a wide variety of conformations and residues (Zhao and Garcia, 2015). Such modifications are highly dynamic and establish a complex network of crosstalk interactions that ultimately define the chromatin/transcriptional state of the cell. In the past few years, several histone PTMs and their associated mechanisms have been described in a wide range of conditions. However, the map of histone modifications is far from complete, especially regarding histone modifications in the context of the transcriptional stress response.

In this PhD thesis, we aimed to identify the histone residues required for properly inducing transcription upon stress. We performed a high throughput screening measuring the activity of a fluorescent reporter under the control of promoters activated by heat and/or osmotic stress. We used a complete library of histone mutant strains in each histone residue. Using this approach, we evaluated the implication of each individual residue upon osmotic and heat stress-induced transcription.

From this work, we generated a catalog of histone residues required for a proper transcriptional reprograming upon stress. We demonstrated that such residues depend on the particular promoter and stress studied. We also defined novel histone regions required for stress-induced transcriptional response.

From the screening, we selected and validated some interesting candidates for further analysis: the histone H4 serine 47 and threonine 30 (H4-S47 and the H4-T30). We demonstrated their relevance for properly modulating stress driven transcription upon osmotic and heat stress respectively. Additionally, we identified and characterized the kinases Cla4 and Ste20 that target H4-S47 and the kinase Ste11 for H4-T30.

Our research adds new valuable information to the yeast histone regulation. Despite our contribution, more research is needed to fully complete and understand such a complex biological response. However, our screening represents a solid starting point to characterize novel histone PTMs involved on the regulation of the transcriptional stress response.

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INTRODUCTION

1. YEAST RESPONSE TO OSMOTIC AND HEAT STRESS

In nature, *Saccharomyces cerevisiae* are saprophytes that grow in plant or animal tissues. Because these environments are highly variable, yeast cells have evolved a cohort of complex and specific mechanisms to overcome extracellular changes and to maintain their internal homeostasis. These sudden changes such as fluctuations in temperature and osmolarity or increases of oxidizing agents, radiation, toxic chemicals and others are commonly known as environmental stresses. Any of these alterations is sensed and the signal is transduced intracellularly by signaling-transduction pathways that coordinate the cellular response. Part of this response involves the activation and accumulation of stress-responsive molecules, the remodeling of the gene expression program and the repression of unnecessary functions such as protein biogenesis. All these dramatic intracellular changes aim to adapt cells to the new extracellular conditions and guarantee their survival (Gasch *et al.*, 2000; Gasch and Werner-Washburne, 2002; Hohmann, 2002; Richter, Haslbeck and Buchner, 2010; Morano, Grant and Moye-Rowley, 2012).

In the laboratory, the conditions of yeast growth are tightly controlled. The optimal temperature is maintained between 25 and 30ºC; osmolarity between cells and media is balanced; and other parameters such as pH and nutrient content are also optimized. To study stress responses, it is necessary to promote their induction by stressing yeast cells, for instance by switching the growth temperature to 39ºC or by adding high concentrations of salt or any other osmolyte to the media.

1.1 Osmotic stress signaling

In yeast's natural niche, one common stress is the change in extracellular osmolarity. Because of the permeability of the plasma membrane, an extracellular increase of the ionic force generates a flux of intracellular water out of the cell. In addition to the obvious damaging effects of losing intracellular free water, there is also a dangerous reduction in cell volume and membrane pressure (Mager and Varela, 1993). All these phenomena affect the performance of all essential biochemical reactions inside the cell, putting cell viability at risk.

In the event of osmotic stress, the high osmolarity glycerol (HOG) pathway and the mitogen-activated protein kinase (MAPK) Hog1 sense and coordinate the response. The HOG pathway senses osmotic stress through two distinct but partially redundant mechanisms: the Sln1 and the Sho1 branches (Fig 1) (Brewster *et al.*, 1993; Saito and Posas, 2012). The Sln1 branch is composed of the osmo-sensor histidine kinase Sln1. In non-stress conditions, Sln1 is active and phosphorylates Ypd1, which in turn transfers the phosphate to Ssk1 (Posas *et al.*, 1996). Phosphorylated Ssk1, is not able to activate the down-stream MAPK kinase kinases (MAPKKK or MAP3K) Ssk2/Ssk22 preventing the activation of the rest of the pathway. Upon osmotic stress, the loss of membrane turgor inhibits Sln1, consequently unphosphorylated Ssk1 is now able to activate Ssk2 and Ssk22 (Posas *et al.*, 1996; Posas and Saito, 1998).

On the other hand, the Sho1 branch initiates with the activation of the GTPase Cdc42 by the extracellular sensors Msb2

and Hkr1 (Maeda, Takekawa and Saito, 1995; Yang *et al.*, 2009). At the same time, Sho1 and Opy2 anchor the downstream components of the pathway to the membrane in close proximity between each other (Reiser, Salah and Ammerer, 2000; Tatebayashi *et al.*, 2006). Cdc42 activates MAPKKK kinases (MAPKKKK or MAP4K) Ste20 and Cla4 that in turn phosphorylate the MAPKKK Ste11 (Raitt, 2000; Van Drogen *et al.*, 2000; Lamson, Winters and Pryciak, 2002).

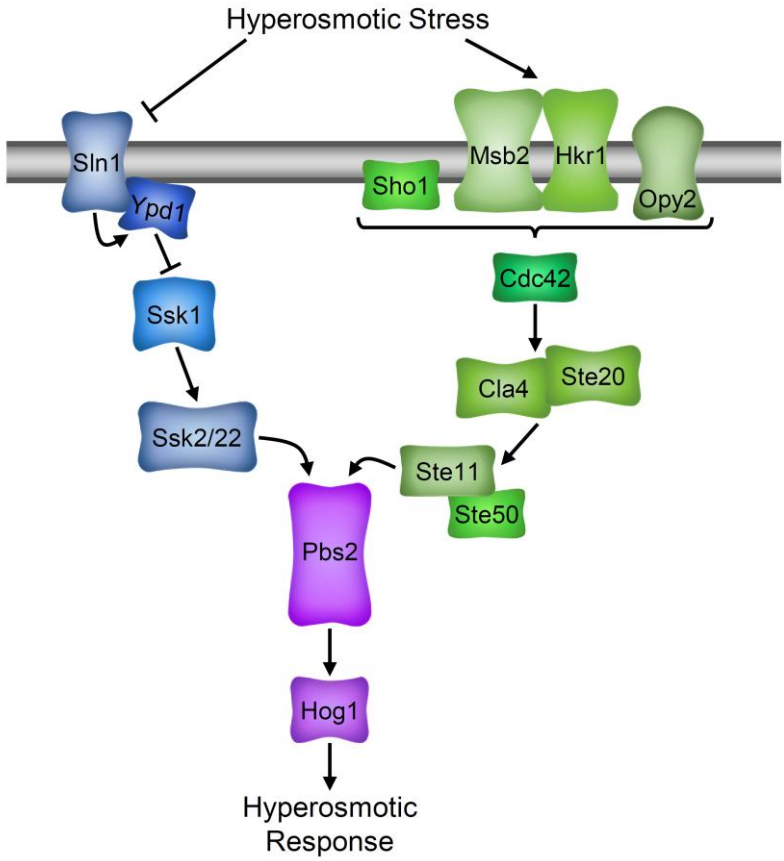


Figure 1. Schematic outline of the HOG pathway.
Osmotic stress activates the HOG pathway through two distinct osmo-sensing branches Sln1 and Sho1. Both branches converge through Ssk2/22 and Ste11 MAPKKK respectively that activate the MAPKK Pbs2 that in turn activates the MAPK Hog1. Hog1 generates and coordinates the intracellular response upon osmostress.

At this point, both branches converge. Ssk2/Ssk22 and Ste11 bind to their respective docking sites on the MAPK kinase (MAPKK or MAP2K) Pbs2. Once bound, any of these MAPKKKs phosphorylate Pbs2 on their Ser514 and Thr518. Activated Pbs2 further transmits the signal by dually phosphorylating the core element of the pathway, the MAPK Hog1 at Thr174 and Thr176 (Brewster *et al.*, 1993; Posas and Saito, 1997).

Hog1 is the kinase responsible for coordinating the osmostress response. It activates a short-term response devoted to accumulate glycerol inside the cell, which restores the osmotic balance with the exterior (Nevoigt and Stahl, 1997). It does so by directly regulating the activity of the 6-phosphofructo-2-kinase Pfk2, inhibiting glycolysis and redirecting the carbon metabolism to produce glycerol (Dihazi, Kessler and Eschrich, 2004). In parallel Hog1 also promotes the closure of the glycerol export channel Fps1 by inhibiting its activators, facilitating the increase of intracellular glycerol (Lee *et al.*, 2013).

Besides Hog1 roles in the cytosol, right after its activation, Hog1 translocates inside the nucleus to activate the transcriptional response (Ferrigno *et al.*, 1998). Once in the nucleus Hog1 phosphorylates several targets such as transcription factors, elongation factors, chromatin remodelers and mRNA processors to induce a complete change in the protein content of the cell (reviewed in de Nadal, Ammerer and Posas, 2011 and further discussed in section 2).

During osmotic stress, Hog1 also controls cell cycle

progression. Because cell cycle is a very sensitive and coordinated process, upon osmotic stress, cells delay its progression to allow a proper adaptation before proceeding to a new division. Hog1 directly regulates the expression and activity of several cell cycle factors during all cell cycle phases (G1, S, G2 and M). This control mechanism ensures a safe and organized cell cycle progression upon the damaging effects of osmotic stress (Escoté *et al.*, 2004; Zapater *et al.*, 2005; Clotet *et al.*, 2006; Adrover *et al.*, 2011; Duch, De Nadal and Posas, 2013; Nadal-Ribelles *et al.*, 2014; Chang *et al.*, 2017; Canal *et al.*, 2018).

Osmotic stress adaptation puts yeast cells out of their comfort zone. Besides the evident delay on cell cycle progression, other fundamental functions such as protein synthesis and catabolism are also compromised. Therefore, once cells are adapted to the new situation, Hog1 signaling needs to be attenuated to reestablish the normal cellular functions. The down-regulation of the pathways is directly triggered by the recovery of cell volume and membrane turgor due to the accumulation of glycerol (Nevoigt and Stahl, 1997; Lee *et al.*, 2013). On the other hand, there are also internal negative feedback loops that attenuate Hog1 signaling such as the phosphorylation of upstream components of the pathway by Hog1 (Hao *et al.*, 2007, 2008). Hog1 also promotes the expression of the phosphatases Ptc1, Ptp2 and Ptp3 that dephosphorylate Hog1 activating sites modulating the activity of the pathway (Jacoby *et al.*, 1997; Warmka *et al.*, 2001).

1.2 Heat stress signaling

In nature, temperature is constantly fluctuating between day and night but also throughout seasons. Despite this variability, the optimal growth temperature for *Saccharomyces cerevisiae* is around 25-30ºC while any sudden increase above these temperatures represents an insult for normal cell growth. The molecular and physiologic effects of heat are diverse. Typically, temperatures above 37ºC cause defects on protein kinetics and folding, which may lead to an increase in protein aggregation. The proteotoxic effect of heat stress reduces the quantity and availability of functional proteins, having an impact on many cellular processes such as cytoskeleton organization, organoid positioning, mRNA processing and translation among others. Additionally, heat shock also increases membrane and cell wall structure, mobility and permeability, affecting its stability and composition (Richter, Haslbeck and Buchner, 2010; Verghese *et al.*, 2012).

In order to overcome the undesired effects of heat, there is no a unique signaling pathway, but several that in combination restore cellular homeostasis (Fig 2). Despite current knowledge, some of these pathways are not completely understood and many molecular mechanisms have not been well defined yet.

One of the main regulators of the heat stress response is the heat shock factor 1 (Hsf1). Hsf1 is a highly conserved transcription factor in eukaryotes that induces gene expression of heat-responsive genes. In the absence of heat stress, Hsf1 is already bound to promoters allowing certain levels of basal transcription. However,

the protein chaperones Hsp90 and Hsp70 bind Hsf1, sequestrating its activity. Upon heat stress, Hsp90 and Hsp70 are required to bind unfolded proteins. This relaxes their inhibition towards Hsf1 and allows it to initiate thermo-responsive transcription (Fig 2) (Hottiger *et al.*, 1992; Morano, Grant and Moye-Rowley, 2012; Verghese *et al.*, 2012; Brown *et al.*, 2017). A far less understood layer of regulation is the post-translational modification of Hsf1, mainly by

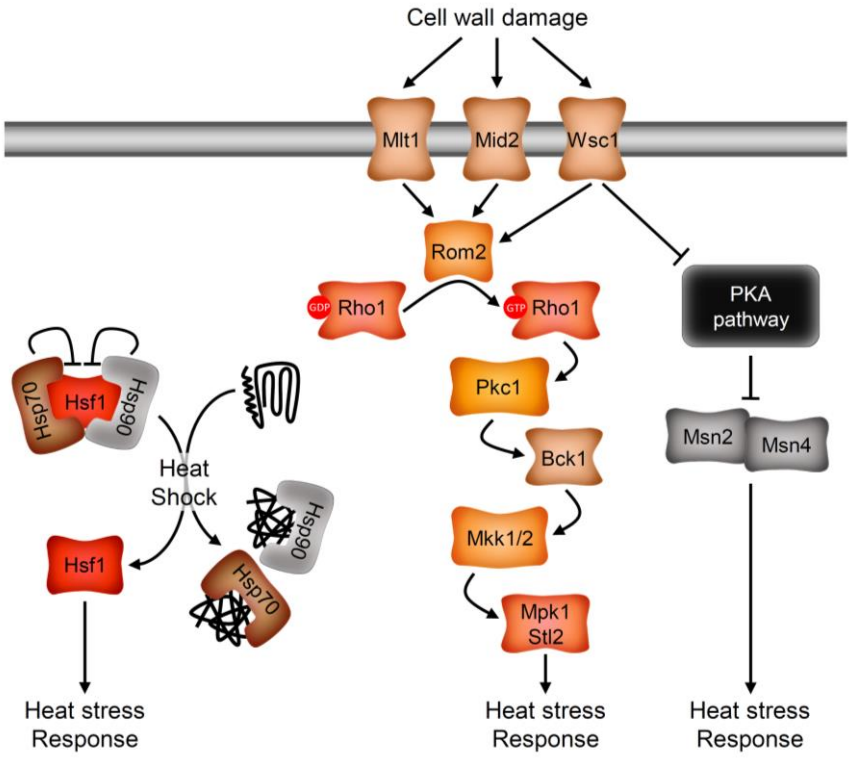


Figure 2. Outline of the three main mechanisms that coordinate the heat stress response in yeast.
From left to right. Upon heat shock, protein chaperones Hsp70 and Hsp90 bind unfolded proteins liberating Hsf1 repression. Free Hsf1 activates the transcriptional heat stress response. Thermal stress damaging effects on the cell wall activate the CWI pathway. Once activated, the MAPK Mpk1/Stl2 coordinate the intracellular heat stress response. Simultaneously, heat shock inhibits the PKA pathway releasing the transcription factors Msn2/4 from their cytosolic inhibition, allowing them to activate the transcriptional response.

phosphorylation and sumoylation. Despite the fact that such modifications are identified in humans and *Xenopus* Hsf1 (not in yeast), their role in the context of the heat stress response has not been well defined yet (Hong *et al.*, 2001; Guettouche *et al.*, 2005).

Besides Hsf1, the functionally redundant transcription factors Msn2 and Msn4 (from now on Msn2/4) are also activated upon heat stress. Once again, the precise molecular mechanism that activates both transcription factors has not been completely elucidated yet. Current models hypothesize that the protein kinase A (PKA) pathway inhibits Msn2/4 to avoid activation of any stress-responsive program during favorable conditions. Upon heat stress, the PKA pathway is inhibited releasing Msn2/4 to activate transcription in the nucleus (Fig 2) (Fuchs and Mylonakis, 2009; Morano, Grant and Moye-Rowley, 2012; García *et al.*, 2017; Sanz *et al.*, 2018).

In addition to the activation of these transcription factors, heat stress also causes defects on cell wall dynamics, which activate the cell wall integrity pathway (CWI), another MAPK pathway in yeast (Fig 2). In brief, heat-induced cell wall damage is sensed by a cohort of extracellular sensors (mainly Wsc1, Mlt1 and Mid2). These sensors transmit the signal to a guanine nucleotide exchange factor Rom2, whose catalytic activity activates the GTPase Rho1. Then GTP-bound Rho1 activates the protein kinase C (Pkc1) that in turn activates the MAPKKK Bck1. Bck1 phosphorylates the two redundant MAPKKs Mkk1 and Mkk2 which finally transmit the signal to the MAPK Stl2 also named Mpk1. Once activated, the MAPK further transmits the signal by activating several

transcription factors, chromatin remodels and other downstream targets (Levin, 2011; Verghese *et al.*, 2012; Sanz *et al.*, 2018).

These three mechanisms aim to protect the cell from the undesired effects of the increase in temperature. A fraction of the response consist on the activation of the synthesis of trehalose. This disaccharide has a thermo-protective effect on proteins and membranes (Magalhães *et al.*, 2018). The heat stress response also includes an increased synthesis of chaperons and co-factors that stabilize unfolded proteins and try to diminish protein aggregates (Morano, Grant and Moye-Rowley, 2012; Verghese *et al.*, 2012).

2. TRANSCRIPTIONAL RESPONSE UPON STRESS

Transcription is a fundamental cellular process that produces messenger RNA from a DNA template. Newly synthetized mRNA is modified and translated into poly-amino acid chains that ultimately form proteins. The yeast genome is composed of around 6600 genes, but not all of them are simultaneously transcribed. In basal conditions, cells are devoted to cell growth and, as a consequence, the transcription machinery is particularly focused on massively transcribing ribosomal related genes such as rRNAs, ribosomal proteins and tRNAs (Gasch *et al.*, 2000). There is another set of genes known as housekeeping genes that are constitutively transcribed independently of the cellular status. This subset of genes encode for structural proteins, metabolic enzymes, some rRNAs, etc., all necessary to sustain cell viability (Huisinga and Pugh,

2004). On the other hand, the transcription of another subset of genes fluctuates in a timely-regulated manner according to the cell cycle in which their products are required (McInerny, 2016). In optimal conditions, the transcription of these three groups of genes coexists. However, upon a sudden environmental change, cells massively reconfigure their transcriptional program to adapt to the new situation. Indeed, stress represses the transcription of the above-mentioned genes and induces the transcription of a subset of stress-responsive genes (Gasch *et al.*, 2000; Capaldi *et al.*, 2008; Nadal-Ribelles *et al.*, 2012). Expression regulation of all these types of genes is a key but also complex process that shapes the transcriptional landscape of cells depending on the particular requirements of each environmental condition.

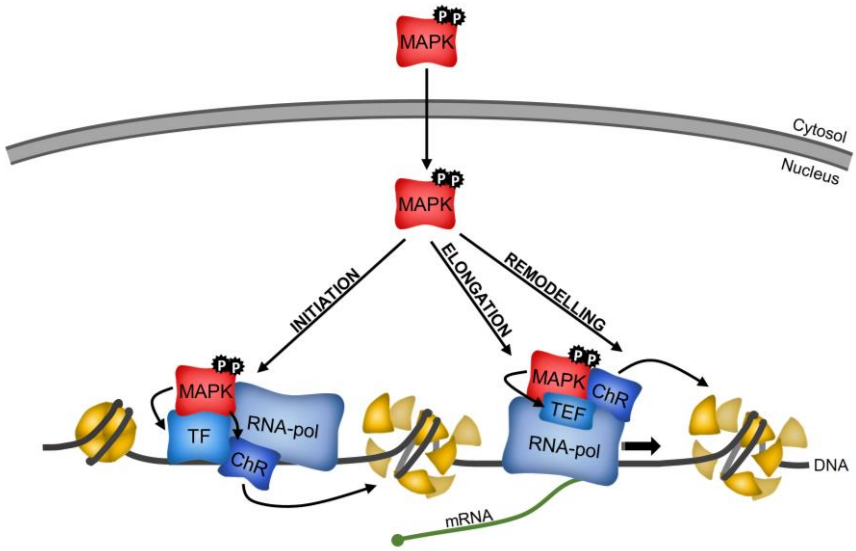


Figure 3. Control of transcription by MAPKs upon stress.
Stress activated MAPKs shuttle from the cytosol to the nucleus. In the nucleus, MAPKs promote transcription initiation by interacting with transcription factors (TF) and chromatin remodelers (ChR). During mRNA elongation MAPKs also target transcription elongating factors (TEF) and chromatin remodelers to remove nucleosomes and facilitating RNA-polymerase II (RNA-pol) passage across the gene.

Upon osmotic and heat stress, there is an exhaustive control of all the processes that lead to protein synthesis. This multi-layered response implicates the regulation of transcription initiation, elongation and chromatin remodeling among other processes of mRNA biogenesis (Fig 3) (de Nadal, Ammerer and Posas, 2011). This transcriptional control by MAPKs builds a complex and robust regulatory network that complements the cytosolic response against heat and osmostress.

2.1 Stress-induced transcription reprogramming

Upon stress, the expression pattern of 10 to 20% of genes in yeast are altered depending on the type and strength of the stress (Gasch *et al.*, 2000; Posas *et al.*, 2000; Causton *et al.*, 2001; Pelet *et al.*, 2011; Ho and Gasch, 2015; Brown *et al.*, 2017). Growing and biogenesis genes are no longer a priority and are quickly down-regulated. Instead, cells induce a new subset of stress-responsive genes in order to survive and adapt to that particular stress. Each stress has its own transcriptional program depending on the strategies developed by the cell to better cope with the damaging effects of the stress. The duration and intensity of the transcriptional response also depends on the type and intensity of the stress. However, there is also a common transcriptional response for different stresses known as the environmental stress response (ESR) (Gasch *et al.*, 2000; Pelet *et al.*, 2011; Brown *et al.*, 2017).

The ESR includes approximately 300 genes that are up-regulated, mainly related to mechanisms of general stress defense

such as carbohydrate metabolism, protein folding/degradation, redox regulation and DNA damage. Whereas around 600 genes are down-regulated most of them related to ribosomal and translational functions (Gasch *et al.*, 2000; de Nadal, Ammerer and Posas, 2011; Brown *et al.*, 2017).

The advantages of such a general response seems to go further than simpler stress survival. Surprisingly, most of the genes that are induced in the ESR are not essentially required for cell survival upon stress (Giaever *et al.*, 2002). Instead, it has been postulated that the synthesis of proteins from the ESR serves as a mechanism of cross-protection for future and diverse stresses, adapting yeast cells to their highly variable natural niches (Berry and Gasch, 2008; Berry *et al.*, 2011).

Besides the ESR, each stress induces its own transcriptional program. Part of this specific response also includes the transcriptional modulation of positive and negative feedback loops. Indeed, transcription factors, phosphatases and other regulatory elements are transcriptionally regulated to finely control the dynamics of stress responses (Brown *et al.*, 2017).

2.2 Transcription initiation upon stress

Transcription begins with the formation of the pre-initiation complex (PIC). The PIC is composed of transcription factors, some of them are general as the TFII family and others are gene-specific. Subsequently, the transcription factors recruit and coordinate the

interaction of co-activators, chromatin remodelers and the RNA-polymerase II (RNA-Pol II) (Shandilya and Roberts, 2012).

The transcription factors Msn2/4 are the main activators of the general stress-induced ESR, activated by different means upon both heat and osmotic stress (Görner *et al.*, 1998; Gasch *et al.*, 2000; Rep *et al.*, 2000; García *et al.*, 2017). Msn2/4 bind to the STRE sequence in stress-induced genes. It is generally accepted that Msn2/4 regulation is mainly mediated by its nuclear/cytosolic shuttling. This model fits with the observations in which upon heat stress the inhibition of the PKA pathway favors their nuclear accumulation (Fig 2) (Görner *et al.*, 1998; García *et al.*, 2017). A similar increase on Msn2/4 nuclear localization is observed upon Hog1 activation by osmotic stress (Schüller *et al.*, 1994; Rep *et al.*, 2000). Besides its nuclear localization, Hog1 also increases the occupancy of Msn2/4 and other initiation factors on osmo-responsive genes by directly up-regulating the ubiquitin protease Ubp3 and thus preventing their polyubiquitin-mediated degradation (Solé *et al.*, 2011).

In addition to the ESR, each stress requires a particular subset of transcription factors to generate the appropriate transcriptional response (Capaldi *et al.*, 2008; Ni *et al.*, 2009). Upon osmotic stress, Hog1 regulates the transcription activators Smp1, Hot1, Rtg1/Rtg3 and Sko1 (Alepuz *et al.*, 2001, 2003; Proft *et al.*, 2001; de Nadal, Casadome and Posas, 2003; Ruiz-Roig *et al.*, 2012). The mechanisms of transcription factor activation are diverse. For instance, Hog1 directly mediates Smp1 and Rtg1/Rtg3 activation through direct phosphorylation (de Nadal, Casadome and Posas,

2003; Ruiz-Roig *et al.*, 2012). Sko1 activity is also regulated by Hog1 phosphorylation. However in this case, Sko1 phosphorylation frees it from its repressor complex with Ssn6 and Tup1, allowing Sko1 to act as a transcription activator (Kuchin and Carlson, 1998; Proft and Struhl, 2002). Finally, Hog1 also phosphorylates Hot1, although such phosphorylation is not necessary for transcription activation (Alepuz *et al.*, 2003). Each of the mentioned transcription factors target a particular subset of genes, but some of them also work in combination with others, including Msn2/4, in order to properly initiate the transcriptional response upon osmotic stress (Capaldi *et al.*, 2008; Ni *et al.*, 2009; de Nadal and Posas, 2015).

Transcription initiation and the activity of transcription factors is also tightly regulated upon heat stress. As described before, the transcription factor Hsf1 is the major inductor of the specific transcriptional heat stress response. Hsf1 regulation directly depends on chaperones Hsp70 and Hsp90 although in some organisms, it can also be modulated by post-translational modifications such as phosphorylation and sumoylation (Hong *et al.*, 2001; Hilgarth *et al.*, 2004; Guettouche *et al.*, 2005). Hsf1 binds DNA at heat shock elements (HSE) to mediate transcription activation. The HSE are composed of several tandem repeats of the sequence nGAAn at promoters of heat responsive genes. Hsf1 can bind in multiple combinations (alone, di- or trimerizing) with different specificities and outcomes (Erkine *et al.*, 1999; Hahn *et al.*, 2004; Erkina and Erkine, 2006).

On the other hand, the CWI pathway and its MAPK Mpk1 also target several transcription factors. Mpk1 phosphorylates the

transcription factor Rlm1 that in turn activates transcription of the majority of genes up-regulated by the CWI (Jung *et al.*, 2002; Levin, 2011; Sanz *et al.*, 2018). This transcriptional response includes the transcription of the *RLM1*, *MPK1* and other genes of components of the CWI pathway as a positive auto-regulatory feedback loop. At the same time, Mpk1 promotes the expression of several phosphatases (mainly Ptp2 and Msg5) that attenuate the response, balancing the activation/repression state of the pathway (Mattison *et al.*, 1999; Hahn and Thiele, 2002). In addition, Mpk1 also activates the transcription factors SBF composed of Swi4 and Swi6 through a non-catalytic mechanism (Baetz *et al.*, 2001). Active Mpk1 binds to Swi4 replacing its inhibitors; once freed, Swi4 and Swi6 can dimerize and activate transcription (Kim and Levin, 2011).

Once the heat and osmo-activated transcription factors are bound to the promoters of stress-responsive genes, other regulatory elements such as Spt-ADA-Gcn5 acetyltransferase complex (SAGA) and Mediator interact with these promoters (de Nadal, Ammerer and Posas, 2011), leading to the recruitment of the RNA-Pol II to initiate transcription.

2.3 Chromatin remodeling

Generally speaking, stress-responsive genes are basally silenced and their promoters and coding regions are condensed within nucleosomes that act as natural barriers for the transcription machinery. Upon stress, chromatin remodelers disrupt the existing

DNA-histone interactions to evict or displace nucleosomes facilitating the PIC formation and transcription initiation. Once transcription is initiated, to facilitate RNA-Pol II passage during RNA elongation, chromatin remodelers and histone modifiers are also required to remove nucleosome across the gene body (Shandilya and Roberts, 2012). On the other hand, once environmental stresses end or cells have managed to adapt, the stress transcriptional program is also terminated while chromatin remodelers reposition histones back and chromatin is re-condensed on stress-responsive genes (de Nadal, Ammerer and Posas, 2011).

In budding yeast, there are three main families of chromatin remodelers involved in the transcriptional stress response: SWI/SNF, ISWI and INO80. Besides these families, other complexes such as SAGA also act as chromatin remodelers in stress-responsive genes.

The SWI/SNF family is composed by the SWI/SNF (Snf2, Swi3, Swi1, Snf5, Swp82, Snf12, Arp7, Arp9, Snf6, Snf11 and Taf14) and the RSC (Rsc1-10, Sht1 and others) complexes (Smith *et al.*, 2003; Mas *et al.*, 2009; Lorch and Kornberg, 2017). Both complexes are low abundant and have an intrinsically none-specific mode of action. They require the binding of transcription factors such as Rlm1 and Sko1 or other effectors such as Hsf1 and Msn2/4-dependent activators, in order to specifically interact with promoters (Proft and Struhl, 2002; Erkina, Tschetter and Erkine, 2008; Mas *et al.*, 2009; Sanz *et al.*, 2018). Once targeted, the mechanism of action, specificity and regulation of the SWI/SNF and RSC complexes differs. However, the activity of both results in a similar

increased accessibility for the RNA-Pol II at promoters of stress-responsive genes (Shivaswamy and Iyer, 2008; Mas *et al.*, 2009). Both complexes also mediate the nucleosome removal during RNA elongation.

MAPKs Mpk1 and Hog1 also regulate SWI/SNF and RSC activity to promote a complete transcription cycle of the stress-induced genes (de Nadal and Posas, 2011; Kim and Levin, 2011; Silva *et al.*, 2017). As example, Hog1 directly contacts RSC to tether the remodeler complex to osmo-induced genes (Mas *et al.*, 2009; Nadal-Ribelles *et al.*, 2015).

SAGA is a polypeptide complex with different subunits that specifically remodels chromatin preferentially in highly regulated and stress-induced genes (Huisinga and Pugh, 2004; Rodríguez-Navarro, 2009). SAGA mediates chromatin remodeling through the histone acetyl transferase activity of its subunit Gcn5 (Grant *et al.*, 1997; Wu *et al.*, 2004). In order to ensure an adequate transcriptional response, Hog1 targets SAGA to osmo-responsive genes (Zapater *et al.*, 2007). Similarly, SAGA is also required in heat shock genes for proper transcription initiation (Kremer and Gross, 2009).

The complexes ISWI and INO80 compose another family of chromatin remodelers. In contrast to the activating function of SWI/SNF and RSC, ISWI and INO80 catalyze the repositioning of nucleosome on stress-responsive genes (Klopf *et al.*, 2009, 2017; Krietenstein *et al.*, 2016). Consequently, their activity is associated with a repression of transcription once the stress is finished or the

negative feedback loops take control to limit toxic levels of stress-responsive genes that may compromise cell viability.

3. HISTONE AND NUCLEOSOME DYNAMICS IN YEAST

In eukaryotic cells, DNA fibers are packed inside the nucleus. This packaging is highly dynamic and chromatin is compacted or decompacted depending on a variety of scenarios such as cell cycle progression, replication or transcription. Typically, highly condensed regions known as heterochromatin are transcriptionally silenced, while open chromatin (euchromatin) correlates with active genes. The main proteins that physically condensate chromatin and define its accessibility are histones (Luger *et al.*, 1997). Eight histone proteins form octamers that wrap DNA into structures known as nucleosomes (Fig 4). Nucleosomes in turn aggregate and form a higher order of condensed chromatin to fully compact chromosomes during cell division (Jansen and Verstrepen, 2011; Luger, Dechassa and Tremethick, 2012). As mentioned before, nucleosome compaction has a profound effect on chromatin accessibility for the transcription machinery. Thus, yeast has evolved several mechanisms to modulate nucleosome positioning on chromatin, also upon extracellular stresses, when a fast and intense transcriptional response is required.

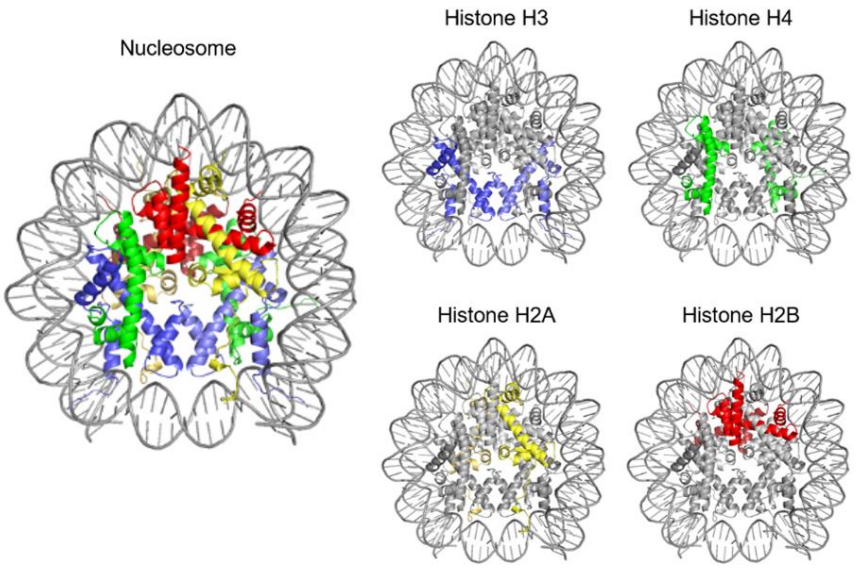


Figure 4. Cristal structure of the yeast nucleosome.
A nucleosome is composed of an histone octamer of one histone H3 (blue) and H4 (green) tetramer plus two H2A (yellow) and H2B (red) heterodimers that wraps 147 base pairs of DNA. Coordinates for nucleosome structure were obtained from White *et al.*, 2001 (Protein Data Bank accession number 1ID3) and analyzed with Pymol software.

3.1 Histone and nucleosome structure

Histone are highly conserved proteins across species, from yeast to humans. *Saccharomyces cerevisiae* has four core histones: H3, H4, H2A and H2B. In budding yeast, each histone protein is encoded by two different genes: H3 (*HHT1* and *HHT2*), H4 (*HHF1* and *HHF2*), H2A (*HTA1* and *HTA2*) and H2B (*HTB1* and *HTB2*) (Hereford *et al.*, 1979; Choe, Kolodrubetz and Grunstein, 1982; Smith and Andrésson, 1983). Regarding their sequence, there are some nucleotides that differ between gene pairs, but most of the mutations are silent and do not change the amino acid composition. Histone genes are organized in pairs separated by a bidirectional

promoter, each of them located in a different region of the genome: *HHT1-HHF1* (ChrII) and *HHT2-HHF2* (ChrXIV) for H4 and H3; *HTA1-HTB1* (ChrIV) and *HTA2-HTB2* (ChrII) for H2A and H2B (Hereford *et al.*, 1979; Choe, Kolodrubetz and Grunstein, 1982; Smith and Andrésson, 1983). Despite their high level of homology and redundancy, the transcription level between gene pairs are not equal. For instance, the *HHT2-HHF2* pair contributes around 80% to the final level of H3 and H4 whereas the remaining corresponds to *HHT1-HHF1* (Cross and Smith, 1988). Despite their differences, one gene pair can compensate the deletion of the other with only minor phenotypic effects in some particular conditions (Norris and Osley, 1987; Norris, Dunn and Osley, 1988; Liang *et al.*, 2012). In addition, transcription of the core histone genes is highly regulated in a cell cycle manner. During late G1, transcription of histones is activated to cope with DNA doubling during S phase (Osley, 1991). All core histone genes share the same regulatory elements and their transcription rates are coordinated to maintain the stoichiometry necessary for proper nucleosome composition (Sittman, Graves and Marzluff, 1983; Eriksson *et al.*, 2012).

Unlike higher eukaryotes such as mouse or humans, *Saccharomyces cerevisiae* has a much more limited number of core histone genes and non-canonical histone variants (Marzluff *et al.*, 2002; Henikoff and Smith, 2015). Budding yeast known histone variants are the linker protein H1 (gene *HHO1*); the H2A.Z (gene *HTZ1*) that substitutes the canonical H2A in some specific regions; and the centromeric H3-like protein cenH3 (gene CSE4) (Stoler *et al.*, 1995; Santisteban, Kalashnikova and Smith, 2000; Li *et al.*,

2005; Panday and Grove, 2017). The function of these variants seems to be restricted under certain conditions in specific locus.

Histones are relatively small proteins ranging from the shortest H4 with only 103 amino acids to the 136 of the largest H3. All four histones have an intrinsically disordered and flexible N-terminal tail (around 20 to 30 amino acids long) and a shorter C-terminal tail, whereas the rest of the protein has a more globular and rigid structure (Luger *et al.*, 1997; White, Suto and Luger, 2001).

Histones form complexes known as nucleosomes. Nucleosomes are composed of one H3-H4 tetramer and two H2A-H2B dimers. These octamers wrap 147 base pairs of DNA in an almost two-turn (1.65) superhelix (Fig 4) (Luger *et al.*, 1997). Nucleosome structure is stabilized by several protein-protein interactions within the histone octamer and by electrostatic interactions and hydrogen bonds between DNA and proteins (Luger *et al.*, 1997; Richmond and Davey, 2003; Luger, Dechassa and Tremethick, 2012). Histone N-terminal tails protrude out from the globular nucleosome and, due to their enrichment in positively charged lysines and arginines, help to stabilize the intra-nucleosome structure with DNA (Iwasaki *et al.*, 2013). Histone tails also make contacts with other nucleosomes to generate more compacted forms of chromatin through inter-nucleosome interactions (Kan, Caterino and Hayes, 2009). Because of their higher accessibility, histone tails also serve as platforms for several histone-binding proteins. These tails are also heavily post-translationally modified and subjected to intense regulation as further described in section 3.4 (Krebs, 2007; Zhao and Garcia, 2015).

3.2 Histone chaperones

According to the high degree of conservation between histones, the nucleosome structure and conformation is also similar throughout eukaryotes. Despite their natural affinity for DNA, histones do not self-assemble into nucleosomes. Due to their enrichment in positive charges, free histones tend to form aggregates. For this reason, histones are bound to negatively charged proteins (chaperones) that prevent their aggregation and un-coordinated interactions with the acidic DNA (Pardal, Fernandes-Duarte and Bowman, 2019). The histone chaperones mediate the correct assembly and disassembly of nucleosomes, not only during DNA replication but also upon DNA damage or during transcription (Akey and Luger, 2003; Keck and Pemberton, 2012; Gurard-Levin, Quivy and Almouzni, 2014). First, the chromatin assembly factor (CAF-1) complex and other factors such as Asf1 and histone acetyltransferases incorporate the H3-H4 tetramer to DNA (Enomoto and Berman, 1998; English *et al.*, 2006; Kumar *et al.*, 2012). Then two H2A-H2B dimers are tethered to both sides of the H3-H4 tetramer by the nucleosome assembly protein-1 (NAP-1) to form a complete histone octamer (Ito *et al.*, 1996; Andrews *et al.*, 2010). Once assembled, nucleosomes are slid through DNA to be properly spaced according to the cellular requirements (Bowman, 2010).

As mentioned before, chromatin remodelers are required to remove nucleosomes during transcription. In this complex process, histone chaperones also contribute to effectively disassembly nucleosomes or reposition them once the activating signal is

terminated (Korber *et al.*, 2006). An illustrative example is the interaction of the histone chaperon Asf1 with the chromatin remodeler INO80 to restore nucleosomes in stress-responsive genes after transcription (Klopf *et al.*, 2009).

3.3 Histone post-translational modifications

Histones, as many other proteins, are post-translationally modified to finely tune their molecular properties and biological functions. Typically, histone modifications involve the addition through a covalent bond of a small group of atoms that changes the biochemical properties of the modified residue. The most common histone PTMs are acetylation, methylation and phosphorylation. Larger PTMs are also incorporated to histones such as ubiquitination, sumoylation or ADP-ribosylation. In recent years, with advances in mass spectrometry and with improvements on the production of new antibodies, several novel types of histone PTMs have been described such as propionylation, butyrylation, succinylation or crotonylation (a complete list is shown in Fig 5) (Zhao and Garcia, 2015). Other rarer and less frequent types of modifications such as glutarylation or benzoylation have also been reported (Tan *et al.*, 2014; Huang *et al.*, 2018). As previously stated, histone N- and C-terminal tails are heavily modified, but in some cases PTMs are also deposited on their core globular domain (Bannister and Kouzarides, 2011; Kebede, Schneider and Daujat, 2015; Zhao and Garcia, 2015).

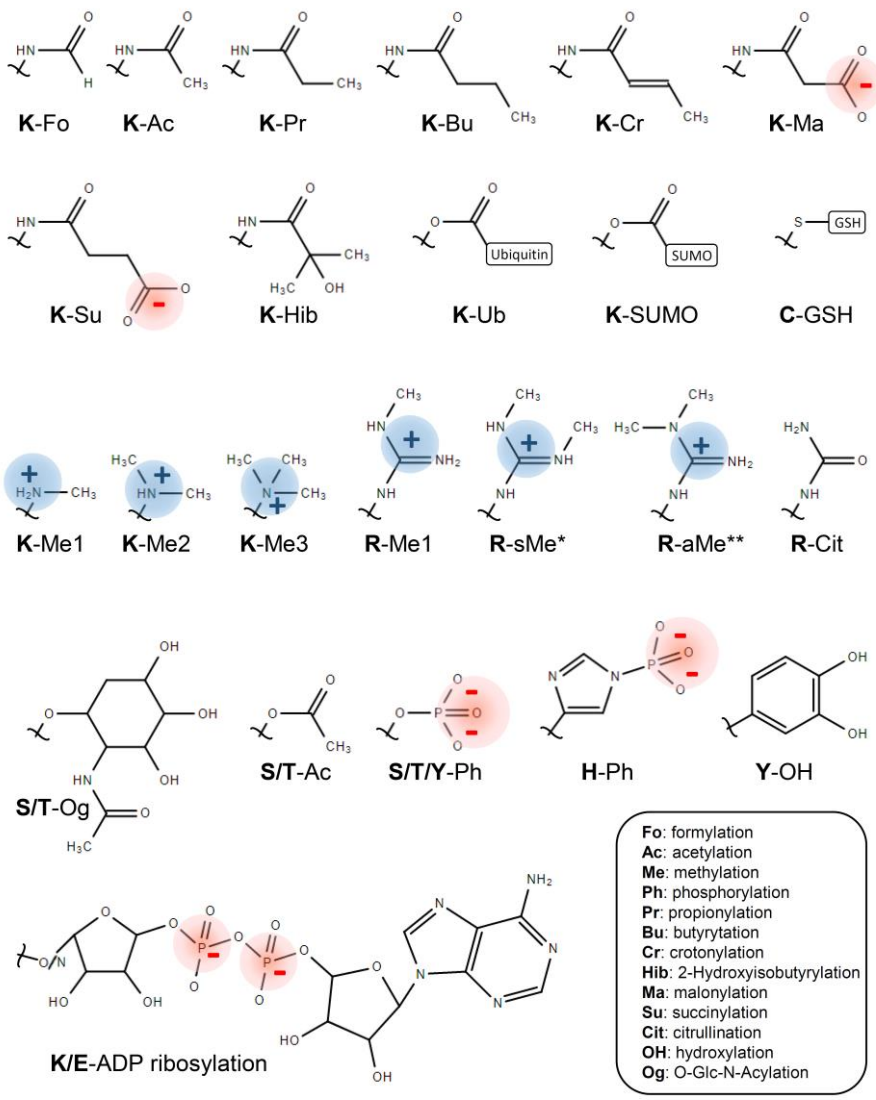


Figure 5. Chemical structures of histone PTMs.
Molecular structures and charges of described histone PTMs in their respective modified residues in a single letter code. SUMO: sumoylation, GSH: glutathionylation, *symmetric or **asymmetric methylation. Chemical structures were constructed with the webpage: www.chemspider.com/StructureSearch.aspx.

Histone acetylation was first reported in the 60s and since then, it has been detected in a wide variety of histone residues (Allfrey, Faulkner and Mirsky, 1964). Typically, histone acetyltransferases (HATs) transfer the acetyl group from the cofactor acetyl-CoA to the ε-amino group of lysine. By this addition, the positive charge of the unmodified lysine is neutralized. The loss of charge may, for instance, destabilize nucleosomes by weakening the interactions between DNA and histones or change the binding affinity of other proteins for histones (Deckert and Struhl, 2001; Shogren-Knaak *et al.*, 2006; Williams, Truong and Tyler, 2008; Zhang *et al.*, 2018). If HATs add the acetyl group, the proteins in charge of removing the modification are histone deacetylases (HDAC) (Chen, Zhao and Zhao, 2015; Barnes, English and Cowley, 2019). Dynamic regulation of HATs and HDACs activity has a profound effect on transcription.

Another histone mark that has been intensively studied is the methylation of lysines and arginines. The ε-amino group of lysine can be either mono-, di-, or tri-methylated, whereas the ω-guanidino group of arginine can be mono-methylated and symmetrically or asymmetrically di-methylated (Fig 5). Methylations tend to decorate several residues on histone tails and globular domains (Morillon *et al.*, 2005; Du, Fingerman and Briggs, 2008; Zhao and Garcia, 2015; Worden *et al.*, 2019). The enzymes in charge of transferring the methyl group from the SAM cofactor to histones are the histone lysine/arginine methyltransferases (HKMT and PRMTs respectively) (Bannister and Kouzarides, 2011). Unlike acetylation, methylation does not alter the positive charge of the modified

residues but instead affects its conformation and structure. Thus, the exact effect of such modification greatly depends on the residue modified. However, in general, methylation or the lack of it often alters the docking site of histone binding proteins. Hence methylation status ultimately defines the cohort of proteins that interact with nucleosomes and their different effects on chromatin (Bernstein *et al.*, 2002; Santos-rosa *et al.*, 2002; van Leeuwen, Gafken and Gottschling, 2002; Kirmizis *et al.*, 2007; Worden *et al.*, 2019). Besides the canonical lysine and arginine methylation, glutamine methylation has also been reported in yeast histone H2A-Q105, demonstrating once again the variability within histone PTMs (Tessarz *et al.*, 2014).

Phosphorylation is also a relevant histone PTM despite the fact that it is less abundant than acetylation and methylation (Zhao and Garcia, 2015). Highly dynamic, the addition of a phosphate group from ATP by a kinase can occur mainly in the hydroxyl residue of serines, threonines and tyrosines, and less frequently on histidines. The phosphate group adds a strong negative charge in that particular residue altering the biochemical properties of the surrounding region. Histone phosphorylation is associated to apoptosis, DNA damage responses and transcription activation or silencing depending on the modified site (Berger, 2010; Bannister and Kouzarides, 2011; Haase *et al.*, 2012; Zhao and Garcia, 2015; Millan-Zambrano *et al.*, 2018).

Finally, there is a more complex catalogue of less frequent but relevant histone modifications, as shown in figure 5. The function of some of them is not clear yet, whereas others such as

ubiquitination and sumoylation are known to serve as interacting platforms for other proteins (Ng *et al.*, 2002; Bannister and Kouzarides, 2011; Patel and Wang, 2013; Zhao and Garcia, 2015). It is worth mentioning that despite the high degree of homology between species and that some histone PTMs are conserved from yeast to humans, not all these types of modifications are found in all organisms nor the modified amino acids are the same.

3.4 Histone regulation

The numerous PTMs that decorate histones have a profound effect on nucleosome dynamics and are scrupulously regulated. In the early 90s with the increasing reporting on histone PTMs, researchers in the field started to speculate with the idea that a sort of rational "code" could exist for histone PTMs (Tordera, Sendra and Pérez-Ortín, 1993; Turner, 1993). This idea was accompanied by the identification of a complex cohort of nucleosome binding proteins that require specific histone PTMs for proper binding. Later, with the acknowledgement that histone PTMs often co-exist and establish a complex network of crosstalk interactions among them, the term "histone code" was set (Turner, 2000; Imhof and Becker, 2001; Jenuwein and Allis, 2001). Up to date, the number of different histone PTMs described are in the order of hundreds (Zhao and Garcia, 2015; Sidoli *et al.*, 2019). Some of them have been intensively studied and functional pathways are associated to them. Despite the current knowledge, the histone code is far from being fully understood. Several histone modifications were only detected

in mass spectrometry screenings and no function has been found yet (Zhao and Garcia, 2015; Janssen, Sidoli and Garcia, 2017). Furthermore, it is still unclear how most of the histone PTMs interact with each other and with other regulatory elements and which is their relevance in the context of cell biology.

Among the well-defined functions of histone PTMs is the regulation of transcription. One hallmark modification associated with active transcription is H3-K4 tri-methylation (H3-K4me3) in promoters and coding regions from yeast to humans (Santos-rosa *et al.*, 2002; Schneider *et al.*, 2004). In yeast, H3-K4me3 is mediated by the methyltransferase Set1 and serves as a platform to recruit factors that promote transcription such as the SAGA complex (through its Chd1 domain) or the NuA4 complex (Morillon *et al.*, 2005; Pray-Grant *et al.*, 2005; Berger, 2007; Williamson *et al.*, 2013). Once the SAGA complex recognizes the H3-K4me3, its HAT Gcn5 acetylates preferentially the H3 N-terminal tail (K9, K18, K23 and K27), whereas the HAT Esa1 in the NuA4 complex does it on H4, H2A and H2B N-terminal tails (Fig 6A) (Suka *et al.*, 2001; Vogelauer *et al.*, 2003; Doyon and Côté, 2004; Morillon *et al.*, 2005; Krebs, 2007). These acetylations weaken histone-DNA interactions facilitating RNA-Pol II passage across coding regions (Lee *et al.*, 2000; Sanz *et al.*, 2016; Bruzzone *et al.*, 2018; Church and Fleming, 2018).

As exemplified in the previous case, histone PTM crosstalk is an essential mechanism to control gene expression. Another example of multiple histone PTMs interaction is the methylation of K79 in the globular domain of H3. This mark regulates transcription

elongation and it is necessary in the DNA damage response (Krogan *et al.*, 2003; Giannattasio *et al.*, 2005; Steger *et al.*, 2008). H3-K79 methylation requires the prior H2B-K123 (K120 in humans) ubiquitination by Rad6 (Robzyk, Recht and Osley, 2000; Ng *et al.*, 2002). Once the H2B-K123ub is set, the methyltransferase Dot1 binds to the nucleosome in close proximity with the H3-K79 side chain. To fulfill the methylation, the residues R17 and R19 in H4 N-terminal tail are required to induce a conformational change in the neighboring region of the H3-K79 side chain. This reorients its ε-amino group that is then accessible for the methyltransferase Dot1 (Fingerman, Li and Briggs, 2007; Worden *et al.*, 2019). Once established, H3-K79 methylation blocks the binding of the silent

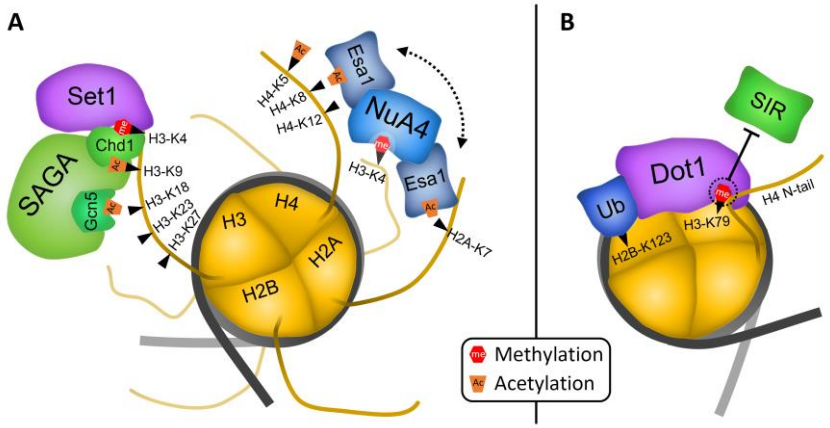


Figure 6. Outline of two distinct mechanisms that modulate nucleosome dynamics through histone PTMs in yeast.
(A) Set1 tri-methylates N-terminal H3-K4. Methylated H3-K4 allows SAGA complex to bind nucleosomes though its Chd1 domain. HAT Gcn5 in SAGA acetylates several residues in the N-terminal tail of H3. H3-K4me3 also allows NuA4 complex binding while its HAT Esa1 acetylates H4 and H2A N-terminal tails. **(B)** H4 N-terminal tail induces a conformational change on H3-K79 that alongside H2B-K123 ubiquitination allows H3 lysine 79 methylation by the methyltransferase Dot1. H3-K79 methylation in the globular domain inhibits chromatin silencing by SIR proteins.

information regulator (SIR) proteins, repressing gene silencing in that particular region (Fig 6B) (van Leeuwen, Gafken and Gottschling, 2002; Steger *et al.*, 2008).

The two examples presented above illustrate the complex interplay between histone PTMs, chromatin remodelers and the transcription machinery. As reviewed in Zhao and Garcia *et al* 2015, the list of histone PTMs is long and the known mechanisms behind such modifications are also diverse.

In addition to the classic catalogue of "writers", "readers" and "erasers" that regulate and interact with histone PTMs, in recent years, histone tail clipping has emerged as a novel mechanism to regulate nucleosome dynamics. From yeast to mammals, the specific clipping of the N-terminal tails by proteases imposes a complete erase of histone tail PTMs and impairs the formation of intra- and inter-nucleosome interactions, adding another layer of histone/nucleosome regulation (Santos-Rosa *et al.*, 2009; Nurse *et al.*, 2013; Azad and Tomar, 2016; Azad *et al.*, 2018).

Histone PTMs are highly dynamic and are added or removed according to cellular requirements. Upon stress, an intense rewiring of the histone PTMs and the activation of new players are associated to the transcriptional outburst generated by the different signaling pathways (Weiner *et al.*, 2012; Magraner-Pardo *et al.*, 2014). As detailed before, histone tail acetylation promotes nucleosome weakening and induces gene expression. However, the histone deacetylase Rpd3 is recruited in both heat and osmo-induced genes and is required for proper stress-induced

transcription (de Nadal *et al.*, 2004; Ruiz-Roig *et al.*, 2010). Upon stress, another histone mark that switches roles is the well-known "activating" H3-K4me3. As previously described, H3-K4 methylation is deposited in nucleosomes across genes, which correlates with active transcription. Oppositely to its activating role, upon stress Set1 and H3-K4 methylation mediate transcription repression of ribosomal biogenesis genes (Weiner *et al.*, 2012). In addition, alternative methylation patterns of H3-K4 upon stress change the cohort of chromatin remodelers binding to stress-responsive genes, promoting a specific stress response (Nadal-Ribelles *et al.*, 2015). These two examples demonstrate the specificity of the histone PTM regulation upon stress that it can even confer to activating marks a completely opposite role.

Histone PTMs are also involved in a wide variety of other chromatin related processes, such as the DNA damage response (Ahn *et al.*, 2005; Muñoz-Galván *et al.*, 2013; Wang *et al.*, 2017; Millan-Zambrano *et al.*, 2018), DNA replication (Baker *et al.*, 2010; Rivera *et al.*, 2014) or chromosome segregation (Haase *et al.*, 2012; Ishiguro *et al.*, 2018), among others. Histone PTM rewiring is also crucial in several human diseases such as cancer or neurodegenerative diseases, highlighting their relevance in a wide variety of contexts (Anderson and Turko, 2015; Noberini *et al.*, 2018).

4. STRATEGIES TO STUDY HISTONE MODIFICATIONS

The histone code is still far from being deciphered. The relatively small catalogue of histone proteins and its easy genetic manipulation and modification has made yeast a very attractive model organism to study histone and nucleosome dynamics compared with more complex organisms (Marzluff *et al.*, 2002; Henikoff and Smith, 2015). Additionally, the low number of histone variants and genes has also allowed researchers to generate histone mutant strains that facilitate the functional study of a particular histone residue.

4.1 Histone mutant libraries

A common practice when studying the function of any protein of interest is to generate mutants of specific regions or residues. Taking advantage of their reduced size, first Nakanishi *et al* 2008 (SHIMA) and shortly after Dai *et al* 2008 (Open Biosystems YSC5105/6) generated two complete sets of yeast histone mutant libraries. Right from the beginning, these libraries were instrumental to study the implication of individual residues in transcription, general fitness, chromosome integrity or chemical sensitivity among other phenotypes (Dai *et al.*, 2008; Nakanishi *et al.*, 2008).

In the SHIMA's library, the endogenous histone genes were deleted and substituted by a plasmid harboring mutant versions of the four core histones *HTA1-HTB1* or *HHT2-HHF2*. More precisely, it contains a set of strains with single amino acid mutations to

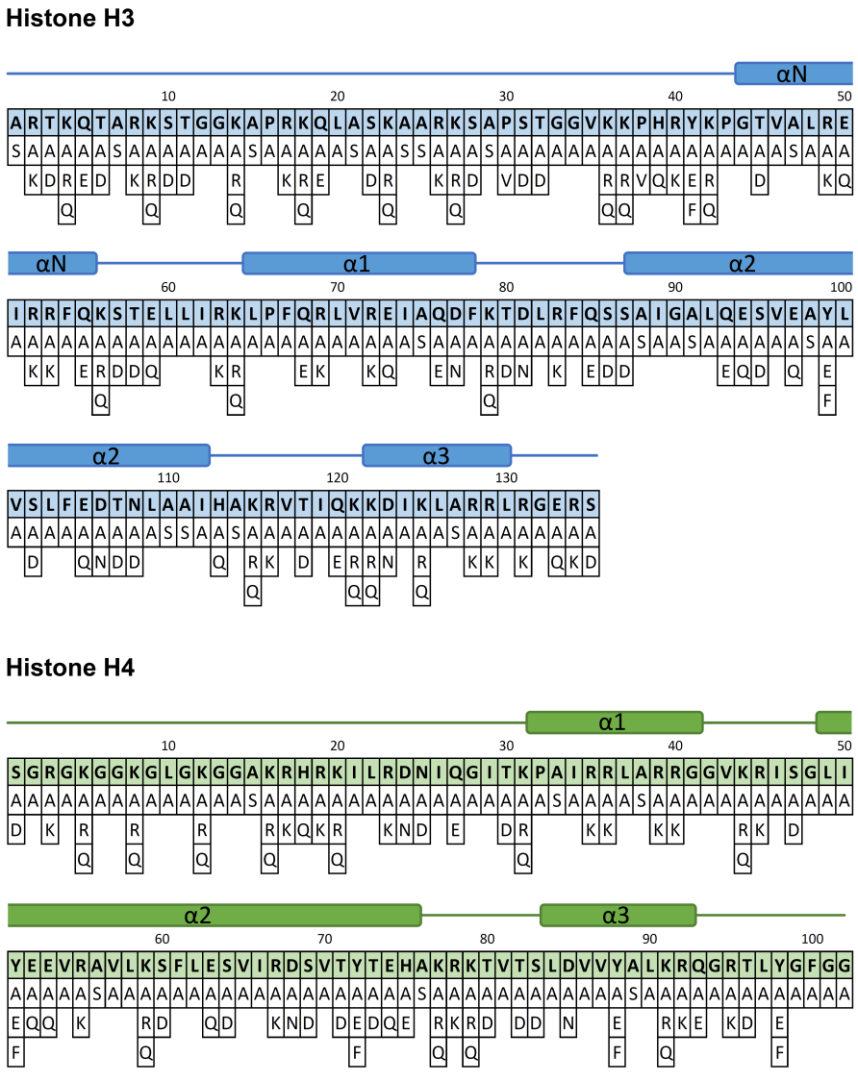
alanine, a small amino acid with a non-reactive methyl side chain (Fig 7). These alanine substitutions aim to abolish any function or interaction that the endogenous residue was sustaining.

On the other hand, the collection from Dai *et al* 2008 is only composed of H3 and H4 mutants with the corresponding alanine substitutions and other mutants that aim to mimic possible modifications of the endogenous residues. For instance, threonines and serines were mutated to aspartic acid that adds a negative charge that mimics a phosphorylation (Fig 7). Also differing from the SHIMA's collection, in the H3-H4 collection histone mutated genes were integrated into the *HHT2-HHT2* locus in the genome instead of being carried in a plasmid.

For our experiments, we used the H2A and H2B mutants from Nakanishi *et al* 2008 and the H3 and H4 mutants from Dai *et al* 2008, as their collection is richer in the variety of histone mutants available. A complete list of all mutants used for this project is depicted in figure 7.

These two pioneer libraries have been instrumental for researchers in the field that have used them to screen histone residues required for a wide variety of conditions. A useful tool to retrieve valuable information regarding histone mutant screenings is the web page-based database HistoneHits (available at http://histonehits.org). This database compiles the results from several systematic screenings assessing a wide range of phenotypes (Huang *et al.*, 2009).

One criticism of these libraries is that the two copies of the endogenous genes were deleted. The rationality at the time was that histone genes dose compensate the deletion of the other copy. However, this approach underestimates the mild but well-documented defects of histone gene deletions under certain conditions (Norris and Osley, 1987; Norris, Dunn and Osley, 1988; Liang *et al.*, 2012). As a response, recently, the same authors of the



Open Biosystems collection constructed a dose-matching histone library for the two copies of the four core histones. This new collection is more similar to the genetic background of wild type cells (Jiang, Liu, Wang, *et al.*, 2017). This increases the number of available tools to study histone residue for future research in yeast.

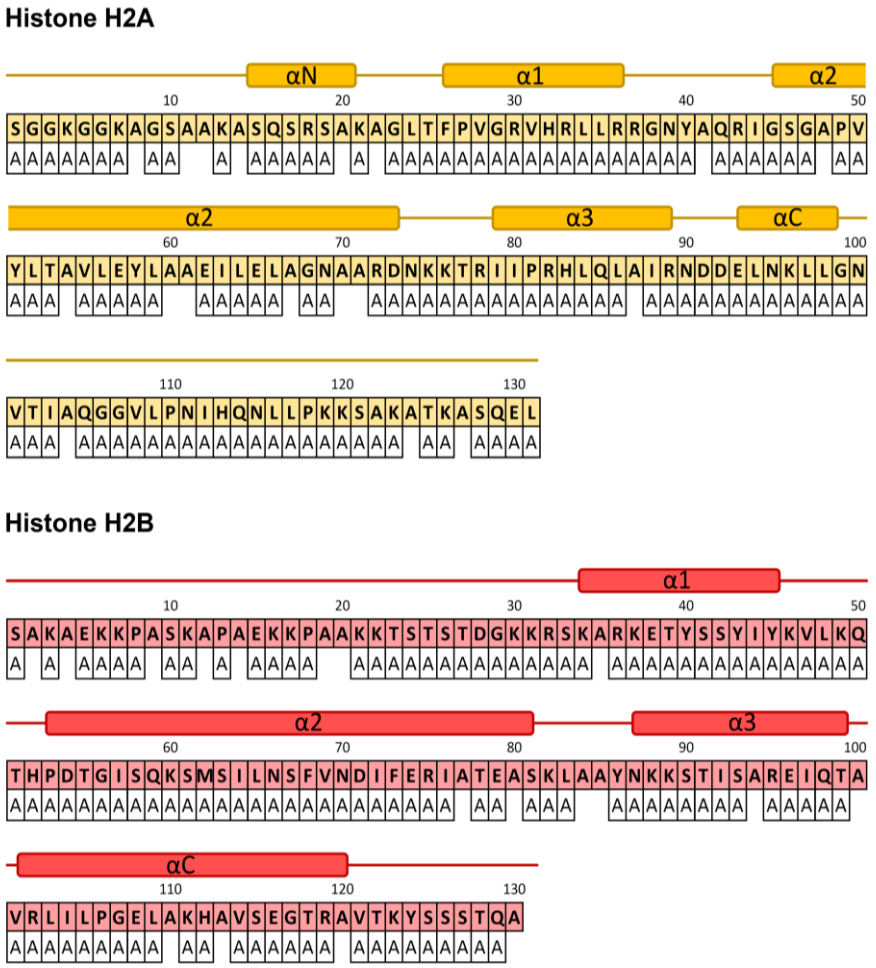


Figure 7. Histone mutant libraries used in this thesis.
Upper colored row contains the amino acid primary sequence for the four yeast core histones. Lower rows indicate the amino acid mutants available for each position in the mutant libraries from Dai *et al* 2008 (H3 and H4) and Nakanishi *et al* 2008 (H2A and H2B). Above each primary sequence, schematic representation of histones' secondary structure (helix α) from Luger *et al* 1997.

4.2 Mass spectrometry approaches

Histone mutants, as the ones described before, provide useful insides on the function of several histone residues. However, it does not demonstrate the *in vivo* existence of a PTM on a particular site.

A widely used approach to study histone PTMs is mass spectrometry (MS). MS allows a high throughput and *in vivo* detection of histone PTMs that has been instrumental to screen novel modifications in a wide range of organisms (Bonaldi, Imhof and Regula, 2004; Hyland *et al.*, 2005; Arnaudo and Garcia, 2013; Zhao and Garcia, 2015; Noberini *et al.*, 2018). One drawback of classic MS approaches is the use of trypsin to digest histones as part of the MS pipeline. Trypsin targets lysines and arginines and generates histone peptides around 4-20 amino acids long. However, lysines and arginines are highly enriched on histone tails, this generates smaller digested fragments that cannot be visualized by MS (discussed in El Kennani *et al.*, 2018; and Simithy, Sidoli and Garcia, 2018). Several strategies have been recently implemented in order to avoid such limitation. One straightforward approach is to use other proteases that target less abundant amino acids such as aspartic acid (AspN) or glutamic acid (GluC). These proteases generate peptides around 40-50 amino acids that include the histone tails (Sidoli and Garcia, 2017b). Another approach is to chemically derivate unmodified lysines by the incorporation of a propionyl group that reduces the cleavage sites of trypsin, also generating bigger fragments (Garcia, Mollah, *et al.*, 2007; Simithy, Sidoli and Garcia, 2018). Because both strategies generate bigger peptides, middle-down proteomics is required in order to resolve these

fragments by MS, instead of the classic bottom up approach used when digesting with trypsin (Garcia, Mollah, *et al.*, 2007; Sidoli and Garcia, 2017b; Simithy, Sidoli and Garcia, 2018). These two methods allow to resolve histone PTMs on histone tails. Another clear advantage is that by analyzing bigger fragments, coexisting PTMs are easily detected than in smaller peptides (Sidoli and Garcia, 2017a).

A pitfall of these MS strategies is that they tend to assess the global picture of histone PTMs. As previously mentioned, there is a whole range of histone PTMs coexisting in the same chromatin at the same time, with variable frequencies and locations. Hence, in bulk MS approaches, low abundant modifications are not efficiently detected and are diluted among the most abundant. Some strategies were developed to purify specific chromatin regions followed by a proteomic analysis. DNA binding proteins such as dCas9, TALENS or LexA are tethered to the desired loci, then the whole region is purified and the associated proteins, including histones, are analyzed by MS. These strategies allow to detect specific modifications occurring for instances in specific promoters or in close proximity to double strand breaks (Byrum *et al.*, 2012; Byrum, Taverna and Tackett, 2013; Waldrip *et al.*, 2014; Dai *et al.*, 2017; Wang *et al.*, 2017).

4.3 Antibodies based approaches

Antibodies are also a very powerful tool to study histone PTM dynamics both *in vitro* and *in vivo*. Companies such as ABCAM,

Covalab or Santa Cruz offer hundreds of different antibodies against histone modifications in a wide variety of formats and specificities. Antibodies are very instrumental not just to demonstrate the *in vivo* existence of a given modification, but also to determine its localization (ChIP or ChIPseq), its dynamics (Western blot techniques) or interactions (Co-IP). These techniques are less time consuming and often cheaper than proteomic strategies. However, the repertoire of available antibodies is often limited to the previously described histone modifications, lagging the study of less studied or novel histone PTMs. Moreover, the generation of new specific antibodies against histone PTMs is controversial and not always successful, especially regarding the specificity in recognizing the modified epitope (Egelhofer *et al.*, 2011; Peach *et al.*, 2012; Baker, 2015; Cornett, Dickson and Rothbart, 2017). Furthermore, the lot-to-lot differences between antibodies and their promiscuity often compromise the experimental reproducibility of antibody-based discoveries in the field. Despite the current efforts to address this issue, the use of antibodies is still problematic and open for discussion (Hattori *et al.*, 2013; Baker, 2015; Kungulovski, Mauser and Jeltsch, 2015). Nevertheless, besides the above-mentioned drawbacks, in the past years, antibodies against specific histone PTMs have been a very useful tool to study histone modifications and nucleosome dynamics *in vivo*.

OBJECTIVES

Our group is interested in understanding the mechanisms by which transcription is regulated in response to environmental stresses. Because histones are known to play a key role on transcriptional regulation, the objectives of this project were to give insights into the role of histone post-translational modifications in response to stress.

Specifically, the main objectives of this PhD were:

1. Validation of the high throughput screening used to identify novel histone residues relevant for stress-mediated transcription.
2. Characterization of the transcription phenotypes of H4-S47 and H4-T30 mutants upon osmotic and heat stress respectively.
3. Study the effects of H4-S47 and H4-T30 mutation on cell viability.
4. Characterization of the H4-S47 and H4-T30 phosphorylation *in vivo*.
5. Identification and characterization of the kinases modifying both residues *in vitro* and *in vivo*.
6. Selection and characterization of additional residues from the screening relevant for stress-mediated transcription.

RESULTS AND DISCUSSION

A genetic analysis reveals the histone residues required for transcriptional reprogramming upon stress

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RESULTS AND DISCUSSION

Environmental stresses induce a dramatic reconfiguration of the yeast transcriptional program. This reprograming consists in the activation and repression of several hundreds of genes in a few minutes scale, which depends on the stress, its intensity and duration (Gasch *et al.*, 2000; Causton *et al.*, 2001; Capaldi *et al.*, 2008; Pelet *et al.*, 2011). MAPKs Hog1 and Mpk1 and the transcription factors Hsf1 and Msn2/4, among others, are in charge of coordinating such transcriptional response (Richter, Haslbeck and Buchner, 2010; de Nadal, Ammerer and Posas, 2011; Saito and Posas, 2012). Histone modifications and nucleosome dynamics are key to properly modulate stress-induced transcription. In stress-responsive genes, nucleosomes impose a physical barrier that impairs transcription factor and RNA-Pol II binding during transcription initiation; as well as they slow down polymerase elongation in the gene bodies during mRNA elongation (Joshi and Struhl, 2005; Shandilya and Roberts, 2012; Weiner *et al.*, 2012; Bruzzone *et al.*, 2018). Hence, to allow transcription, histones are transiently displaced from actively transcribed genes (Kulaeva, Hsieh and Studitsky, 2010; Petesch and Lis, 2012; Weiner *et al.*, 2015).

Numerous factors control nucleosome dynamics. Chromatin remodelers such as SWI/SNF, RSC, INO80 and ISW1 use ATP to displace, evict or position histones across chromatin, facilitating or restricting transcription (Erkina, Tschetter and Erkine, 2008; Mas *et*

al., 2009; Weiner *et al.*, 2012; Klopf *et al.*, 2017). Another critical layer of regulation involves the post-translational modification of histones in a wide variety of forms and sites (see introduction sections 3.3 and 3.4). The different combinations of PTMs decorating histones and their intricate network of crosstalk interactions have been associated to active or repressed transcription. Upon stress, there are some examples of histone PTMs that experience drastic changes, with specific marks being dynamically written or erased. More strikingly, some histone PTMs such as acetylation or H3-K4 tri-methylation change their role to a completely opposite function, from activating marks in steady-state conditions, to repressive marks upon stress (Weiner *et al.*, 2012; Magraner-Pardo *et al.*, 2014; Nadal-Ribelles *et al.*, 2015).

Researchers in the field have used histone mutant libraries to study the function of specific residues in a wide variety of contexts. A classic and straight-forward approach is to assess mutant viability upon different stress conditions such as changes in temperature, diamide (reducing agent), acetic acid or upon DNA damage (Dai *et al.*, 2008; Huang *et al.*, 2009; Liu, Zhang and Zhang, 2014; Weiner *et al.*, 2015). However transcriptional studies have mainly focused on steady-state transcription of specific genes or by measuring telomere and rDNA silencing (Huang *et al.*, 2009; Hainer and Martens, 2011). Despite some studies also tested the incidence of such histone mutants on stress-induced transcription (Liu, Zhang and Zhang, 2014; Weiner *et al.*, 2015), much little is known regarding the role of specific histone residues in such transcriptional regulation. To fill this gap, we conducted a high throughput genetic

screening assessing the histone residue requirements for proper osmotic and heat stress-induced transcription in *Saccharomyces cerevisiae*.

Global map of histone residues required for stress-induced transcription

In order to systematically assess stress-induced transcription, we engineered yeast strains harboring a fluorescent reporter driven by different stress-responsive promoters. We chose the promoter of *STL1* (p*STL1*) that activates transcription upon osmotic stress, the promoter of *HSP82* (p*HSP82*) that responds to heat stress and the *ALD3* promoter (p*ALD3*) that induces transcription upon both heat and osmotic stress. These three promoters are targeted by different transcription factors: Hot1 and Sko1 regulate p*STL1*, Hsf1 regulates p*HSP82* and Msn2/4 regulates p*ALD3* (Eastmond and Nelson, 2006; Capaldi *et al.*, 2008; Ni *et al.*, 2009). By using these three promoters, we were able to assess stress-induced transcription driven by different transcription factors and stresses. We mated these strains with a library of histone mutants (described in introduction, section 4.1) carrying mutations in all amino acids in histones H2A, H2B, H3 and H4. This new collection of mutant strains was stressed with either osmotic stress (0.4M NaCl) or heat stress (39ºC) to activate the expression of the reporter (Fig 1A from manuscript). The fluorescence signal of each histone mutant was compared to its wild type to identify those that exhibited differences in such stress-induced transcription.

In our screening, 209 out of the total 498 histone residues mutated (approximately 42%) displayed transcriptional levels of the fluorescent reporter at least 10% different when compared with their wild type strain, for any type of mutation and promoter in response to osmotic and heat stress (Fig 1B and Table S1). This high number of residues relevant for transcription contrasts with the low ratio of essential residues for cell viability (around 5-10%) (Dai *et al.*, 2008; Nakanishi *et al.*, 2008). This data suggests that despite the high degree of conservation between species, nucleosome structure can accept a high degree of residue variation without compromising cell viability in optimal growing conditions. In contrast, dynamic transcription reprograming required by stress seems to tolerate less variation in the amino acid composition of histones. Other screenings using similar histone mutant libraries, but measuring rDNA or telomere silencing, obtained similar percentages of histone residues with phenotypic alterations (Dai *et al.*, 2008; Jiang, Liu, Xu, *et al.*, 2017).

Transcriptionally altered histone amino acid mutants were similarly distributed among the four core histones: 37.7% of H3 (51 of its 135 residues), 47% of H4 (48 of its 102 residues), 37.4% of H2A (49 of its 131 residues) and 46.9% of H2B (61 of its 130 residues) (Fig 1 and Table S1). These data suggest a similar contribution of the four proteins to nucleosome dynamics upon stress. When the three different promoters were analyzed, each of them had a similar number of affected residues, except for p*HSP82*. *HSP82* promoter showed significantly altered basal transcription levels in 56 mutants whereas none other promoter had such basal

transcription defect. A plausible explanation for this phenomena may be the specific features of the *HSP82* promoter. Although transcription of *HSP82* is specifically induced upon heat stress, in basal conditions the transcription factor Hsf1 is already bound to the promoter (Gross *et al.*, 1993; Erkine *et al.*, 1999). This relaxes the chromatin blockade and primes the transcription machinery for a rapid activation in case of a sudden increase on temperature. As a consequence, this also results in higher levels of basal transcription compared to the less leaky promoters such as p*STL1* and p*ALD3*.

Furthermore, we observed an enrichment on H2A and H2B residues with defects on basal transcription for the p*HSP82* reporter compared to the lesser cases of H3 and H4. This could be explained by the fact that H2A-H2B dimers are more easily removed from histone octamers during transcription, compared to H3-H4 tetramers that are more stable on chromatin (Zhao, Herrera-Diaz and Gross, 2005; Kulaeva, Hsieh and Studitsky, 2010; Arimura *et al.*, 2012; Cole *et al.*, 2014). Therefore, mutants for H2A and H2B may be more prone to abandon the histone octamer. This in turn would destabilize the nucleosome and promote higher levels of permissive basal transcription as observed in our screening.

Histone residues required for transcription upon stress are specific for each stress condition and promoter, potentially modifiable and mostly located on the nucleosome surface

In addition to a global picture of the relevant amino acids of the four core histones and the specificities for each promoter, our

screening also offered valuable information regarding the nature of the histone residues required for proper stress-induced transcription. Strikingly, there was little overlap between residues affected in different promoters for the same stress. Only 1 mutant residue displayed transcriptional alterations in both p*ALD3* and p*HSP82* reporter strains upon heat stress. Similarly, just 8 mutant residues overlapped between p*ALD3* and p*STL1* reporters upon osmotic stress (Fig 3A). It is well known that each promoter is regulated by different transcription factors: Msn2/4, Hsf1, Sko1 or Hot1 (reviewed in introduction, section 2.2); which can explain such a poor overlap. Along these lines, our results suggest that each promoter requires the modification of a unique and specific set of histone residues in order to modulate transcription despite being activated by the same extracellular stimuli.

Moreover, we also observed a modest overlap in the residues required for heat or osmotic stress for the same promoter (p*ALD3*-qV heat stress *vs* osmostress*,* Fig 3A), as only 16 out of 60 heat and 55 osmo-affected histone mutants were transcriptionally affected in both stresses. Albeit each stress seems to require a specific set of histone residues, there is at least some degree of common residues, which seem to be involved in a shared histone regulation between heat and osmotic stress. Msn2/4 drives *ALD3* expression for heat and osmotic stress (Navarro-Aviño *et al.*, 1999) and work done by our group already described Rpd3 as a common regulatory histone deacetylase required for both stresses (de Nadal *et al.*, 2004; Ruiz-Roig *et al.*, 2010). Thus, it is likely that factors associated to these regulatory elements may be the ones requiring such a specific set of

common histone residues. To further understand these specific histone requirements, it would be interesting to assess if these are shared between similar regulated genes (by either Msn2/4, Hsf1 or Hot1 and Sko1) by analyzing a broader panel of promoters.

How these histone residues modulate stress-induced transcription remains an open question. In some cases, it is tempting to assume that histone modifiers could target these residues. Certainly, modifiable residues such as lysines, arginines, threonines and serines rank at the top of the residues that were identify in our screening (Fig 3B). Individual histone mutants for residues whose modifications are known to modulate transcription were also identified in our screening. For instances: H3-K4me3 (Santos-rosa *et al.*, 2002), H3-K36me3 (Strahl *et al.*, 2002), H4-K20me (Nishioka *et al.*, 2002; Garcia, Hake, *et al.*, 2007), H3-K56ac (Williams, Truong and Tyler, 2008), H3-K122ac (Tropberger *et al.*, 2013), H4-K31ub (Kim *et al.*, 2013) and H3-K64ac/me3 (Di Cerbo *et al.*, 2014), these last two described in mammals. These examples validate the potential of our screening to detect novel histone modifiable residues and new undescribed PTMs.

Remarkably, most of the histone mutant residues affecting transcription were located in theoretically accessible areas of the nucleosome, in either the tails, lateral or the disk surfaces according to Dai *et al* 2008 classification (Fig 3C). Only around 20% of the identified residues are buried inside the nucleosome and presumably holding a more structural function rather than regulatory.

Histone residue mutants that displayed altered transcription of the reporters were not randomly distributed. When mapped in the tridimensional structure, we can observe some residues that cluster together in some specific regions (Fig 2 and 3D). Interestingly, some of these regions are maintained whereas others differ between promoters and stresses. These regions could be docking sites for nucleosome binding proteins, create a catalytic pocket for a modifying enzyme or be necessary for histone-DNA or histone-histone interactions. Indeed, there are some regions known to be relevant for transcription that are among these clusters. As an example, the residues in the nucleosome acidic patch H2A-E57, H2A-D65 and H2B-E116, which were identified in our screening, are known to be necessary to form higher order of chromatin compaction and for FACT-mediated nucleosome assembly/disassembly (Hodges, Gloss and Wyrick, 2017). Another known group of residues derived from our screening are arginines that directly contact with DNA (for example: H2A-R43, H2A-R78, H2B-R36, H3-R83 and H3-R63) whose mutations were described previously to affect transcription (Hodges *et al.*, 2015). Another example is the region comprising residues G15 to K20 in histone H4 N-terminal tail that it is necessary for proper H3-K79 methylation, that when mutated, also caused defects on stress-induced transcription (see introduction section 3.4 and introduction Fig 6) (Fingerman, Li and Briggs, 2007; Worden *et al.*, 2019). Overall, the identification of mutants in these histone regions exemplify the potential of our approach to detect new

uncharacterized sites relevant for nucleosome dynamics upon heat and osmotic stress.

The phosphomimetic mutation of H4-S47 is detrimental for proper activation of genes responsive to osmostress

After a detailed analysis of the screening results, we selected individual residues relevant for stress-induced transcription for further investigation aiming to report novel histone PTMs. We chose the H4-S47 based on the transcriptional defects displayed by its replacement to aspartic acid (H4-S47D mutant) upon osmotic stress and its accessible position within the nucleosome. This serine is located in the L1-L2 loop of the H3-H4 tetramer at the lateral surface of the nucleosome that closely contacts DNA (Hall *et al.*, 2009). The phosphomimetic H4-S47D mutant rendered cells with a decreased expression of the p*ALD3* reporter upon osmotic stress (Fig 4A). In contrast, the H4-S47 mutation to alanine (H4-S47A) had no appreciable phenotypic effect.

It is worth mentioning, that the constructs expressing the stress-dependent promoter reporter used in the histone mutant collections for the screening were not integrated in their endogenous loci (see materials and methods). For this reason, and to validate screening results, we analyzed the mRNA expression of endogenous osmo-responsive genes by northern blot. Similarly to the results obtained from the screening, the H4-S47D mutant showed a clear down-regulation of *ALD3* expression upon stress, whereas the H4-S47A behaved as the wild type strain (Fig 4B). The

same pattern of expression was observed for *HSP12*, another osmo-responsive gene. To broaden our perspective, we performed RNA sequencing for the H4-S47D mutant upon osmostress (0.4M NaCl for 10 minutes). From the 577 stress-induced genes observed in the H4 wild type strain, the H4-S47D mutant displayed down-regulation (at least by 2-fold change) of 385 of them (66.72%) (Fig 4C and D). The reference genes *ALD3* and *HSP12* genes were among those down-regulated genes. On the other hand, there was also a high degree of stress-repressed genes whose expression was up-regulated in the H4-S47D mutant (53.84%). Regarding the nature of these genes, there was no specific enrichment for a particular subcategory, nor any common preference for a transcription factor or regulatory element, except that all of them are stress-responsive genes. This lack of a clear pattern within the altered genes suggests that the H4-S47D mutant interferes with stress induced and repressed genes in a general manner.

Upon osmotic stress, there is a global redistribution of the transcriptional machinery on the genome that depends on the duration and intensity of such stress (Gasch *et al.*, 2000; Pelet *et al.*, 2011; Nadal-Ribelles *et al.*, 2012). On housekeeping genes, the occupancy of RNA-Pol II and other transcription activators is reduced upon stress, whereas the polymerase machinery and stress-dependent transcriptional activators are specifically recruited to osmo-responsive genes (Proft and Struhl, 2004; Miller *et al.*, 2011; Nadal-Ribelles *et al.*, 2012). This redistribution results in a stress-dependent expression pattern of genes that are up and down-regulated (Fig 4C and Audrey P Gasch *et al.*, 2000). Our RNA

sequencing results suggested that the phosphomimetic mutation of the H4-S47 does not only affect stress-induced gene expression but rather transcription regulation in a more general manner as it also alters the expression pattern of the down-regulated genes.

These transcriptional defects correlated with impaired growth of the H4-S47D mutant upon osmotic stress as assayed by growth curves and spot assays (Fig 4E and F). This observation seems to indicate that the transcriptional defects of the H4-S47D mutant ultimately limit its ability to adapt and restore growth upon osmotic stress when compared to wild type cells.

In addition to the effects of the H4-S47D mutant on stress-dependent transcription, RNA sequencing analysis also uncovered differences on non-stress genes (basal) compared to the wild type (Fig S1A). Interestingly, the H4-S47D mutant was notoriously prone to promote abnormally induced basal transcription (373 genes were up-regulated at least 2-fold compared to wild type) whereas only 23 genes were down-regulated. Of note, according to our analysis (Fig 4C), approximately 37% (138 of the 373 genes) of these basally induced genes fall into the category of osmo-induced genes. This suggests that despite the H4-S47D mutation affects transcription in a general manner it is prominently biased to stress-responsive genes. From our approach, we cannot discriminate the exact impact of such basal defects on the later transcriptional response.

If phosphorylation-mimic mutant is rendering a negative effect on stress-induced transcription, we could speculate that the

impairment of such modification in a H4-S47A mutant may result in an enhanced up-regulation of osmo-induced genes. So far, we did not observe such effect in the screening nor in the northern blot assays (Fig 4A and B). This lack of up-regulation by the H4-S47A mutant could be attributed to other negative regulators that modulate transcription as described in the introductory sections 2.2 and 2.3. We have not addressed by RNA sequencing the overall gene expression pattern of the H4-S47A mutant. Elucidating the phenotype of the A mutation would help to decipher the role of the H4-S47 residue in the transcriptional response upon osmotic stress.

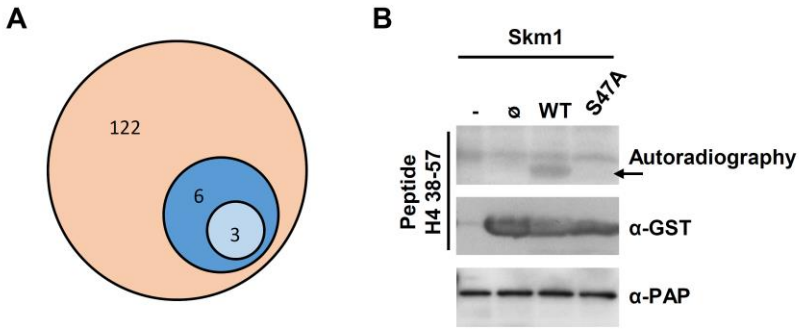
We have not conducted structural assays to determine the effect of the H4-S47D mutant on nucleosome structure. However, due to its close proximity to DNA, one could speculate that the addition of a negative charge in this residue may have a destabilizing effect on the nucleosome structure. Indeed, a previous work demonstrated that the phosphomimetic H4-S47D mutant decreases nucleosome occupancy and causes promiscuous transcription (Hainer and Martens, 2011). This fits with our observation for stress-repressed genes and for the basal gene induction. On the other hand, other authors also reported that a similar phosphomimetic mutant (H4-S47E) causes an increase in telomere and rDNA silencing (Hyland *et al.*, 2005). These regions are composed of less accessible chromatin for the transcriptional machinery in a way, similar to the down-regulation that we observed for stress-induced genes. These divergent transcriptional phenotypes suggest that the H4-S47D mutation has a specific effect depending on the genomic region. Further research is required to

determine the specificities of these genomic regions and how the residue H4-S47 interacts with them upon osmotic stress.

Cla4 and Ste20 PAKs phosphorylate H4-S47 and regulate the transcription of osmo-responsive genes upon stress

In mammals, phosphorylation of H4-S47 has been previously detected and characterized *in vivo* by mass spectrometry (Zhang *et al.*, 2003; Kang *et al.*, 2011; Zhang, Wang and Zhang, 2013). None of these previous studies neither assessed H4-S47 phosphorylation in response to osmostress nor in yeast. To characterize the role of H4-S47 phosphorylation upon stress, we screened the yeast kinome looking for potential kinase(s) performing such modification. We designed and purified from *E. coli* short H4 peptides (from amino acids 38 to 57) containing either the wild type H4-S47 or the non-phosphorylable alanine substitute (H4-S47A peptide). Then we assayed these peptides against TAP-tagged kinases purified from yeast in an *in vitro* kinase assay with radiolabeled ATP. Out of the 122 kinases assayed, 6 of them phosphorylated the histone peptide containing the H4-S47. 3 of these 6 kinases, Cla4, Ste20 and Skm1, were not able to phosphorylate the H4-S47A peptide, indicating that the phosphorylation was specific for the H4-S47 (Fig 5A and additional Fig 1A from this thesis). Cla4 and Skm1 are paralogs in yeast, for this reason and because Skm1 showed less phosphorylation than Cla4, we chose to further characterize only Cla4 and Ste20. We then confirmed that both kinases, Cla4 and

Ste20, phosphorylated *in vitro* the full-length histone H4 and not the full-length H4-S47A protein (Fig 5B).



Additional figure 1. Kinase assay screening for H4-S47.
(A) A screening based on a kinase assay identified 6 kinases out of 122 kinases assayed (dark blue circle) that phosphorylated a fused GST-short histone peptide (amino acids 38 to 57) containing the H4-S47. From these, 3 kinases (Cla4, Ste20 and Skm1) phosphorylated the H4-S47 peptide and not the H4-S47A peptide. **(B)** Skm1 phosphorylates H4-S47 *in vitro* (arrow). Fused GST-short peptides containing the H4-S47 (WT) or the mutated version (S47A) were used as substrates. Empty GST protein (Ø) and no substrate (-) were used as negative controls. Radiolabeled peptides were resolved by SDS-PAGE, transferred to a nylon membrane, and detected by autoradiography. TAP-tagged kinases and GST-tagged histone peptides were detected by western blot. Similar results were obtained for Cla4 and Ste20 (manuscript figure 5B).

To assess the phosphorylation of H4-S47 *in vivo*, we tested several commercial and custom-made antibodies against the modification but none was specific enough to monitor the phosphorylation. Currently, we are addressing this question by using MS approaches, however, up-to-date we were not able to show direct *in vivo* evidences of H4-S47 phosphorylation.

Kinases Cla4 and Ste20 are integral components of the HOG pathway that orchestrates the response to osmotic stress by phosphorylating the MAP3K Ste11 (see introduction section 1.1 and

Fig 1). Of note, these kinases are the yeast orthologs of the human PAK2 kinase family that were previously described to phosphorylate the H4-S47 residue in humans (Kang *et al.*, 2011). In 293T and HeLa cells, H4-S47 phosphorylation by PAK2 increases the affinity of HIRA (histone chaperones) for the H3.3-H4 tetramer. This generates a redistribution of H3.3 variant across the genome in detriment of the canonical H3.1 (Kang *et al.*, 2011). Several chaperones mediate H3-H4 positioning in *Saccharomyces cerevisiae*, some of them are orthologs of the human HIRA complex (Gurard-Levin, Quivy and Almouzni, 2014), but their role in H4-S47 mediated gene silencing still needs to be determined. Another relevant question to address is how H4-S47 phosphorylation mediates transcription down-regulation upon osmotic stress.

Because the phosphomimetic H4-S47D showed reduced mRNA levels for several osmo-responsive genes upon stress, we then tested if a constitutively active kinase Cla4 (Cla4-ΔN) had similar effects on stress-induced transcription. Indeed, stressed cells expressing the Cla4-ΔN were not able to equally induce transcription of the selected osmo-responsive genes as cells expressing wild type Cla4 (Fig 5C). This result supports our conclusion for the effect of the phosphorylation of H4-S47 on stress-induced transcription (Fig 4B).

Of note, both kinases Cla4 and Ste20 localized at the promoters of *ALD3* and *HSP12* upon 5 minutes of osmotic stress (Fig 5D). Interestingly, such binding was dependent on upstream activation of both kinases by the Sho1 branch and transcription activation by Hog1, but it was independent of the down-stream

activation of Ste11 (Fig 5D). A previous study already reported that under anaerobic conditions both kinases translocate to the nucleus and regulate transcription (Lin *et al.*, 2009). This suggests that upon osmotic stress, once Cla4 and Ste20 are activated by their upstream factors (introduction Fig 1), both kinases translocate to the nucleus to localize to osmo-responsive promoters where they may make contact with histones.

Cla4 and Ste20 are not only activated upon osmotic stress. Polarized growth and the mating pheromones response pathways also result in Cla4 and Ste20 activation (Holly and Blumer, 1999; Bardwell, 2005; Park and Bi, 2007). These pathways share several elements with the HOG pathway such as the Cla4 and Ste20 activation by the GTPase Cdc42 and the downstream phosphorylation of Ste11 by the two kinases. Moreover, the activation of these two pathways results in changes of the transcriptional program in yeast (Cullen and Sprague, 2012; Merlini, Dudin and Martin, 2013). Despite these similarities, the polarized growth pathway and the mating pheromone pathway are activated by different means than the HOG pathway. Interestingly, as part of the response to anaerobic conditions, Cla4 and Ste20 translocate to the nucleus, associate with the transcription activator Sut1 and inhibit expression of sterol uptake genes (Lin *et al.*, 2009). Cla4 and Ste20 kinase activity is required to mediate such transcriptional inhibition whereas downstream signaling by Ste11 is not necessary (Lin *et al.*, 2009). These observations in sterol uptake partially resemble our discoveries upon osmotic stress. It would be interesting to assess if Cla4/Ste20 regulate the transcriptional

program upon osmotic stress through H4-S47 by a similar mechanism than the one modulating the sterol uptake.

Human phosphatases PP1α, PP1β and Wip-1 were proposed to remove H4-S47 phosphorylation *in vivo* (Zhang, Wang and Zhang, 2013). The only annotated yeast ortholog of these phosphatases is the serine/threonine phosphatase Glc7 (Offley and Schmidt, 2019). No direct evidence has ever linked this phosphatase with the yeast H4-S47 nor osmotic stress. However, histones are a well-known target of Glc7 (Hsu *et al.*, 2000; Ramaswamy *et al.*, 2003; Bazzi *et al.*, 2010). It would be interesting to test if Glc7 or any other yeast phosphatases remove H4-S47 phosphorylation.

Overall, our results suggest that Cla4 and Ste20 phosphorylate H4-S47 and that such modification has an effect on the remodeling of the transcriptional program upon osmotic stress.

H4-T30A and H4-T30D mutants are not able to properly regulate transcription upon heat stress

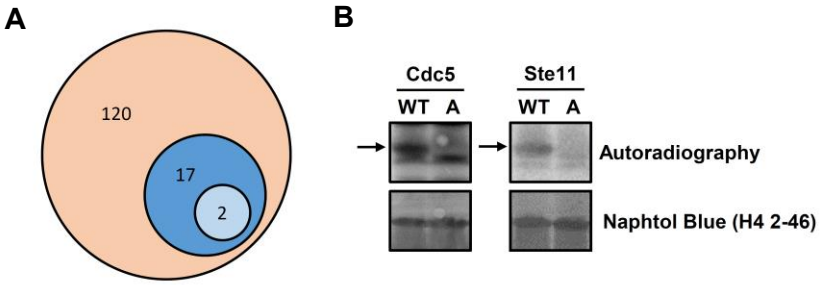
Another interesting residue uncovered from the transcriptional screening is the H4-T30. Following the same rational as for the H4-S47, we focused on the H4-T30 because it displayed clear transcriptional defects upon heat stress and its location within the nucleosome. More precisely, this threonine is located next to the junction between the N-terminal tail and the helix α1 of histone H4 (Luger *et al.*, 1997). In our screening, the non-modifiable alanine mutant H4-T30A showed reduced expression of the fluorescent reporter from the *ALD3* promoter upon heat stress. In contrast, the

phosphomimetic mutant (H4-T30D) had no detectable defects when compared to wild type (Fig 6A).

Following the same strategy developed for the characterization of the H4-S47 mutants, we validated H4-T30A defects on transcription in response to heat stress on the endogenous expression of *ALD3* and *CTT1* by northern blot (Fig 6B). However, when we analyzed the H4-T30A mutant by RNA sequencing, we found a very modest number of significantly affected genes. Only 15 of the 736 heat-induced genes (2% of the total) had reduced levels of mRNA compared to wild type, *ALD3* was among these few genes (Fig 6C and D). Next, based on the data from the northern blot analysis of the H4-T30D mutant, which indicated some degree of *ALD3* down-regulation (Fig 6B *ALD3* at 15 minutes), we also performed RNA sequencing on the phoshomimetic mutant. This genome wide analysis demonstrated that the H4-T30D mutation caused more severe defects on transcription upon heat stress, with a high number of stress-induced genes down-regulated (approximately 27%), while 29% of the stress-repressed genes were up-regulated (Fig 7A). Selected genes from RNA sequencing were validated by northern blot (Fig 7B). The H4-T30D mutant had a modest effect on basal transcription, but clear effects on stress-induced transcription (Fig S1C). Thus, the addition of a negative charge on the residue H4-T30 massively deregulates transcription of heat-responsive genes upon heat stress. Corresponding to its transcriptional alteration, the H4-T30D mutant showed impaired growth upon heat stress when compared to wild

type and H4-T30A mutant both in growth curves and spot assays (Fig 7C and D).

To further characterize the role of H4-T30 upon heat stress, we screened for kinases that phosphorylate the threonine 30 in a kinase screening similar to the one performed for the H4-S47. We generated and purified from *E. coli* a peptide containing wild type H4-T30 or the non-phosphorylable H4-T30A mutant (from amino acids 2 to 46). The kinase screening yielded, 2 kinases (Ste11 and Cdc5) that were able to phosphorylate the H4-T30 and not the alanine substitute (additional Fig 2). However, we could only reproduce in dedicated experiments the phosphorylation by the MAPK3 Ste11 (Fig 7F). We then tested the effect of *STE11* deletion on gene expression. Correspondingly, we found that the *ste11Δ* strain showed similar defects on *ALD3* and *CTT1* transcription than the H4-T30A mutant (Fig 7F), suggesting a possible link between the kinase and this histone residue.



Additional figure 2. Kinase assay screening for H4-T30.
(A) A kinase assay screening identified out of 120 kinases assayed 17 kinases (dark blue circle) that phosphorylated a fused GST-short histone peptide (amino acids 2 to 46) containing the H4-T30. From these 17, 2 kinases (Cdc5 and Ste11) phosphorylated the H4-T30 peptide (WT) and not the H4-T30A **(B)**. Radiolabeled peptides were resolved by SDS-PAGE, transferred to a nylon membrane, and detected by autoradiography. TAP-tagged kinases and GST-tagged histone peptides were detected by western blot.

As mentioned before, the kinase Ste11 is a core component of the HOG, mating pheromone and the polarized growth pathways. Besides its well-known roles as MAP3K in these pathways, several papers describe that Ste11 is also activated and has an activating crosstalk role on the CWI pathway upon thermal and cell wall stress by phosphorylating the MAP2K Mkk1 (see introduction section 1.2 and Fig 2) (García *et al.*, 2009; Jin *et al.*, 2015; Leng and Song, 2016). These studies and our data suggest that Ste11 may have a role on modulating the transcriptional response generated by the CWI pathway through H4-T30 modification.

Of note, the kinases modifying residues H4-S47 and H4-T30 are integral parts of the MAPK pathways that respond upon osmotic and heat stress. As described in the instruction section 1, upon stress, MAPKs Hog1 and Mpk1 translocate to the nucleus to activate transcription. Our results suggest that other upstream members of their pathways as MAP4K Cla4, Ste20 and MAP3K Ste11 are also implicated in transcription regulation upon stress. Their exact regulatory role still needs to be determined, but these kinases may be part of novel regulatory feedback loops regulating stress-induced transcription by targeting histone dynamics upon heat and osmotic stress.

To date, nothing has been reported on the modification or specific function of H4-T30. Our genome wide analysis suggested that the H4-T30 phosphorylation has a deleterious effect on heat stress-transcription. The neighboring H4-K31 is a well-known residue, which is modified mainly by ubiquitination, methylation and acetylation among other modifications in humans and yeast

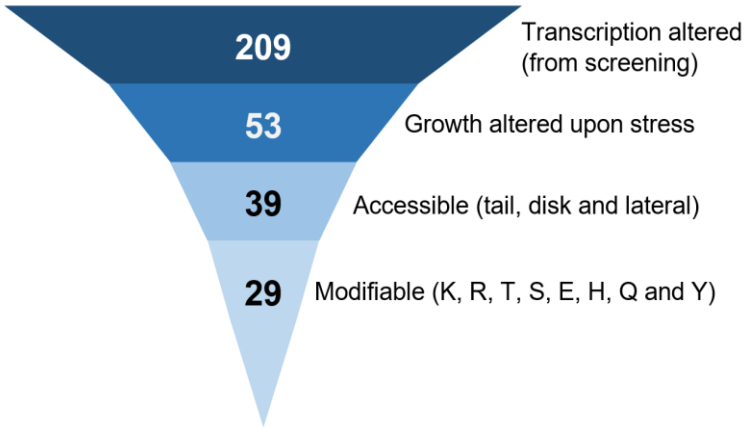
(Freitas, Sklenar and Parthun, 2004; Garcia, Hake, *et al.*, 2007; Xie *et al.*, 2012; Kim *et al.*, 2013). The exact role of such a broad panel of modifications has not been well established yet. Some reports suggest that human H4-K31 ubiquitination has a destabilizing effect on nucleosomes and hence it acts as a transcription activating mark (Kim *et al.*, 2013; Machida *et al.*, 2016). In contrast, yeast H4-K31 mutants have increased levels of telomeric and rDNA silencing and decreased binding of the transcription elongation factor Spt16 (Hyland *et al.*, 2005; Nguyen *et al.*, 2013). Thus, due to its close proximity, H4-T30 phosphorylation may interfere with H4-K31 modification by altering its docking for modifying enzymes. Alternatively, a specific H4-T30 post-translational modification could also act as an active regulatory mechanism that modulates H4-K31 modification.

Moreover, in our screening, the residues close to the H4-T30, from H4-I26 to H4-I29, have similar altered transcription of the reporter upon heat stress (Fig 2). Some previous screenings reported phenotypic alterations of histone mutants in this region but no particular function was associated to them (Huang *et al.*, 2009). Instead, work done on humans suggests that this particular H4 N-terminal region may have a structural function in the histone octamer by interacting with several other histone residues through hydrogen bonds (Montellier *et al.*, 2013). Therefore, the transcriptional phenotypes of the H4-T30 may also be caused by the interplay with its surrounding region.

Further characterization of novel histone residues relevant for stress-induced transcription

Our screening also highlighted the relevance of other individual residues required for proper stress-induced transcription. This opens the possibility to describe novel histone PTMs that define specific mechanisms for histone dynamics. To narrow down the list of 209 histone residues that showed altered transcription (Table S1), we conducted a high throughput viability assay upon stress. We spotted in agarose plates the mutants of the 209 histone residues and grow them for 3 days either upon osmotic (1.2M NaCl) or heat stress (39ºC). Viability was scored by comparing to histone wild type strains. This viability assay indicated that 53 out of the 209 histone residues that were identified for having transcriptional alterations had also growth defects upon stress. Next, we selected the residues that were more accessible to regulatory elements, based on their location within the nucleosome (lateral, tail and disk). We discarded the histone residues classified as buried assuming that the phenotypes observed in their mutants could be due to alterations of the nucleosome structure. Finally, because we aim to identify novel histone modifications, we also filtered out residues that cannot be post-translationally modified. Our selection process rendered 29 histone residues that were identified in the transcriptional screening for having transcriptional defects and impaired growth upon stress, that are accessible and modifiable (Additional Fig 3). Selected residues were systematically assayed by northern blot to validate the screening results and to confirm such transcriptional defects upon stress. In addition, we conducted growth curve assays to

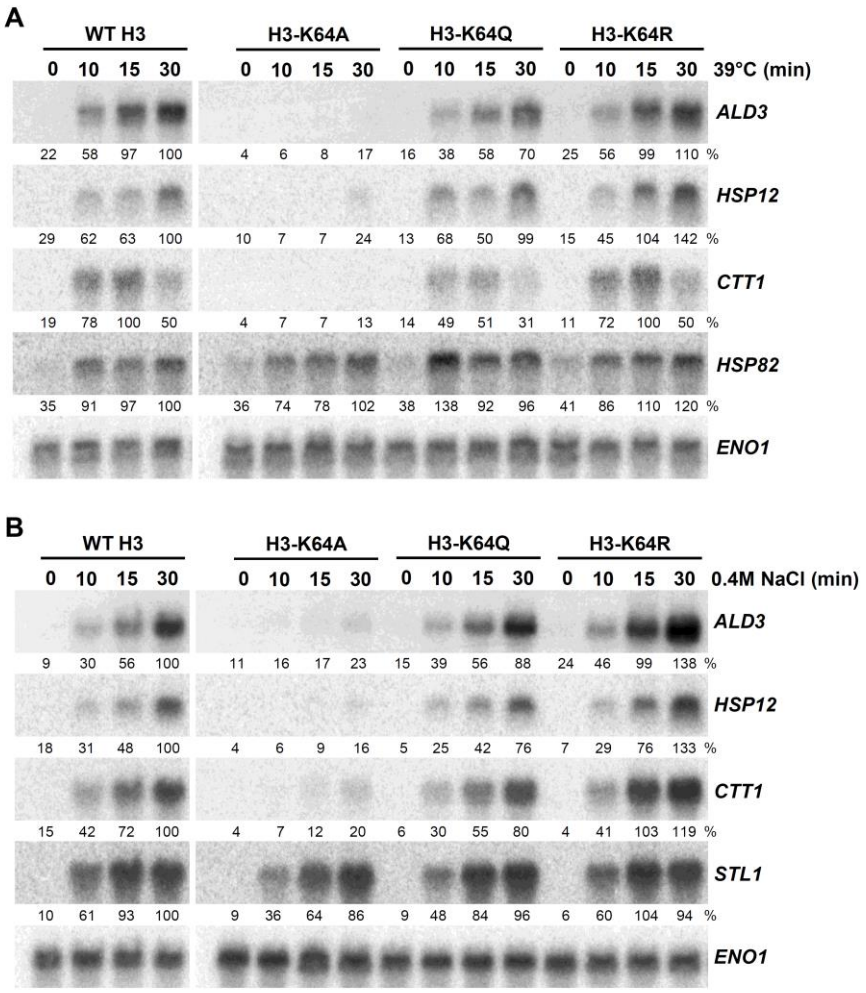
selected residues to add complementary information of these histone mutants.



Additional figure 3. Schematic pipeline to select histone residues for further study from the transcriptional screening.
From top to bottom. From the 209 histone residues identified in our high throughput transcriptional screening, 53 of them showed altered growth upon heat or osmotic stress. From these 53 histone residues, 39 were located in accessible regions according to die *et al* 2008 classification. From these, 29 are histone residues that can be post-translationally modified.

One interesting new candidate to follow up is the lysine 64 in H3 (H3-K64). This lysine is located on the lateral surface of the histone octamer in close proximity with the inner gyre of DNA (Luger *et al.*, 1997). In mouse embryonic stem cells, H3-K64 is found acetylated in actively transcribed regions (Di Cerbo *et al.*, 2014). The addition of this acetyl group neutralizes the positive charge in the lysine residue destabilizing the nucleosome structure. On the other hand, during mouse embryo development, H3-K64 methylation has an opposite effect and correlates with

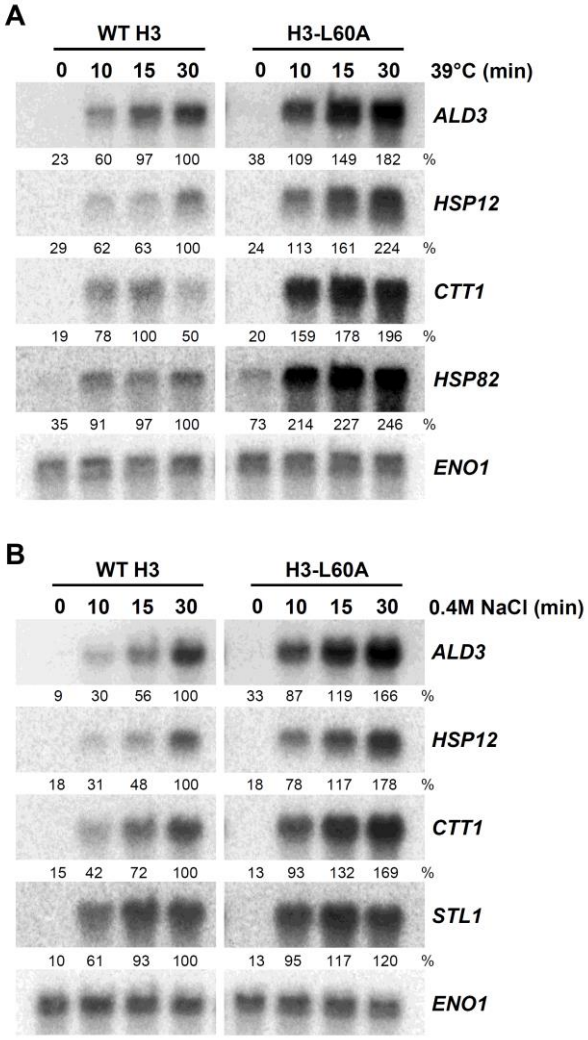
heterochromatin and transcriptionally inactive regions (Daujat *et al.*, 2009). Of note, up to date, there is no evidence for H3-K64 modification in yeast. By northern blot analysis, the non-modifiable H3-K64A mutant caused almost no *ALD3*, *HSP12* and *CTT1* gene expression, while having a much modest effect on *HSP82* and *STL1* in response to heat and osmostress (additional Fig 4A and 4B). These results suggested that the modification of this histone residue in yeast has a profound effect on stress-induced transcription for a particular subset of genes, while others remain largely unchanged. Following, the H3-K64Q acetyl mimetic mutant resulted in a much milder down-regulation of stress-induced genes (additional Fig 4A and 4B). On the other hand, the H3-K64R mutant, that retains the positive charge and can be methylated but not acetylated, causes a transcription up-regulation of the selected genes (additional Fig 4A and 4B). Overall, these results seem to indicate that the dynamic modification of this histone residue has a profound effect on stress-induced transcription. It would be worth testing by RNA sequencing the genome wide degree of such transcriptional defects to clearly define the genes regulated by the H3-K64 modification. Moreover, addressing by MS or antibody based techniques (if available) if any PTMs can be identified on H3-K64 and their dynamics upon stress would help better characterize the role of this histone residue. Additional investigation will be also required to elucidate the exact role of this residue in the transcriptional reprograming shared upon heat and osmotic stress.



Additional figure 4. Stress-induced transcription analysis of H3-K64 mutants upon heat and osmotic stress.
H3 WT and the H3-K64A, Q and R mutant strains were subjected to heat stress (39ºC) **(A)** or osmostress (0.4M NaCl) **(B)** for the indicated times. Total mRNA was assayed by northern blot with radiolabeled probes for *ALD3*, *HSP12*, *CTT1*, *HSP82* and *STL1* (stress-responsive genes) and *ENO1* (loading control). RNA quantification is expressed in percentage as the ration of mRNA levels normalized by *ENO1*.

In addition to amino acids that may be potentially modified, the transcriptional screening also offered valuable information about typically unmodifiable histone residues whose function may be also relevant in the context of stress. One example of such residue is the leucine 60 in H3 (H3-L60), which is located in the disk surface of the nucleosome. The leucine substitution to alanine highly increased the expression of both heat and osmo-induced genes (additional Fig 5A and 5B). Such clear effects on stress-induced transcription may be caused by a structural role of this leucine within the nucleosome. Therefore, its mutation may destabilize nucleosome structure and thus facilitate higher rates of transcription. Alternatively, the observed effects of the H3-L60A mutant could be caused by a deficient interaction with other regulatory elements. For instance, H3-L60 mutation may alter a docking site for proteins that modulate nucleosome dynamics. In order to fully decipher the role of H3-L60 in stress induced transcription further studies assessing its structural relevant will be required. Overall, these observations and other published data (Hainer and Martens, 2011; García-Pichardo *et al.*, 2017; Hodges, Gloss and Wyrick, 2017) point out that unmodifiable residues may have functions equally relevant for transcription than modifiable amino acids.

These additional two residues further exemplify the potential of our transcriptional screening to identify novel histone residues relevant for stress-induced transcription. The study of these and other novel residues opens new possibilities for future research, that will ultimately contribute to add new information to the current understanding of histone dynamics and regulation upon stress.



Additional figure 5. Stress-induced transcription analysis of H3-L60 mutants upon heat and osmotic stress.
H3 WT and H3-L60A mutant strains were subjected to heat stress (39ºC) **(A)** or osmostress (0.4M NaCl) **(B)** for the indicated times and analyzed by northern blot as in additional figure 4.

PERSPECTIVES

The transcriptional screening presented in this thesis has been instrumental to identify the histone residues required to drive proper stress-induced transcription upon osmotic and heat stress. The high number of residues identified revealed the complex interplay of histone requirements to finely tune transcription in response to stress. It also uncovered several histone regions that are important for such transcriptional outburst. These regions may be relevant due to their physical properties, for instance, by interacting with DNA or by stabilizing the nucleosome structure. However, they could also function as landing platforms for proteins specifically required for stress-mediated transcription. The poor overlap of histones residues between p*ALD3*, p*HSP82* and p*STL1* adds another layer of specificity to the already known regulatory differences of the three stress-dependent promoters analyzed here. Moreover, the modest overlap observed between type of stresses (osmotic and heat stress) also suggests that the mechanisms governing both transcriptional responses differ also at the histone level. The mechanisms and molecular players behind such differences still need to be fully characterized.

To further demonstrate the potential of our screening, we characterized two new residues relevant for transcription regulation upon osmotic and heat stress, the H4-S47 and H4-T30 respectively. Our discoveries pointed out the possibility of two novel negative feedback loops modulating stress-induced transcription in yeast. Further research is required in order to complete the mechanisms behind such observations. From the screening, we also selected

other histone residues and started to characterize them in order to identify novel histone PTMs relevant for stress-mediated transcription.

In the thesis presented here, we were not able to demonstrate the *in vivo* presence of H4-S47 nor H4-T30 phosphorylation. We assayed a number of commercial and custom-made antibodies but none of them was specific enough for the identification of such modifications. To solve this drawback, we started to implement a single locus proteomic technique named CRISPR-ChAP-MS developed in Waldrip *et al.*, 2014. As described in the introduction, we chose this technique for its potential to retrieve a single genomic locus by affinity purification of a TAP tagged death Cas9 (dCas9) directed with a guide RNA (gRNA) to a locus of interest. Then, proteins associated to this locus, including histones and their modifications, are identified by MS. We plan to design gRNAs specific for stress-induced promoters such as *ALD3*, *HSP12*, *STL1* or *HSP82* and perform the technique on cells treated with heat and osmotic stress. By this approach, we will be able to define *in vivo* the histone PTMs that are specifically placed or removed upon stress in these specific loci.

Because of the high homology between yeast and human histones and some common regulatory mechanisms, our results are also relevant to better understanding histone dynamics in mammals. Moreover, as defects on histone modifications and their miss-regulation are associated to several high impact disease such as cancer or neurodegenerative diseases (Soragni *et al.*, 2012; Anderson and Turko, 2015; Anderson *et al.*, 2015; Noberini *et al.*,

2018), our work can also bring new insights relevant for human health research.

Personal contribution to this work: the initial transcriptional screening was performed by CV and CS. I participated in the validation of the screening, and the experimental design, execution and discussion of the results described in this article for the residues H4-S47 and H4-T30 and the characterization of additional potential interesting histone residues.

CONCLUSIONS

The following conclusions can be reached from this PhD thesis:

- A high throughput screening has served to reveal histone residues required for proper heat and osmotic stress-induced transcription.

- In the screening, mutants of 209 histone residues show defects on stress-induced transcription.

- In general, the subset of histone residues identified in the screening are promoter and stress specific, suggesting a modest overlap between histone regulation for the three promoters analyzed and for heat and osmotic stress.

- Mutations on modifiable and accessible residues show higher rates of transcriptional defects in response to stress.

- The H4-S47D mutant show transcriptional defects on osmotic stress-mediated transcriptional reprograming and increased levels of basal transcription of several genes. This suggests a detrimental effect on transcription of the H4-S47 modification.

- The yeast homologs of the mammalian PAK2 kinase family, Cla4 and Ste20, phosphorylate the H4-S47 and localize at osmo-responsive promoters upon osmotic stress. Correspondingly, a Cla4 hyperactive mutant mimics the transcriptional defects of H4-S47D.

- The H4-T30A and H4-T30D mutants show altered transcription upon heat stress, pointing out that the dynamic modification of the H4-T30 residue is necessary for a proper transcriptional response upon heat stress.

- The yeast HOG pathway MAP3K Ste11 phosphorylates H4-T30 and its deletion mimics H4-T30A down-regulation in heat-induced transcription.

- The transcriptional validation and characterization of novel histone residues identified in the screening serves to select additional histone residues, such as the H3-K64 and H3-L60, for further studies of their role on stress-mediated transcription.

SUPPLEMENTARY ARTICLE

Shaping the transcriptional landscape through MAPK signaling

Mariona Nadal-Ribelles*, Carme Solé*, Gerard Martínez-Cebrián*, Francesc Posas** and Eulàlia de Nadal**

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DISCUSSION

In eukaryotes, MAPK pathways modulate a wide variety of cellular behaviors. These pathways sense extracellular stimuli, integrate and transmit the information to coordinate the appropriate response to inputs. Part of this response is devoted to modulate and control specific gene expression programs. In this review, we compiled and discussed current knowledge regarding the control of MAPK pathways on transcription regulation from yeast to mammals.

MAPKs regulate gene expression all the way from transcription initiation, elongation and termination to mRNA modification, export and translation (Cargnello and Roux, 2011; Seger and Wexler, 2015). Their activities modulate the final transcriptional outcome and the levels of protein expression in numerous and different ways as detailed in the review. MAPKs can act as transcriptional repressors or activators depending on the cellular needs and context. Due to their central role, MAPK pathways miss-regulation is relevant in several human pathologies such as cancer, inflammatory and immunologic diseases (Gubern *et al.*, 2016; Seidel *et al.*, 2016; Maik-Rachline *et al.*, 2018).

Central components of the MAPK signaling cascades are generally well defined. In contrast, detailed characterization of several down-stream and up-stream elements is still lacking. As an example, in mammals the cohort of upstream sensors activating MAPKs and they interplay have not been complete characterized

yet. Also, the trans-activating mechanisms that modulate MAPK activation through other MAPKs pathways are poorly understood. Additionally, the gaps in understanding MAPK-mediated gene termination and the control of non-coding RNA synthesis are also significant. Another research gap is the elucidation of the exact role of the MAPK2 (for instance MEK1/2 and MKK6) in gene expression. Although it is well accepted that they associate to chromatin, their role there is still controversial.

MAPK pathway activation, outcomes and regulations often vary in a cell-type specific manner. However, MAPK pathways and their control on gene expression have been studied in multiple model organisms and in different cell lines. Therefore, although such mechanisms may be conserved across cell lines and organisms, an integrative view of all these observations is still missing.

Further investigation is required to fully understand the above-mentioned gaps to understand the role of MAPK pathways in the context of disease. It is also necessary to tackle the high degree of variable behaviors and responses depending on cell type to develop treatments that are more specific and effective. Ultimately, MAPK pathway control represents an opportunity to develop novel targeted therapeutic treatments to life-threatening diseases.

Personal contribution to this work: I have taken part in all steps of the writing, editing and revision of this review article.

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LIST OF ABBREVIATIONS

CWI pathway: cell wall integrity pathway.

dCas9: death Cas9

ESR: environmental stress response.

gRNA: guide RNA.

HATs: histone acetyl transferases.

HDAC: histone deacetylases.

HOG pathway: high osmolarity glycerol pathway.

HSE: heat shock elements.

MAPK: mitogen-activated protein kinase.

MAPKK or MAP2K: mitogen-activated protein kinase kinase.

MAPKKK or MAP3K: mitogen-activated protein kinase kinase kinase.

MAPKKKK or MAP4K: mitogen-activated protein kinase kinase kinase kinase.

mRNA: messenger RNA.

MS: mass spectrometry.

Msn2/4: Msn2 and Msn4.

PIC: pre-initiation complex.

PKA pathway: protein kinase A pathway.

PTM: pots-translational modification.

RNA-Pol II: RNA polymerase II.

SAGA: Spt-ADA-Gcn5 acetyltransferase complex.

SIR: silent information regulator.